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Fourier Transform Infrared (FTIR) Characterization, Antioxidant Potential, and Anti-Parkinsonian Activity of *Dipteracanthus patulus* (Jacq.) Leaf Extracts in a 6-OHDA-Induced Rat Model

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ABSTRACT

Parkinson's disease (PD), a debilitating neurodegenerative disorder that involves degenerative changes to the dopaminergic neurons in the nigrostriatal cortex, affects millions worldwide. Males are believed to be more prone to PD than females, with limited therapeutic options. Aim of the study in search of novel therapeutic agents, investigate the FT-IR characterization, antioxidant potential, and anti-Parkinsonian activity of Dipteracanthus patulus (Jacq.) leaf extracts in a 6-hydroxydopamine (6-OHDA)-induced rat model. The leaf extracts were characterized using FT-IR spectroscopy, and their antioxidant potential was evaluated using various in vitro assays. The anti-Parkinsonian activity was assessed in a 6-OHDA-induced rat model, where the extracts were administered to evaluate their neuroprotective effects on motor functions, dopamine levels, and oxidative stress. The results showed that the leaf extracts exhibited significant antioxidant activity and attenuated oxidative stress, motor deficits, and dopamine depletion in the 6-OHDA-induced rat model, indicating their potential as a therapeutic agent for PD. This study provides evidence for the potential of Dipteracanthus patulus leaf extracts as a natural remedy for the management of PD, offering a promising alternative to conventional therapies.

Keywords: Parkinson's, Dopamine, FT-IR, 6-OHDA, Oxidative stress, Antioxidant, Dipteracanthus patulus (Jacq.).

INTRODUCTION

The world suffers from neurological disorders, like Alzheimer's, Parkinson's, and epilepsy. Parkinson's disease (PD) was first described by James Parkinson in 1817 and is the second most common neurodegenerative disorder of the elderly. It is a progressive neurodegenerative motor disorder characterized by degenerative changes to the dopaminergic neurons in the nigrostriatal pathway[1,2]. Worldwide, approximately 1% of people above 70 have PD, and only a small number of people suffer from familial PD or Parkinsonism. Males are believed to be more prone to PD than females[3]. In PD, motor skills and cognitive abilities are affected and can only be treated with conventional therapeutic approaches that alleviate symptoms. Levodopa (l-DOPA) is the most effective therapy for PD; however, the disease progresses with significant side effects in the long term, so finding a treatment that inhibits its progression is necessary. A wide range of current investigations are focused on finding novel substances that may provide therapeutic benefits for patients with PD [4]. There is no drug currently available for the treatment of Parkinson's disease that offers permanent benefits for patients. However, Nutritive Scientists have found that natural products can reduce the incidence of Parkinson's drugs with a comparatively low rate of adverse events while attenuating the symptoms of the disease[5]. Phytochemicals such as flavonoids, phenolic acids, stilbenes, and lignans, along with terpenes, are the most abundant types of phytochemicals with antiparkinsonian effects. There are several mechanisms through which phytochemicals exert their anti-Parkinsonian effect. Among them, widely accepted mechanisms are, suppression of apoptosis (by lowering Bax/Bcl-2 and caspase-3, -8, and -9, as

Relevant conflicts of interest/financial disclosures: The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

well as the accumulation of α-synuclein), reduction in the loss of dopaminergic neurons, dopamine depletion, and a decrease in proinflammatory cytokines (Eg; prostaglandin E2, interleukin-6, etc.,). The anti-Parkinsonian effect can also be attributed to the modulation of cellular and nuclear inflammatory signaling elevation of neurotrophic and antioxidant factors. Thus, plant-derived natural products could potentially be used as pharmaceutical drugs or adjuvant treatments to conventional therapies [6].

Dipteracanthus patulus (D.patulus) belongs to the family Acanthaceae. The major phytoconstituents found in *D.patulus* are Tannins, Saponins, Alkaloids, Steroids anthroquinones, Triterpenoids, and Flavonoids. It has been used as a medicine for a variety of diseases including Gonorrhea, Sore eyes, Syphilis, Renal infection, Cough, Scalds, Stomach ache, Kidney stones, Tumors, Rheumatic complaints, Dental problems, Itches, Insect bites, Paranchia, Veneral diseases for Wound healing, Cardiotonic, Antiulcer agent Sedative, Antipyretic agent, Anti-inflammatory and Anti-diabetic and also antidote of poison to Kaduvachilandhi[7,8].

MATERIALS AND METHODS:

Experimental design:

Preparation of Ethanol Extracts of *Dipteracanthus patulus* (EEDP) and Chloroform Extracts of *Dipteracanthus patulus* (CEDP):

D. patulus Leaves collected were dried at room temperature under shade for 15 days and coarsely powdered. The powdered materials were extracted with ethanol and chloroform. The last traces of the solvent were removed and concentrated to dryness under vacuum using a rotary evaporator. The dried extract was weighed and then kept at -4 ºC until ready for use. The yield of the extract was EEDP 78.63 % (w/w) and CEDP 45.74 % (w/w). In each experiment, the extract was diluted with water to the desired concentration[9].

Animal:

Adult male albino rats (150 to 200 gm) were used in this study. Animals were housed in an environmentally controlled room following the guide for care and use of laboratory animals, maintained at a constant temperature of 22 + 1 degrees Celsius with a 12/12 h light/dark cycle. They were maintained in clean, sterile, and polypropylene cages and fed with commercial pellet rat chow (M/S Hindustan Lever Limited, Bangalore, India) and water ad libitum.

Approval:

The study was approved by the Institutional Ethical Committee, which follows the guidelines of the focuses on therapy with medicinal plants which are effective with minimal side effects. About half a million plants have medicinal values of which many are to be investigated.

Characterization of Phytoconstituents using spectroscopy techniques:

FT-IR stands for Fourier Transform Infra Red, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted). The resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. All the separated compounds from ethanol and chloroform extract of *D.patulus* leaf was characterized by FTIR spectroscopy technique[10].

In-vitro Evaluation of Antioxidant Activity:

Total antioxidant activity:

When taken EEDP and CEDP extracts different concentrations ranging from 100 µl to 500 µl to each test tube individually containing 3 ml of distilled water and 1 ml of Molybdate reagent solution. Test tubes were kept incubated at 95 °C for 90 min. After incubation, these tubes were normalized to room temperature for 30 min and the absorbance of the reaction mixture was measured at 695 nm measured using a UV-VIS spectrophotometer. **Gallic acid was** standard.

Nitric oxide scavenging assay:

Various concentrations of EEDP and CEDP extracts (100-500 µg/ml) were mixed with 0.5 mL of sodium

nitroprusside and incubated at 37°C for 2 hours. After incubation, 0.5 mL of Griess reagent was added and the absorbance was read at 540 nm using a spectrophotometer. The percentage of nitric oxide scavenging was calculated. Trolox **was** standard.

Estimation of total reducing power:

Various concentrations of EEDP and CEDP extracts (100-500 µg/ml) was mixed with 2.0 mL of 2.5 mol/L phosphate buffer pH 6.6 and 2.0 mL of 1% K_3Fe solution. The mixtures were incubated at 50° C for 20 min. After incubation adds 2.0 mL 10% trichloroacetic acid, this mixture was centrifuged at 3000g for 15 min. The supernatant 2.5 mL was collected and mixed with 2.5 mL of deionized water containing 0.5 mL of 0.1% ferric chloride. The absorbance was measured at 700 nm using a spectrophotometer, with distilled water as the blank. **Ascorbic acid** was used to construct the standard curve[11,12,13].

Anti-Parkinson's Activity

Study by using Induction of Parkinsonism by 6-OHDA

- Adult healthy male albino rats aged 10 to 12 weeks and 150-200 weighing grams.
- The animal were divided into seven groups, each group contain six animals **(Table: 1)**

Table 1: Groop of Animals

Group	Drug administered	Dose (mg/kg)/ Route
Group I	Normal control (Normal saline)	5 ml/kg (orally)
Group II	Disease control 6 - OHDA Induction of Parkinsonism	20 µg (i.SNc)
Group III	6 - OHDA + Standard treatment (L-DOPA)	6 mg/kg (orally)
Group IV	6 - OHDA + *Ethanolic extract of Dipteracanthus patulus*	100 mg/kg (orally)
Group V	6 - OHDA + *Ethanolic extract of Dipteracanthus patulus*	100 mg/kg (orally)
Group VI	6 - OHDA + C*hloroform extract of Dipteracanthus patulus*	200 mg/kg (orally)
Group VII	6 - OHDA + C*hloroform extract of Dipteracanthus patulus*	200 mg/kg (orally)

Procedure:

- Starting the study desipramine (25 mg/kg, IP) was administered 30 min before surgery, to protect nor-adrenaline-containing terminals from the effects of 6-hydroxydopamine.
- All animals were anesthetized with Thiopentone sodium (3 mg/kg i.v), and were fixed in a stereotaxic apparatus.
- After the skull was exposed, a burr hole was drilled for the accommodation of the needle. The needle was inserted into the substantia nigra on brain with an injection of 6-hydroxydopamine 20 µg was then made over 5 min and the needle was left "Figure 1"
- Then the skull was secured with stainless metallic screws and the wound area was covered by dental cement.
- Each rat was housed individually following the surgical procedure.
- *EEDP and CEDP extracts* was given orally at dose was 100 mg/kg and 200 mg/kg after 48 hrs of induction for sixty days. L-DOPA was given orally at a dose of 6 mg/kg as a standard drug.
- The after the 60th day of treatment following Parameters were evaluated Muscle rigidity Test[14,15,16,17].



Figure 1: Substantia nigra on brain with an injection of 6-hydroxydopamine

Muscle rigidity Test (Rota Rod Test)

The main symptom of the Parkinsonism disease is muscle rigidity. The loss of muscle grip is an indication of muscle rigidity.Muscle rigidity test was conducted using rota rod apparatus. On the day before the first day of testing, the animal was placed on the rotating rod individually and trained for 3 minutes at 25 rpm. The animal naturally tries to stay on the rotating rod to avoid falling to the ground. Three separate trials were conducted by each animal at five-minute intervals with a fixed cut off time of 180s. To alleviate stress and fatigue, a 5- minute rest period was given after each trial. Motor coordination was tested by comparing the latency to fall on the very first trial between treatment groups. The time taken by animals to fall from the rotating rod was noted[18].

RESULTS:

FT-IR studies of separated compound

Table 2: IR Spectroscopic data of Ethanolic extract of *D. patulus* (EEDP)

S. No	Group	Stretching Frequency (cm-1)
1	C-H stretching of CH_3	1045
2	C-H stretching CH_2	1087
3	C=C stretching	1381
4	C-O bending	1649

Table 3: IR Spectroscopic data of Chloroform extract of *D. patulus* (CEDP)

S. No	Group	Stretching Frequency (cm-1)
1	C-H stretching of CH_3	3020
2	C-H stretching CH_2	2927
3	C=C stretching	2148
4	C-O bending	2364

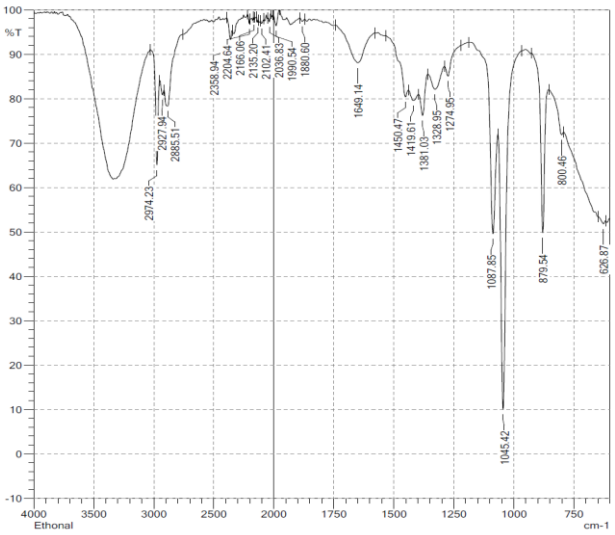


Figure 2: FT-IR Studies on *D. patulus (Jacq)* Ethanolic extract (EEDP).

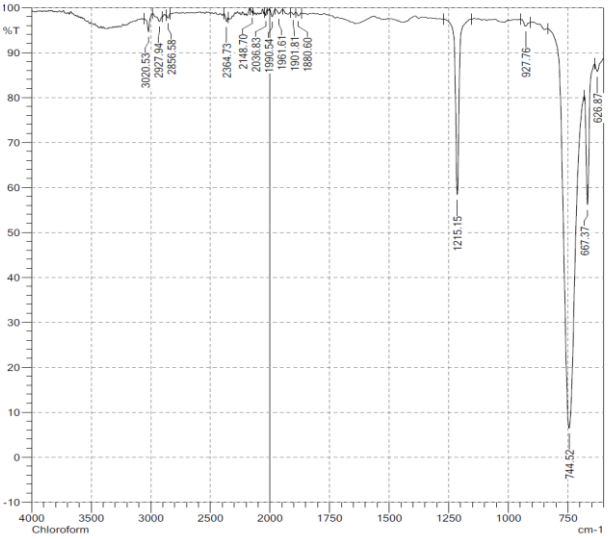


Figure 3: FT-IR Studies on *D. patulus (Jacq)* Chloroform extract (CEDP).

***In-vitro* Evaluation of Antioxidant Activity**

The results of Total antioxidant activity is Phosphomolybdenum spectroscopic method for the quantitative determination of antioxidant capacity **(Table 4)**

Table 4: Phosphomolybdenum Assay of Ethanolic and Chloroform extracts of *D.patulus* compared with gallic acid was Standard.

S.No	Concentration (µg/ml)	Absorbance (nm)		
		Standard	EEDP	CEDP
1	100	0.457	0.108	0.098
2	200	0.589	0.138	0.149
3	300	0.678	0.275	0.256
4	400	0.694	0.381	0.346
5	500	0.632	0.254	0.232

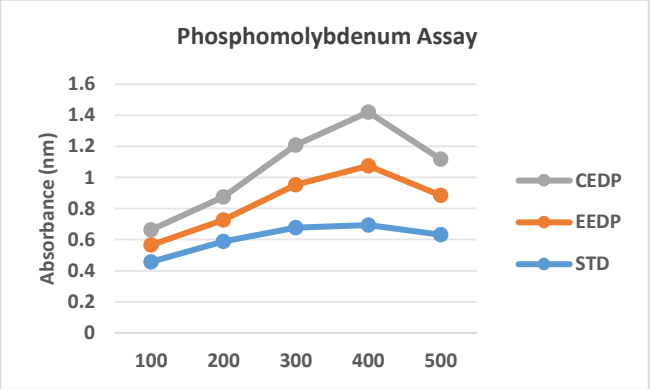


Figure 4: Phosphomolybdenum Assay of Ethanolic and Chloroform extracts of *D.patulus* compared with gallic acid

The results of antioxidant activity by nitric oxide assay indicated a significant scavenging capability with an increase in the concentration when compared to the standard **(Table 5)**

Table 5: Nitric oxide Assay of ethanolic and chloroform extracts of *D.patulus* compared with Trolox was standard.

S.No	Concentration (µg/ml)	Absorbance (nm)			% Scavenging		
		Standard	EEDP	CEDP	Standard	EEDP	CEDP
1	100	1.113	1.122	1.118	2.27	1.48	1.83
2	200	1.109	1.107	1.104	2.62	2.80	3.06
3	300	0.963	1.102	1.101	15.44	3.24	3.33
4	400	0.852	0.951	0.948	25.19	16.50	16.76
5	500	0.753	0.862	0.846	33.88	24.31	25.72

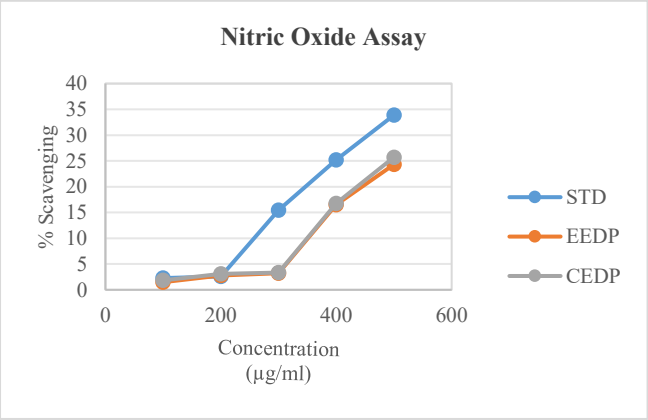


Figure 5: Nitric oxide Assay of ethanolic and chloroform extracts of *D.patulus* compared with Trolox

The results of antioxidant activity by Reducing Power Assay of increase in absorbance with an increase in the concentration of EEDP and CEDP compared to the standard Ascorbic acid. **(Table 6)**

Table 6: Reducing Power Assay of ethanolic and chloroform extracts of *D.patulus* compared with Ascorbic acid was standard.

S.No	Concentration (µg/ml)	Absorbance (nm)		
		Standard	EEDP	CEDP
1	100	0.925	0.911	0.914
2	200	0.986	0.956	0.967
3	300	1.119	0.984	0.991
4	400	1.868	1.127	1.138
5	500	1.972	1.134	1.181

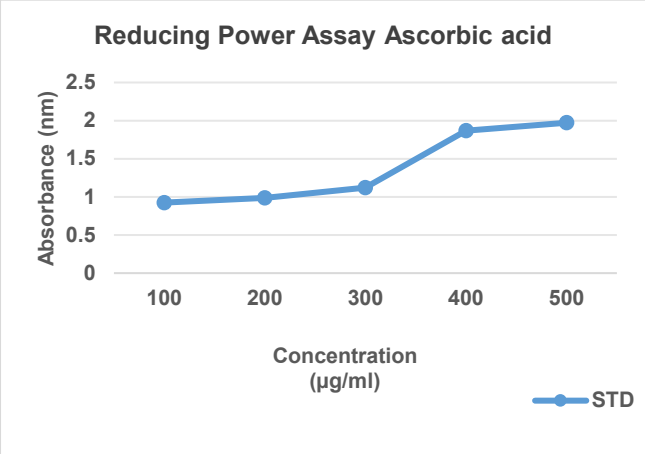


Figure 6: Reducing Power Assay of Ascorbic acid was standard.

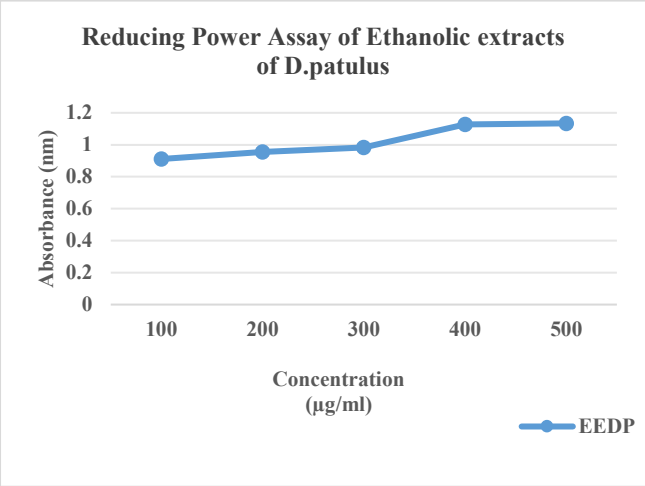


Figure 7: Reducing Power Assay of Ethanolic extracts of *D. patulus*.

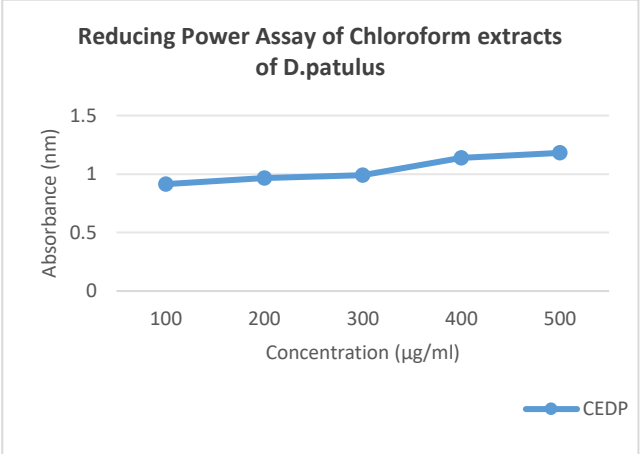


Figure 8: Reducing Power Assay of Chloroform extracts of *D. patulus*

Anti-Parkinson's Activity

6-OHDA - induced Parkinson's Disease used in Rota Rod Apparatus

In Rotarod test, the group which received only 6-OHDA, significantly, decreases fall off time ($P<0.001$) compared to the Normal group (Group I). In standard treated group (Group III) a significant increase in fall off time was recorded, while in extract treated groups (Group IV & V), is less significant increases in fall off time compared to the 6-OHDA treated group. Where as significant difference in fall off time was seen when 200mg/kg treated groups (Group VI & VII) and standard treated group (Group III) **(Table 7)**.

Figure 9 represents the difference in muscle strengths exhibited in all seven groups. The standard group with L-dopa (6 mg/kg) exhibited better muscle strength after 6-OHDA administration followed test group administered with *Ethanolic and Chloroform extracts of Dipteracanthus patulus* 200 mg/kg.

Table 7: Effect of *Ethanolic and Chloroform extracts of Dipteracanthus patulus* on Rota Rod Test in Rats

S. No	Group	Rota Rod Test (sec) (Mean ± SD)		
		Day 1	Day 4	Day 7
1	Group I	68.737 ± 3.113	69.492 ± 2.977	68.667 ± 2.349
2	Group II	23.634 ± 2.863*	22.783 ± 2.632*	21.982 ± 2.91*
3	Group III	61.234 ± 3.241***	63.524 ± 2.832***	65.632 ± 1.252***
4	Group IV	28.321 ± 2.672*	32.632 ± 2.541*	35.731 ± 2.887*
5	Group V	28.537 ± 2.461*	31.542 ± 2.492*	35.592 ± 2.652*
6	Group VI	53.981 ± 1.386**	55.432 ± 1.892**	59. 431 ± 2.327**
7	Group VII	53.763 ± 1.482**	54.986 ± 1.991**	57.862 ± 1.392**

Group 1 (Normal Saline 5ml/kg); Group 2 (6 - OHDA 20 ug); Group 3 (L-DOPA 6 mg/kg); Group 4 (EEDP 100 mg/kg); Group 5 (CEDP 100 mg/kg); Group 6 (EEDP 200 mg/kg); Group 7 (CEDP 200 mg/kg); SD-Standard deviation.

Results are expressed as mean ± SEM from four observations as compared to the standard group the one-way ANOVA is Graph Pad"s software method, ($P< 0.001$) by conventional criteria; this difference is extremely statistically significant.

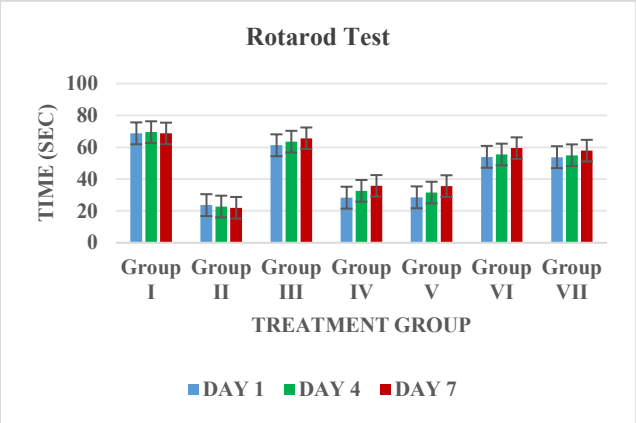


Figure 9: Effect of EEDP and CEDP on Rota Rod Test in Rats

DISCUSSION

In this study, the *in vitro* antioxidant and neuroprotective effects of *Dipteracanthus patulus* on 6 - OHDA induced animal models were investigated. The antioxidant property evaluated by total antioxidant activity, nitric oxide radical scavenging and total reducing power showed that EEDP and CEDP has potent antioxidant property. The antioxidants contained in the plant exhibited radical scavenging and inhibiting properties. They reported a decrease in oxidative stress markers such as MDA and FRAP and an increase in brain-derived neurotrophic factor.

In the present study, 6 - OHDA (20 ug i.SNc) induced significant rotarod in rats as evidenced by a significant increase in the time spent on the compared to vehicle treated animals and standard animals. Treatment with *D. patulus* significantly increase the locomotor activity and muscle activity in 6 - OHDA treated rats in dose dependent manner. The EEDP and CEDP at doses of 400 mg/kg showed protective effect against 6 - OHDA induced muscles rigidity indicated that this plant has an ability to protect dopaminergic neurotransmission in striatum.

CONCLUSION

In view of the above facts, we are concluding that ethanolic and chloroform extracts of *Dipteracanthus patulus* plant showed to be an antioxidant and showed a promising effect in animals with Parkinson's disease. And we appreciate further detailed molecular studies with this drug in anti-Parkinson's pharmacology and toxicology and also characterization of active constituents responsible for neuroprotective effect.

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