ORIGINAL ARTICLE

Exploring neuroprotective potential of *Cissus quadrangularis* plant extract in Parkinson's disease

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Abstract

Background: Parkinson's disease (PD) caused due to neuroinflammation and oxidative stress associated with motor and non-motor complications. Neuroprotection strategies create a hope for patients. Natural products have been increasing the gain to have specific molecular or pharmacological activities that are likely to supply the development of neuroprotective agents against PD. *Cissus quadrangularis* constitutes all suitable physical and chemical properties and its ability to cross the blood–brain barrier making it a relevant candidate for the treatment of neuroprotection in PD. **Materials and Methods:** The stem part of the plant was extracted with distilled water and ethanol. The antiparkinson's activity of both extracts of *C. quadrangularis* was evaluated using rotenone-induced excitotoxicity model in zebrafish for 96 h resulting in alterations in swimming pattern measured through a video recording system. Further, cognitive improvement activity in PD of both extracts of plant was evaluated using reserpine-induced cognitive impairments in mice for 29 days. Biochemical parameters, that is, levels of catalase and glutathione (GSH) were analyzed using UV-visible spectrophotometer. **Results:** The aqueous extract of *C. quadrangularis* (AECQ) showed a significant and dose-dependent effect against rotenone-induced PD in adult zebrafish's at a dose of 4 and 10 µg/mL and cognitive improvement activity against reserpine-induced cognitive impairment in mice at a dose of 200 mg/kg and 500 mg/kg. The AECQ also showed a significant *in vivo* antioxidant potential by restoring the levels of catalase and GSH. The results of the present study demonstrate the potential antiparkinson's properties of the aqueous extract. **Conclusion:** The result of the present study conclusively shows the Anti-Parkinson's activity of *C. quadrangularis* and improved cognition in PD.

Key words: *Cissus quadrangularis*, cognitive improvement, excitotoxicity, neuroprotective, Parkinson's disease

INTRODUCTION

"Neurodegeneration" is the umbrella term for the gradual loss of structure or function of neurons, inclusive of the death of neurons.[1] It is a complex multifactorial process that aims at neuronal death in the brain and spinal cord, resulting in brain and spinal cord destruction and dysfunction. This degeneration is encompassed by oxidative stress, protein oligomerization, axonal, transport deficits, aggregation, calcium deregulation, mitochondrial dysfunction, abnormal neuron–glial interactions, neuroinflammation, DNA damage, and aberrant RNA processing. Neurodegeneration occurs in neurotraumatic, neurodegenerative, and neuropsychiatric diseases.[2]

Parkinson's disease (PD) is a progressive neurodegenerative disease with motor and non-motor symptoms. PD is characterized by the degeneration of dopaminergic neurons in the substantia nigra pars compacta and deficiency of dopamine in the striatal region. The primary objective of PD research is to understand the pathogenesis, targets, and development of therapeutic interventions to control the progress of the disease.[3]

The elementary symptoms are the results of decreased stimulation of the motor cortex by the basal ganglia, normally caused by the inadequate formation and action of dopamine formed in the dopaminergic neurons of the midbrain (specifically the substantia nigra).[1] The mechanism

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elaborated in the development of PD includes various factors such as the aggregations of misfolded proteins, mitochondrial damage, activation of protein degradation pathways, and oxidative stress, along with certain gene mutations.[4]

The characteristic symptoms of PD are progressive motor deficits, including tremor, bradykinesia, akinesia, rigidity, postural instability, and gait difficulties. Non-motor symptoms, including depression, anxiety, sleep disturbance, cognitive decline, and anosmia are also prevalent in PD patients and often occur before the onset of motor symptoms.[5]

Natural products have been increasing the gain to have a specific molecular or pharmacological activity that is likely to supply the development of neuroprotective agents against PD.[6] This requires testing of the neuroprotective properties of different natural products that could be applied either as independent or adjunctive therapy with conventional drugs.[7]

Cissusc quadrangularis is a climbing herb belonging to the family, vitaceae. It is commonly referred to as hadjod. Phytochemical studies of *C. quadrangularis* have shown the presence of various versatile constituents such as polyphenols, flavonoids, triterpenoids, Vitamin C, and many others, for example, resveratrol, piceatannol, and phytosterols. The *C. quadrangularis* contains a high amount of carotene A and water-soluble glycoside. The plant also shows the presence of δ amyrin and δ amyrone is also reported. The plant has been widely used medicinally as an anticonvulsant, digestive, antioxidant, antiviral, analgesic, and anti-pyretic activity.[8] *C. quadrangularis* constitutes all suitable physical and chemical properties and its ability to cross the blood–brain barrier making it a relevant candidate for the treatment of neuroprotection in PD. Thus, the reason for selecting the plant is its probability of having a neuroprotection activity in PD. Thus, this present study is an attempt to evaluate the neuroprotective activity of the extract of *C. quadrangularis* in rotenone-induced PD in zebrafish and reserpine-induced cognitive impairment in PD in mice. The systemic rotenone model of (PD) accurately replicates many aspects of the pathology of human PD and has provided insights into the pathogenesis of PD.[9] Glutamate-mediated excitotoxicity may be involved in a destructive vicious cycle, which mainly contributes to the worsening of nigrostriatal degeneration in PD.[10] Zebrafish (*Danio rerio*) models of PD have contributed to a better understanding of the role of several genes implicated in the disease.[11] Furthermore, zebrafish is a vertebrate model particularly suited for large-scale drug screenings. The relatively small size of zebrafish, optical transparency, and lifecycle is key characteristics that facilitate the study of multiple compounds at the same time.[11] In summary, this version of the rotenone model is highly reproducible and may provide an excellent tool to test new neuroprotective strategies.[9]

MATERIALS AND METHODS

Plant

The dried powder of the Stem of *C. quadrangularis* was collected from Shree Gajanan Aushadhi Bhandar Borivali, Maharashtra, India. The dried powder of plant *C. quadrangularis* was authenticated by Dr. Harshad Pandit, HOD of Botany, Andheri. The authentication number of the plant is roc p 1019295.

Physicochemical Analysis of Plant[8,12]

Physicochemical analysis of the plant was carried out for determining total ash value, water soluble ash value, acid insoluble ash value, and loss on drying.

Preparation of Extracts

Aqueous extract of C. quadrangularis (AECQ)

The dried stems of *C. quadrangularis* were ground. The powder (250 g) was macerated for 3 days with 2.5 L of distilled water at room temperature. The mixture was filtrated with a Whatman filter paper and the filtrate was evaporated using a Rota vapor at a temperature of 70°C and stored in a refrigerator. The observed yield was 6% w/v.[13]

Ethanolic extract of C. quadrangularis (EECQ)

Plant stem powder (100 g) was tightly packed in a Soxhlet apparatus and extracted with 400 mL of ethanol as a solvent for 4 days at a temperature of 50–60°C using a heating mantle. The extract was filtered and the filtrate was evaporated in a water bath until it gets concentrated and placed in a hot air oven to get dry product that the extract was stored in a refrigerator. The observed yield was 5.5% w/v.[14]

Phytochemical Analysis[8]

Preliminary phytochemical analysis was done for carbohydrates, triterpenoids, polyphenols, flavonoids, glycosides, tannins, etc. A confirmatory test of polyphenols was performed to confirm the presence of polyphenols.

Animals

Protocol approval

The protocol submitted for approval to the IAEC committee was approved having protocol no: PCOL/IAEC/2019/03 (for mice) and PZEB/IAEC/2019/04 (for zebrafish) CPCSEA registration number is (879/PO/Re/S/05/CPCSEA).

Procurement of animals

- Mice (Swiss albino): Bombay Veterinary college Parel, Mumbai, Maharashtra.

- Zebrafish (*D. rerio*): Vikrant aqua culture Bandra, Mumbai, Maharashtra.

The Swiss albino mice, 20–25 g were procured from Bombay Veterinary College, Parel Mumbai-Maharashtra and adult wild type zebrafish, 0.5–1 g were procured from Vikrant Aqua Culture, Bandra. The animals were housed in a well-ventilated room and the animals were exposed to 12 h day and 12 h night cycles, with a temperature between 20°C ± 10°C.[15] All the experiments were carried out after obtaining prior sanction from IAEC. Zebrafish were acclimatized in the zebrafish facility located in M. pharm Pharmacology laboratory under standard husbandry conditions, that is, the temperature of 28°C ± 5°C, optimum pH of 7–8, conductivity of 0.25 –0.75 ppt and 14:10 h light/dark cycle. The institution's animal house is registered with the Government of India, having registration number 879/PO/Re/S/05/CPCSEA and supports the CPCSEA guidelines for the handling and care of experimental animal research. The animals were accommodated in standard propylene cages with wire mesh tops and husks as bedding. The experimental animals had free access to food and water supplied *ad libitum* under stringent hygienic conditions. All experimental groups had a distinct set of animals and care was taken to assure that animals used for one response were not employed somewhere else.

Acute Toxicity testing: Up-and-down Procedure

For mice

Acute toxicity for plant root was earlier carried out according to OECD guideline 425. The highest dose used for testing was 2000 m/kg p.o. There was no mortality recorded in animals and they did not show toxicity or behavioral changes. It was considered non-toxic. Hence acute toxicity was not performed in mice and doses of 200mg/kg and 500 mg/kg has been selected for the study.[16]

For zebrafish

Acute toxicity was performed on the test compound according to OECD guidelines 203 in 8 zebrafish. The fish were exposed to the extracts at a maximum dose of 100 m/mL for 96 h. Mortalities were reported at 24, 48, 72, and 96 h after exposure. If all the fishes survive, 1/5th, 1/10th, 1/20th, and up to 1/25th doses are been selected for the actual study.[17]

In Vivo Study

Rotenone-induced PD in zebrafish model[18]

Fifty-six adult wild-type zebrafish, weighing 0.5–1 g were used for the study. They were divided into seven groups of eight fishes each as given below:

- Group I: Vehicle (10% DMSO)
- Group II: Toxic-rotenone (3 pg/mL)
- Group III: Standard-selegiline (0.03 µg/mL selegiline + 3 pg/mL rotenone)
- Group IV: Aqueous extract (4 µg/mL + 3 pg/mL rotenone)
- Group V: Aqueous extract (10 µg/mL + 3 pg/mL rotenone)
- Group VI: Ethanolic extract (4 µg/mL + 3 pg/mL rotenone)
- Group VII: Ethanolic extract (10 µg/mL + 3 pg/mL rotenone).

Dosing schedule

The fishes were first exposed to standard (selegiline) or test drug for 30 min, after 30 min exposure, the fishes are transferred to fresh aerated water for 15 min. After that, fishes were exposed to an inducing agent, that is, rotenone for 30 min. After exposure to rotenone, fish were placed in an experimentation tank. It consists of a 5L tank with one horizontal line which divides the water portion of the tank into two halves. These vertical lines will use to calculate the speed of fish by measuring the time taken by fish to travel from the first line to the last and the horizontal line will give an idea about the time spend in the upper and lower half of the tank by fish. All behavioral evaluations like latency to travel from one point to another, complete cataleptic time, and time spent near the bottom of the tank will be done using a camera.

Reserpine-induced model for cognition in PD[19]

Fifty-six female Swiss albino mice, 8–12 weeks old, weighing 20–25 g were used for the study. They were divided into seven groups of eight animals each as given below:

- Group I: Vehicle (0.25 mL/kg, p.o.)
- Group II: Disease-reserpine (0.25 mg/kg s.c)
- Group III: Standard-entacapone (0.25 mg/kg s.c + 30 mg/kg i.p)
- Group IV: Aqueous extract (0.25 mg/kg s.c + 200 mg/kg p.o)
- Group V: Aqueous extract (0.25 mg/kg s.c + 500 mg/kg p.o)
- Group VI: Ethanolic extract (0.25 mg/kg s.c + 200 mg/kg p.o)
- Group VII: Ethanolic extract (0.25 mg/kg s.c + 500 mg/kg p.o).

Dosing schedule

Except for animals from the vehicle control group, all animals from other groups were administered with reserpine at a dose of 0.25 mg/kg on the day before 48 h of testing by the subcutaneous route, test drug by oral route, and standard drug by i.p. route for 29 days. The behavioral parameters were recorded on days 2, 8, 15, 22, and 29 after administration of the reserpine in discriminative avoidance plus maze apparatus. *In vivo* study animals were sacrificed and the brains of the animals were removed for the estimation of glutathione (GSH) and catalase.

Discriminative avoidance plus maze test for cognitive impairment in PD[19]

DAVT is a model that allows simultaneous learning, memory, and locomotor activity. The apparatus employed was a modified elevated plus maze; comparing two enclosed arms opposite to two open arms. A speaker of 80 dB and 100 W lights was placed over one enclosed arm (aversive arm).

Procedure

Each animal was placed in the center of the apparatus. The training session was done after 24 h of administration of the dose and after 48 h of test session. The aversion was produced by 80 dB noise and 100 W lights when it entered into the aversive arm. The training session was done for 10 min and the test session was done for 3 min.

Observation

Total time spent in the aversive versus non-aversive arm in a training session. Total time spent in the aversive versus non-aversive arm in a testing session.

Biochemical estimation[19]

Estimation of GSH

Brain tissue homogenate was centrifuged at 16,000 g for 15 min 4°C. The supernatant (0.5 mL) was mixed with 4 mL of ice-cold 0.1 mM solution of 5, 5-dithiobis-[2-nitrobenzoic acid] in 1 M phosphate buffer (pH 8). The optical density was interpreted at 412 nm in a spectrophotometer.

Estimation of catalase

The assay mixture consisted of 50 µL of 1 M Tris-HCl buffer (pH 8.0) containing 5 mM ethylenediaminetetraacetic acid, 900 µL of 10 mM H_2O_2, 30 µL of distilled water, and 20 µL of the brain tissue supernatant. The value of decomposition of hydrogen peroxide was deteced spectrophotometrically at 240 nm.

Statistical Analysis

The results of neuroprotective activity were expressed as mean ± standard error of the mean from eight animals in each group for mice and eight zebrafish in each group. Results were statistically analyzed using the one-way analysis of variance followed by Tukey's multiple comparison test's $^{*}P \leq 0.05$, $^{**}P \leq 0.01$, and $^{***}P \leq 0.001$, when compared with the disease group and $^{\#}P < 0.001$, $^{\#\#}P < 0.01$, and $^{\#\#\#}P < 0.05$ when compared with a standard group.

RESULTS

The present study was performed to carry out the phytochemical and pharmacological evaluation of plant extract of *C. quadrangularis*. The results were presented in tables and graph format. The results displayed include physicochemical, phytochemical evaluation and confirmatory test for plant extract, acute toxicity study, and pharmacological effect of plant extract on rotenone-induced excitotoxicity model in zebrafish and reserpine-induced cognitive impairments in mice.

Physicochemical and Preliminary Phytochemical Analysis

As per the phytochemical test, both extracts showed the presence of alkaloids, glycosides, flavonoids, tannins, diterpene, phenolic compounds, and carbohydrate. A confirmatory test showed the presence of polyphenols. Results obtained for the physicochemical parameters are mentioned in Table 1.

Acute Toxicity Study

In zebrafish: When adult zebrafish were exposed to plant extract at the dose of 100 mg/L for a period of 96 h, no mortality was recorded.

In Vivo Models

Rotenone-induced PD in zebrafish

Antiparkinson's effect on both extracts of *C. quadrangularis* was evaluated in rotenone-induced PD in zebrafish. The effect of the extract on behavioral parameters was determined at doses of 4 µg/mL and 10 µg/mL. 10 µg/mL dose of AECQ showed significantly better results as compared to other test drug groups. The results are summarized in Figures 1–3.

Latency to travel from one point to another

Latency to travel from one point to another decreased significantly ($P < 0.05$) in the group administered with test drug when compared to disease control. 10 µg/mL dose of AECQ showed better results as compared to other test drug groups.

For group vehicle (86.7%), standard (65.3%), AECQ test 1 (68%), AECQ test 2 (72%), EECQ test 1 (43%), and EECQ test 2 (58.75%) were statistically significant when compared to disease control group.

Complete cataleptic time

Complete catalepsy was significantly ($P < 0.05$) absent in all groups administered with the test drug when compared to disease control.

Time spent near the bottom of the tank

Time spent near the bottom of the tank was significantly ($P < 0.05$) less in the group administered with vehicle (31%), standard group (52.7%), AECQ test-1 (24.14%), AECQ test-2 (70%) EECQ test-1 (22.39%), and EECQ test-2 (28%) when compared to disease control. 10 µg/mL dose of AECQ showed better results as compared to other test drug groups.

Table 1: Physicochemical parameter evolution

S. No.	Types of ash value	Results	Standard values
1.	Total ash value	16.5% w/w	NMT 20% w/w
2.	Water soluble ash value	8% w/w	NMT 23% w/w
3.	Acid soluble ash value	4.2% w/w	NMT 5% w/w
4.	Loss on drying	3% w/w	NMT 5% w/w

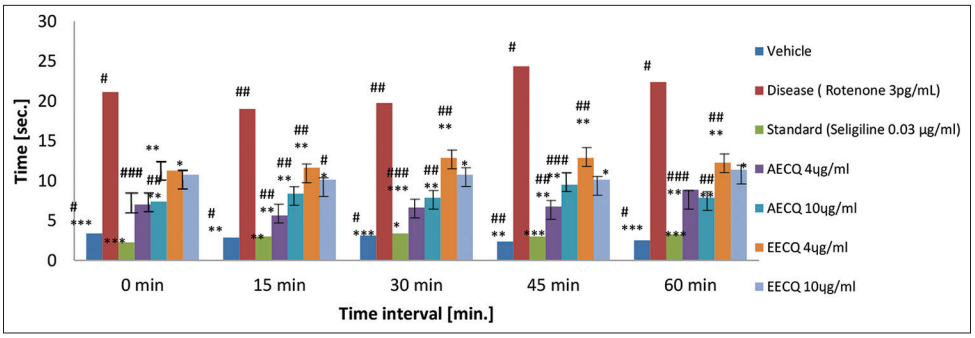

Figure 1: Latency to travel from one point to another

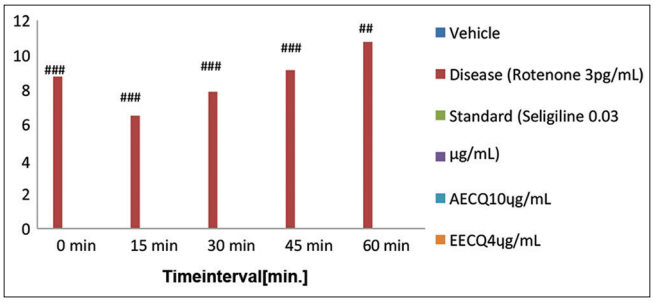

Figure 2: Complete cataleptic time

Reserpine-Induced Model for Cognition in Parkinson Disease

The cognitive improvement activity of both extracts of *C. quadrangularis* was evaluated in reserpine-induced model for cognition in Parkinson disease using the DAVT apparatus. Behavioral parameters were observed on the 2nd, 8th, 15th, 22nd, and 29th day of the main study. The effect of the extract on time spent in aversive versus non-aversive in sec was determined at doses of 200 mg/kg and 500 mg/kg. On the day of observation, the animals were kept for testing session for 3 min. The results show significance increase in time spent in the non-aversive arm in the test drug group which shows significance memory improvement. The results are summarized in Tables 2–6.

On the 2nd day, the animals were kept for testing session for 3 min. The results show significance ($P < 0.05$) increase in time spent in the non-aversive arm which shows memory improvement. 500 mg/kg dose of aqueous extract showed significantly better results as compared to other test drug groups.

The time spent in the non-aversive arm increased in the vehicle (68.24%), standard group (67.88%), AECQ test-1 (60%), AECQ test-2(67%), EECQ Test-1 (64%), and EECQ test-2 (65%) when compared with the disease control group.

On the 8th day, the animals were kept for testing session for 3 min. The results show significance ($P < 0.05$) increase in time spent in the non-aversive arm which shows memory improvement. 500 mg/kg dose of aqueous extract showed significantly better results as compared to other test drug groups.

The time spent in the non-aversive arm increased in vehicle (60%), standard group (49.2%), AECQ test-1 (42.29%), AECQ test-2 (50%), EECQ test-1 (38.47%), and EECQ test-2 (46.14%) when compared with the disease control group.

On the 15th day, the animals were kept for testing session for 3 min. The results show a significance ($P < 0.05$) increase in time spent in the non-aversive arm which shows memory improvement. 500 mg/kg dose of aqueous extract showed significantly better results as compared to other test drug groups.

The time spent in non-aversive arm increased in vehicle (53.8%), standard group (55.4%), AECQ test-1 (50.1%), AECQ test-2 (55%), EECQ test-1 (48.5%), and EECQ test-2 (50.5%) when compared with the disease control group.

On the 22nd day, the animals were kept for testing session for 3 min. The results show a significance ($P < 0.05$) increase in time spent in the non-aversive arm which shows memory improvement. 500 mg/kg dose of aqueous extract showed significantly better results as compared to other test drug groups.

The time spent in the non-aversive arm increases in the vehicle (51.8%), standard group (54.1%), AECQ test-1 (47.3%), AECQ test-2 (53.4%), EECQ test-1 (47.64%), and EECQ test-2 (49.3%) when compared with the disease control group.

On the 29th day, the animals were kept for testing session for 3 min. The results show a significance ($P < 0.05$) increase in time spent in the non-aversive arm which shows memory improvement. 500 mg/kg dose of aqueous extract showed significantly better results as compared to other test drug groups.

The time spent in non-aversive arm increases in the vehicle (56.4%), standard group (61.8%), AECQ test-1 (49.8%), AECQ test-2 (58.3%), EECQ test-1 (52.4%), and EECQ test-2 (54%) when compared with the disease control group.

Biochemical Estimation

On the 29th day, animals were sacrificed by exposure to CO2 chamber, and the brain was isolated. The brains were homogenized and centrifuged to form a supernatant fluid,

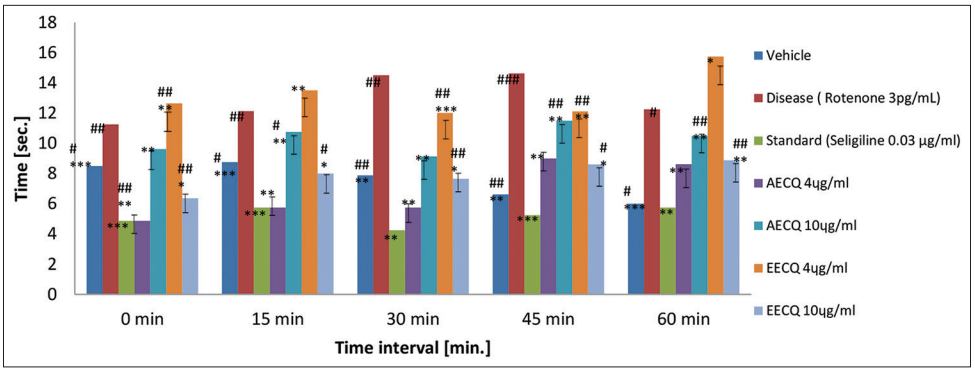

Figure 3: Time spent near the bottom of the tank

Table 2: 2nd day of DAVT apparatus

Group	Aversive arm	Non-aversive arm
Vehicle group	41±5.68	100.37±11.64***###
Disease group (reserpine)	96.12±4.79	31.87±5.54###
Standard (entacapone)	45.87±6.57	99.25±1.64***
Test Group 1 (AECQ)	60±6.09	81±4.14*#
Test Group 2 (AECQ)	50.37±5.51	96.87±2.9**###
Test Group 1 (EECQ)	82.62±3.96	88.62±3.96*#
Test Group 2 (EECQ)	63.62±4.33	93.62±2.47*#

AECQ: Aqueous extract of *Cissus quadrangularis*,
EECQ: Ethanolic extract of *Cissusquadrangularis.*
Results were statistically analyzed using the One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.01, *** p≤0.001, when compared with the disease group and # P<0.001, ## P<0.01, ### P<0.05 when compared with a standard group

Table 3: 8th day of DAVT apparatus

Group	Aversive arm	Non-aversive arm
Vehicle group	37.62±5.58	111.25±6.20***###
Disease group (reserpine)	99.25±4.25	52.37±5.35###
Standard (entacapone)	34.87±6.42	103.25±2.74***
Test group 1 (AECQ)	55.75±3.33	90.75±2.62**###
Test group 2 (AECQ)	45.12±5.47	102.5±3.14***###
Test group 1 (EECQ)	68.37±3.66	85.12±3.2*#
Test group 2 (EECQ)	61.25±4.63	97.25±3.34*#

AECQ: Aqueous extract of *Cissus quadrangularis*,
EECQ: Ethanolic extract of *Cissus quadrangularis*
Results were statistically analyzed using the One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.01, *** p≤0.001, when compared with the disease group and # P<0.001, ## P<0.01, ### P<0.05 when compared with a standard group

which was used for biochemical estimation. Brain level of antioxidants such as GSH and catalase were found to be increased significantly in vehicle and experimental drug groups as compared to the disease control group. The results of catalase and GSH are mentioned in Table 7.

Table 4: 15th day of DAVT apparatus

Group	Aversive arm	Non-aversive arm
Vehicle group	38.87±4.3	101.75±5.07***###
Disease group (reserpine)	104.87±3.55	46.87±4.47###
Standard (entacapone)	33.62±2.67	105.12±3.8***
Test group 1 (AECQ)	51.12±4.42	94±2.78**#
Test group 2 (AECQ)	40.37±5.17	104.3±4.45***###
Test group 1 (EECQ)	64.12±5.9	91.12±6.29**###
Test group 2 (EECQ)	50.87±3.66	94.87±5.19**###

AECQ: Aqueous extract of *Cissus quadrangularis*,
EECQ: Ethanolic extract of *Cissus quadrangularis*
Results were statistically analyzed using the One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.01, *** p≤0.001, when compared with the disease group and # P<0.001, ## P<0.01, ### P<0.05 when compared with a standard group

Table 5: 22nd day of DAVT apparatus

Group	Aversive arm	Non-aversive arm
Vehicle group	43.7±5.82	103.87±5.41**###
Disease group (reserpine)	109.5±2.64	50±4.31###
Standard (entacapone)	33.62±3.37	109.25±3.93***
Test group 1 (AECQ)	45.25±6.76	95±3.37**###
Test group 2 (AECQ)	36.25±4.64	107.5±4.58**###
Test group 1 (EECQ)	50.12±5.84	95.5±2.72*
Test group 2 (EECQ)	45.62±6.73	98.62±4.43**###

AECQ: Aqueous extract of *Cissus quadrangularis*,
EECQ: Ethanolic extract of *Cissus quadrangularis*
Results were statistically analyzed using the One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.01, *** p≤0.001, when compared with the disease group and # P<0.001, ## P<0.01, ### P<0.05 when compared with a standard group

GSH level increased in vehicle (46.1%), standard group (59.8%), AECQ test-1 (45.8%), AECQ test-2 (59%), EECQ test-1 (12%), and EECQ test-2 (45.8%) when compared with disease control group.

Table 6: 29th day of DAVT apparatus

Group	Aversive arm	Non-aversive arm
Vehicle group	36.7±4.89	106.87±5.35***###
Disease group (reserpine)	112.62±5.9	46.5±5.8###
Standard (entacapone)	24.2±3.26	121.75±15.6***
Test group 1 (AECQ)	45.5±7.81	92.7±7.98**##
Test group 2 (AECQ)	25.2±3.53	111.7±5.68***###
Test group 1 (EECQ)	50.75±5.84	97.8±2.4**
Test group 2 (EECQ)	51.37±6.66	101.12±2.81**##

AECQ: Aqueous extract of *Cissus quadrangularis*, EECQ: Ethanolic extract of *Cissus quadrangularis* Results were statistically analyzed using the One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.01, *** p≤0.001, when compared with the disease group and # P<0.001, ## P<0.01, ### P<0.05 when compared with a standard group

Table 7: Observed levels of glutathione, catalase

Group	Glutathione	Catalase
Vehicle group	18.19±0.85	20.44±1.79
Disease group (reserpine)	9.83±1.1	11.16±1.89
Standard (entacapone)	24.19±0.42	22.4±1.12
Test group 1 (AECQ)	18.16±0.81*#	17.16±1.39**##
Test group 2 (AECQ)	23.98±0.51**##	20.18±1.01**##
Test group 1 (EECQ)	11.16±0.98*	15.41±1.36*
Test group 2 (EECQ)	18.16±0.85*#	17.18±1.13**##

AECQ: Aqueous extract of *Cissus quadrangularis*, EECQ: Ethanolic extract of *Cissus quadrangularis* Results were statistically analyzed using the One way ANOVA followed by Tukey's multiple comparison test's * p≤0.05, ** p≤0.01, *** p≤0.001, when compared with the disease group and # P<0.001, ## P<0.01, ### P<0.05 when compared with a standard group

Antioxidant GSH was significantly ($P < 0.05$) found to be more in groups administered with test drug than disease. Hence, it can be concluded that an increase in GSH may help in improving cognition symptoms. 500 mg/kg dose of aqueous extract showed significantly better results as compared to other test drug groups.

Catalase level increased in vehicle (45.4%), standard group (50%), AECQ test-1 (35%), AECQ test-2 (44.6%), EECQ test-1 (27.5%), and EECQ test-2 (35%) when compared with the disease control group.

Antioxidant catalase was significantly ($P < 0.05$) found to be more in groups administered with test drug than disease. Hence, it can be concluded that an increase in catalase may help in improving cognition symptoms. 500 mg/kg dose of aqueous extract showed significantly better results as compared to other test drug groups.

DISCUSSION

PD is one of the fatal and major neurodegenerative disorders in modern society.[1] Modern perception has changed the perception of PD from a pure movement (or motor) disorder to a multifactorial disease.[20] Cognitive impairment in PD is characterized by predominant executive deficits, visuospatial dysfunction, and relatively affected memory.[21] Natural products have definitive molecular or pharmacological effects that are prone to contribute to the development of neuroprotective agents against PD.[6] Glutamate-mediated excitotoxicity may be elaborated in a dangerous vicious cycle, which crucially contributes to the exacerbation of nigrostriatal degeneration in PD.[10] The ample majority of neurodegenerative diseases that are connected to glutamate signaling dysregulation have cognitive comorbities.[22] *C. quadrangularis* plant consists of various phytoconstituents such as phenolic compounds and triterpenoids and flavonoids. It is a plant of the Vitaceae family account for its anticonvulsant effects in traditional medicine.[11] Thus, the reason for selecting the compound from plant is its probability of having a neuroprotection activity in PD by NMDA antagonistic activity.

Aqueous and EECQ are known to exhibit central nervous system activity.[13] Phytochemical screening of both extracts showed the presence of alkaloid, carbohydrate, flavonoids, tannin, and triterpene which may contribute to its activity.[8] Confirmation of the presence of polyphenols gave us assurance that the plant will show activity against excitotoxicity as they are known to be effective against excitotoxicity.

In this study, the neuroprotective activity of *C. qudrangularis* was evaluated in two models, that is, rotenone-induced PD in zebrafish and reserpine-induced cognitive impairments in mice was evaluated.[17,18]

Acute toxicity in zebrafish at a maximum dose of 100 mg/L was carried out by OECD guidelines 203.[17] Both extracts did not show any signs of toxicity or mortality. Acute toxicity in rodents was performed earlier at the maximum dose, that is, 2000 mg/kg.[16] Extracts caused no visible signs of acute toxicity at the maximum dose, hence, were not performed.[16]

Studies have shown that exposure to rotenone altered behavioral and motor deficits, which are similar to human PD, counting muscular rigidity (catalepsy), postural instability, bradykinesia, and unsteady gait.[23]

A decrease in movement, catalepsy was observed when zebrafish was exposed to rotenone at a dose of 3 pg/mL. This toxicity can be related to the mechanism of excitotoxicity induced by rotenone. Treatment with both extract (AECQ and EECQ) at the dose of 4 µg/mL and 10 µg/mL and standard selegiline prevents behavioral parameters like latency to travel from one point to another; times spent near the bottom of the tank and complete cataleptic time in zebrafish. 10 µg/mL doses of AECQ showed significantly better results as compared to other test drug groups may be due to a decrease in excitotoxicity and its capability to antagonize NMDA receptors. According to the observations, AECQ showed a promising effect in improving PD.

Reserpine-induced cognitive impairment model was performed using an elevated plus maze apparatus.[18] The group which received only reserpine, increased the time spent in the aversive arm on the 2nd, 8th, 15th, 22nd, and 29th day as compared to the normal group. Both extracts of plant *C. quadrangularis* showed improvement in activity against reserpine-induced cognitive impairment in mice at a dose of 200 mg/kg and 500 mg/kg due to its NMDA antagonistic activity. Brain antioxidant levels such as GSH and catalase were found to be increased in the vehicle and experimental drug groups as compared to the disease control group. Hence, it can be concluded that an increase in catalase and GSH may help in improving cognition symptoms.[24]

Phytochemical studies of C. quadrangularis have revealed the presence of flavonoids such as quercetins and kaempferols, daidzein, triterpenoids, and Vitamin C.[16] The presence of phenolic compounds and triterpenoids may explain the anticonvulsant and NMDA antagonistic activity of the plant as they are possessing actions against Parkinson disease.[13]

CONCLUSION

Cissus quadrangularis possess all suitable physical and chemical properties and its ability to cross the blood brain barrier making it an appropriate candidate for the treatm loent of neuroprotection in Parkinson's disease. The present study provides evidence that both extract of *Cissus quadrangularis* was found to be effective in improving symptoms of PD in rotenone induced excitotoxicity model. The extract also showed cognitive improvement via DAVT test in reserpine experimental model of cognitive impairment in Parkinson's disease in mice. Thus, the result of the present study conclusively shows the Antiparkinson's activity of *Cissus qudrangularis* and improved cognition in PD.

REFERENCES

1. Redkar RG. Neuropharmacological Studies on *Ocimum sanctum*, Linn for its Therapeutic Role in Parkinson's Disease. Mumbai: Institute of Chemical Technology; 2013. p. 52-61.
2. Farooqui AA. Ischemic and Traumatic Brain and Spinal Cord Injuries: Mechanisms and Potential Therapies. United States: Academic Press; 2018. p. 321.
3. Pingale T, Gupta GL. Classic and evolving animal models in Parkinson's disease. Pharmacol Biochem Behav 2020;199:173060.
4. Patel F, Mandal P. Neurodegenerative diseases and their therapeutic approaches. In: Neurons Dendrites and Axons. London: Intechopen; 2019. p. 49.
5. Konnova EA, Swanberg M. Animal Models of Parkinson's Disease. Brisbane: Exon Publications; 2018. p. 83-106.
6. Essa MM, Braidy N, Bridge W, Subash S, Manivasagam T, Vijayan RK, *et al*. Review of natural products on Parkinson's disease pathology. J Aging Res Clin Pract 2014;3:1-8.
7. Mythri RB, Harish G, Bharath MM. Therapeutic potential of natural products in Parkinson's disease. Recent Pat Endocr Metab Immune Drug Discov 2012;6:181-200.
8. Mishra G, Srivastava S, Nagori BP. Pharmacological and therapeutic activity of *Cissus quadrangularis*: An overview. Int J PharmTech Res 2010;2:1298-310.
9. Cannon JR, Tapias V, Na HM, Honick AS, Drolet RE, Greenamyre JT. A highly reproducible rotenone model of Parkinson's disease. Neurobiol Dis 2009;34:279-90.
10. Ambrosi G, Cerri S, Blandini F. A further update on the role of excitotoxicity in the pathogenesis of Parkinson's disease. J Neural Transm (Vienna) 2014;121:849-59.
11. Vaz RL, Outeiro TF, Ferreira JJ. Zebrafish as an animal model for drug discovery in Parkinson's disease and other movement disorders: A systematic review. Front Neurol 2018;9:347.
12. Dhanasekaran S. Phytochemical characteristics of aerial part of *Cissus quadrangularis* (L) and its *in-vitro* inhibitory activity against leukemic cells and antioxidant properties. Saudi J Biol Sci 2020;27:1302-9.
13. Ngo Bum E, Ngoupaye GT, Talla E, Dimo T, Nkantchoua GC, Pelanken MM, *et al*. The anticonvulsant and sedative properties of stems of *Cissus quadrangularis* in mice. Afr J Pharm Pharmacol 2008;2:42-7.
14. Sheikh S, Siddiqui S, Dhasmana A, Haque E, Kamil M, Lohani M, *et al*. *Cissus quadrangularis* Linn. Stem ethanolic extract liberates reactive oxygen species and induces mitochondria mediated apoptosis in KB cells. Pharmacogn Mag 2015;11 Suppl 3:S365-74.
15. Razali K, Othman N, Mohd Nasir MH, Doolaanea AA, Kumar J, Ibrahim WN, *et al*. The promise of the zebrafish model for Parkinson's disease: Today's science and tomorrow's treatment. Front Genet 2021;12:655550.
16. Feyera T, Assefa S, Mekonnen E, Legesse A. Phytochemical screening and toxicity profiles of crude extracts of *Cissus quadrangularis* L. and *Solunum incanum* L. in mice. Afr J Pharm Pharmacol 2017;11:411-8.
17. OECD. Test No. 203: Fish, Acute Toxicity Test. OECD Guidelines for the Testing of Chemicals; 1992. p. 1-10.
18. Makhija DT, Jagtap AG. Studies on sensitivity of zebrafish as a model organism for Parkinson's disease: Comparison with rat model. J Pharmacol Pharmacother 2014;5:39-46.
19. Naidu PS, Singh A, Kulkarni SK. Effect of *Withania somnifera* root extract on reserpine-induced orofacial dyskinesia and cognitive dysfunction. Phytother Res 2006;20:140-6.
20. Cosgrove J, Alty JE. Cognitive deficits in Parkinson's disease: Current perspectives. J Park Restless Legs Syndr 2018;8:1-11.
21. Mahmoudi Asl A, Mehdizadeh M, Raeesi Roudbari P, Mehdizadeh H, Habibi SA, Niazi Khatoon J, *et al*. Staging of cognitive impairment in Parkinson's disease: Validity

of quick dementia rating system. Disabil Rehabil 2022;44:44-51.
22. Rahn KA, Slusher BS, Kaplin AI. Glutamate in CNS neurodegeneration and cognition and its regulation by GCPII inhibition. Curr Med Chem 2012;19:1335-45.
23. Swathi G. Neuroprotective Role of *Bacopa monnieri* against Rotenone Induced Parkinson's Disease. Shodhganga: A Reservoir of Indian Theses; 2013. p. 43-7.
24. Cruz R, Almaguer Melian W, Bergado Rosado JA. Glutathione in cognitive function and neurodegeneration. Rev Neurol 2003,36:877-86.

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