IJPSR (2021), Volume 12, Issue 10 (Research Article)

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 19 October 2020; received in revised form, 07 February 2021; accepted, 23 June 2021; published 01 October 2021

ANTIPARKINSONIAN ACTIVITY OF HYDROALCOHOLIC EXTRACT OF THE STEMS OF *CAPPARIS DECIDUA* (FORSSK.) EDGEW.

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Keywords:

Capparis decidua, Catalepsy, Haloperidol, Dopamine, DPPH, Ascorbic acid Swiss albino mice

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ABSTRACT: The goal of the research is to analyze the antiparkinsonian activity of hydroalcoholic extract of the stems of *Capparis Decidua* (HECD). Themost common neurodegenerative condition which stand second is Parkinson. It is reported by dopaminergic neuron loss in the striatum's substantia nigra & dopamine loss (DA). Capparis *Decidua* plant is used as anti-inflammatory, laxative, anti-diabetic, anthelmintic, antibacterial, astringent, digestive and for so many other traditional use, we used this research to scientifically verify Parkinson behaviour using suitable animal models. In mice, using haloperidol (1 mg/kg i.p) catalepsy was induced. Bromocriptine (2.5 mg/kg) and HECD were administered orally to the treatment group at the dose of 100 mg/kg, 200 mg/kg, and 300 mg/kg. Bar tests for catalepsy, motor coordination tests by rotarod, and locomotor activity by actophotometer were performed to evaluate behavioural changes. Dopamine and catalase assay were also carried out to assess biochemical parameters. Using standard as ascorbic acid in UV visible spectrophotometer, evaluation of antioxidant activity by DPPH radical scavenging method were performed. The outcome of our work indicates that the hydroalcoholic extract possesses antiparkinsonian activity. It can be reported that because of its antioxidant activity and presence of flavonoids, alkaloids, and polyphenols that may be essential for antiparkinsonian effect, it can be indicated that they may have played an important a role in the treatment.

INTRODUCTION: The second most common neurodegenerative condition is Parkinson's disease (PD) [1, 18]. This usually presents as bradykinesia, stiffness, resting tremor, and unstable posture. In substantia nigra, a midbrain region, PD is primarily characterized by the death of dopaminergic neurons. It is unclear the cause of that selective cell death. Notably, complexes and aggregates of alpha-synuclein-ubiquitine are known for aggregation within affected neurons in Lewy bodies [2].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(10).5388-95

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(10).5388-95

Impaired axonal transport of alpha-synuclein may also induce its build-up in Lewy bodies [3]. Membrane damage caused by alpha-synuclein may be another Parkinson's disease mechanism [4]. Age is the principal recognized risk factor. Mutations in genes such as α-synuclein (SNCA), leucine-rich repeat kinase 2 (LRRK2), glucocerebrosidase (GBA) and tau protein (MAPT) can also induce inherited PD or increase the risk of developing PD [5].

The key clinical symptoms of Parkinson's disease are cell death in the basal ganglia of the brain (affecting by the end of life up to 70 percent of the dopamine secreting neurons in the substantia nigra pars compacta) [6] and the presence of Lewy bodies (protein alpha-synuclein accumulations) in many of the neurons remaining.

The death of astrocytes (star-shaped glial cells) and a substantial increase in the number of microglia (another type of glial cell) in the substantia nigra accompany this loss of neurons [7]. Haloperidol is experimental PD model. Haloperidol is used for the treatment of psychosis as it is neuroleptic drug. It functions by disrupting receptors of dopamine D2 and D1 in medium spiny neurons, which include motor circuit indirect and direct pathways. This leads to blockage of striatal dopamine transmission, which causes abnormal downstream firing in the basal ganglia as symptoms of muscle stiffness, locomotive activity, and catalepsy [8]. Various phyto-constituents that include alkaloids, glycosides, terpenoids, sterols, flavonoids, phenols, and fatty acids have been described and isolated from various parts of *Capparis Decidua*. Some of these compounds like N-triacontane, n-triacontanol, n-pentacosane, 6-(1-hydroxy-non-3-enyl) tetrahydro-pyran-2-one, 2-carboxy-1-dimethylpyrrolidine, β-sitosterol, β-carotene, Glucosinolates, Ascorbic acid, proteins, carbohydrates, Calcium, Potassium, Phosphorous, Zinc, Iron, and Manganeseare located in aerial components of flowers, fruits, stems and seeds [9]. Anti-inflammatory, laxative, anti-diabetic, anthelmintic, antibacterial, astringent, digestive, diaphoretic, and anodyne is shown in the Capparis plant. Numerous diseases, such as rheumatism, asthma, diabetes, liver disorders, hyper-cholesterolemia, hypertension and microbial infections, have beneficial effects [10]. Hence, in this research, we studied the anti-parkinsonian activity of hydroalcoholic extract of the stems of *Capparis decidua* (HECD) (test drug) and bromocriptine (standard drug) in Swiss Albino Mice.

MATERIALS AND METHODS: Fresh, dried stems of *Capparis deciduas* were collected from Jalgaon, and the specimen was submitted to the Department of Botany, authenticated by Harshad M Pandit, Andheri (West), Mumbai-400 058(Ref no.: OCP/2019-20) The stems of *Capparis decidua* were washed, air-dried for 2 days and crushed to a coarse powder. The powder obtained was passed through sieve no. 40 and used for further studies. Dried coarse powder of *Capparis decidua* stem was extracted with a mixture of 90% v/v ethanol (50%) and distilled water (50%) in a 250 ml Soxhlet at 60°C. The solvent obtained was evaporated to remove excess of the solvent, concentrated, and then used for the study. The yield was observed to be 12% w/w. These crude dried extracts were put in a suitable container and kept in refrigerator 4°C until use.

Qualitative Phytochemical Screening: Preliminary chemical tests were carried out on hydroalcoholic extract of *Capparis decidua* stem for determination of the presence of phytoconstituents like alkaloids, flavonoids, saponins, steroids, tannins, proteins, terpenoids, and phenolic compounds.

Experimental Animals: Either sex Swiss Albino Mice (20-25 g) were used for the study. The Swiss Albino Mice were obtained from Bombay Veterinary College Parel, Mumbai-400012. The use of these animals and the study protocols were approved by CPCSEA recognized institutional ethics committee of Oriental College of Pharmacy. Mice were kept at the animal house of Oriental College of Pharmacy, Sanpada, Navi Mumbai. The animals were allowed on a comfortable polypropylene cage with proper bedding followed by a dark and light cycle at a steady temperature of 22 ± 2 °C. They were provided with filtered water and pellets on a daily diet. The IAEC of Oriental College of Pharmacy approved the experimental protocol no. OCP/IAEC/2019-2020/02.

Chemicals and Reagents:

1. Hydroalcoholic extract of the stems of *Capparis decidua (HECD)* (100 mg/kg, 200 mg/kg, 300 mg/kg)
2. Standard drug: Bromocriptine (Aniruddha Medical stores, Navi- Mumbai)
3. Drug to induce catalepsy: Haloperidol (inj. Serenace)
4. 0.1M Perchloric acid
5. Hydrogen Peroxide
6. Levodopa
7. Distilled water
8. Ascorbic acid
9. DPPH

Pharmacological Evaluation: Mice were randomly divided into six groups (n = 6), *viz.*, vehicle control (vehicle-treated), haloperidol control, bromocriptine and HECD treated group low dose (100 mg/kg), intermediate dose (200 mg/kg), high dose (300 mg/kg). Bromocriptine and HECD were administered orally. One hour after the

drug administration, the animals were induced with haloperidol 1 mg/kg intraperitoneal administration.

Estimation of Behavioural Parameters:

1 - Bar Test: [11] To assess catalepsy, the Bar Test was used. The front paw of the animals in the bar test was positioned on a horizontal bar situated 3 cm and 5 cm above and alternately parallel to the base. It recorded the time at which the animal removed its paw from the bar. The rating for catalepsy was given as follows:

Step I: The mice were removed from the home cage and placed on a floor. A score of 0.5 was assigned if the mice failed to move when touched or pressed gently on the back.

Step II: Alternately, the front paws of the mice were placed on a 3-cm-high block. If the mice failed to correct the position within 15 sec, a score of 0.5 for each paw was applied to the score of step I.

Step III: Alternately, the front paws of the mice were placed on a 5-cm-high block, if the mice failed to correct the position within 15 sec, a score of 1 for each paw was applied to the scores of steps I and II.

2 - Motor Co-ordination Test (Rotarod Test): [12] Using a Rotarod apparatus, a motor coordination test was performed. The animals were put on the moving rod prior to treatment, and the mice were selected for the analysis to remain on the rod without dropping for 120 seconds. Before and after the medication, the time taken by animals to fall from the rotating rod was noted. With an acceleration rate of 20 rpm, the starting speed of the rotarod was set to 4 rpm. The maximum velocity was 40 rpm.

3 - Test for Locomotor activity (Acto-photometer): [13] The behavior of the locomotor was measured using the Actophotometer. It consists of a cage with six lights and six photocells positioned in the outer bottom periphery so that only one beam is blocked at a time by single mice. When the light rays fall on the photocells, photocells get activated. The light beam is interrupted when the animal crosses the light beam; the number of cut-off interruptions has been recorded for 10 min.

Biochemical Test:

1- Determination of dopamine by HPLC:

Preparation of Brain Sample: The striatum dissected was instantly frozen on dry ice and placed at -80 degrees Celsius. Striatal tissue was sonicated (about 100 µl / mg tissue) in 0.1 M perchloric acid. The supernatant fluids were taken by HPLC for dopamine level measurements**.** [14].

Assay:

Preparation of Standard Solution: [15]

Levodopa: 50 mg of Levodopa, accurately measured, was transferred to 50 mL of the volumetric flask, added 30 mL of mobile phase, and sonicated for 15 minutes. Make up the volume by the mobile phase. 5ml was taken into the 50ml volumetric flask from the above solution and the mobile phase volume was made up. 2ml was taken into the 10ml volumetric flask from the above solution, and 1µl was injected for HPLC analysis.

Preparation of Sample Solution: Take 5ml of supernatant fluid accurately and transfer to 50ml of volumetric flask dissolved and diluted by mobile phase volume and sonicated for 10 min. The above solution took 5 ml into a 50 ml volumetric flask to make up the mobile phase volume. 2 ml was taken from the above solution into a 10 ml volumetric flask and 1µl was injected into the HPLC for examination. Dopamine concentrations were represented as nanograms per milligram of brain tissues. In general, 20 µl of supernatant fluid was isocratically eluted as the internal standard with a mobile phase containing water and methanol (70:30) respectively via a 4.6-mm C18 column. 1 ml/min was the flow rate.

2-Determination of Catalase (CAT) Principle: [16]

Preparation of Brain Sample: Using the carbon dioxide chamber, the Bar test, Motor Coordination Test and Locomotor Behavior in Haloperidol-induced Parkinson's mice from each group were euthanized after evaluation; brains were rapidly removed and put in ice-cold saline.

The tissues in the 0.1 M Phosphate Buffer (pH 8) were weighed and homogenized. To examine Catalase activity, samples of mice brain homogenates were collected in various test tubes. The supernatant was used for the study of catalase.

Assay: The activity of catalase was measured by UV. 0.1 ml of supernatant was added to the cuvette containing 1.9 ml of 50 mM phosphate buffer (pH 7.0). The reaction was triggered by the addition of 1.0ml of 30 mM H_2O_2 freshly prepared. The spectrophotometric measurement of the rate of decomposition of H_2O_2 was based on changes in absorbance at 240 nm. The activity of catalase was expressed as units/mg protein. The reaction occurs immediately after the addition of H_2O_2. Solutions were well mixed and after 15 seconds (t1) the first absorbance (A1) was read, and after 30 seconds (t2) the second absorbance (A2). The absorbance was read at a wavelength of 240 nm.

Evaluation of Antioxidant Activity by DPPH Radical Scavenging Method: [17] Free radical scavenging activities of the *stem of Capparis decidua* plant were measured by 1, 1- diphenyl-2-picryl hydrazyl (DPPH). Solution of DPPH in ethanol 0.1mM was prepared. This solution (1 ml) was added to 3 ml of different extracts in ethanol at different concentration (5, 10, 20, 40 µg/ml). Here, only those extracts which are solubilise in ethanol were used and different concentrations were prepared by dilution method. The mixture was then shaken vigorously and allowed to for 30 min at room temp. Then, absorbance was measured at 517 nm. by using a spectrophotometer (UV-VIS). The reference standard compound being used was ascorbic acid and the experiment was done in double.

Statistical Analysis: Values were presented as mean ± SEM. Data were statistically evaluated by one-way analysis of variance (ANOVA) followed by Dunnett's test for intergroup comparison using Prism Software by GraphPad. Results were considered to be statistically significant at*P≤0.05, where **** represents significance at P≤0.0001 ***indicated P≤0.001, ** indicated P≤0.01, *indicates P≤0.05 when compared with standard.

RESULTS:

A. Phytochemical Analysis: The phytochemical analysis of the extract revealed that the hydroalcoholic extract of the stems of *Capparis decidua* (HECD) shows the presence of carbohydrates, saponins, flavonoids, alkaloids, phenolic compounds, and tannins.

TABLE 1: RESULT OF QUANTITATIVE PHYTOCHEMICAL ANALYSIS OF POWDERED STEMS OF *CAPPARIS DECIDUA*

Phytochemicals	Observations
Carbohydrates	+
Proteins	-
Steroids	+
Saponins	+
Flavonoids	+
Alkaloids	+
Phenolic compounds	+
Tannins	+

Present (+)/Absent (-)

B. Haloperidol Induced Catalepsy in Mice:

Bar Test: In bar test **Table 2**, haloperidol control group significantly increases the cataleptic score as compared to the vehicle control group. Bromocriptine 2.5 mg/kg and HECD 300 mg/kg showed significant inhibition against catalepsy by decreasing cataleptic score.

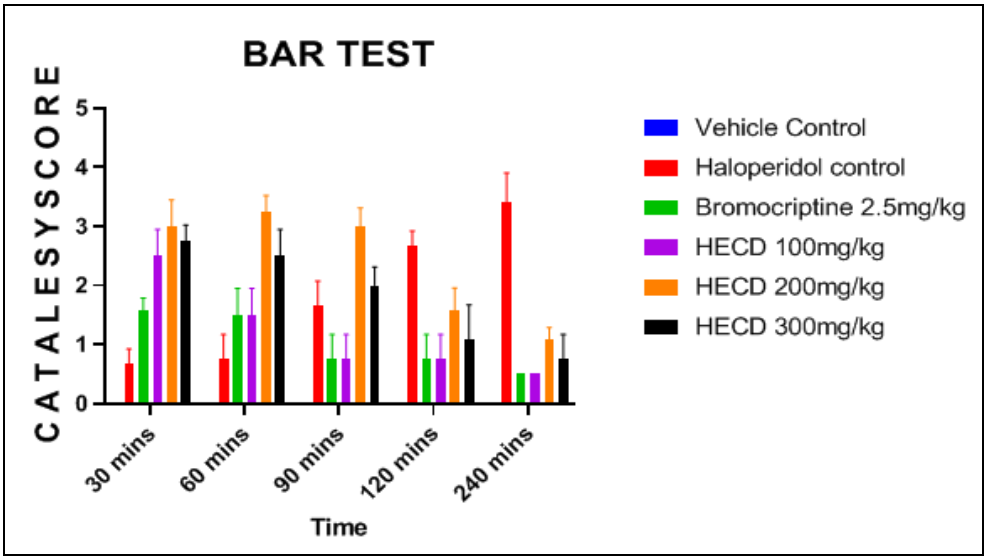

FIG. 1: EFFECT OF BROMOCRIPTINE AND HECD ON CATALEPSY IN BAR TEST

TABLE 2: EFFECT OF BROMOCRIPTINE AND HECD ON CATALEPSY IN BAR TEST

Time interval in min	Mean ± SEM (Cataleptic score)					
	Vehicle control	Haloperidol control	Bromocriptine 2.5 mg/kg	HECD 100 mg/kg	HECD 200 mg/kg	HECD 300 mg/kg
0	0.00±0.00**	0.00±0.00	0.00±0.00**	0.00±0.00	0.00±0.00	0.00±0.00
30	0.00±0.00**	0.66±0.105	1.58±0.083***	2.50±0.183***	3.00±0.183***	2.75±0.112***
60	0.00±0.00**	0.75±0.171	1.50±0.183**	1.58±0.183**	2.75±0.112***	2.50±0.183***
90	0.00±0.00***	1.58±0.167	0.75±0.171***	0.75±0.171***	2.50±0.129***	1.58±0.129***
120	0.00±0.00***	2.50±0.105	0.75±0.171***	0.75±0.171***	1.58±0.154***	1.08±0.239***
240	0.00±0.00***	3.417±0.201	0.50±0.001***	0.50±0.001***	1.08±0.083***	0.75±0.171***

All values are expressed in Mean ± SEM (n = 6). Significance: ****indicated P≤0.0001,***indicated P ≤0.001,** indicated P≤0.01,*indicates P≤0.05 when compared with negative control.

Motor Co-ordination Test: Fall of time from rotarod was significantly decreased in the haloperidol treated group compared to the vehicle control group. It was significantly improved with Bromocriptine 2.5mg/kg, HECD 200 and 300 mg/kg **Table 3**.

TABLE 3: EFFECT OF BROMOCRIPTINE AND HECD ON MOTOR CO-ORDINATION TEST USING ROTAROD

Treatment Groups	Fall of Time (Sec) Mean ± SEM
Vehicle Control	42.67±0.88**
Haloperidol Control	10.67±0.33
Bromocriptine 2.5mg/Kg	43.83±1.24**
HECD 100 mg/kg	23.17±0.90**
HECD 200 mg/kg	38.00±0.73**
HECD 300 mg/kg	49.33±0.49**

All values are expressed in Mean ± SEM (n = 6). Significance: ****indicated P≤0.0001, ***indicated P ≤0.001, **indicated P≤0.01,*indicates P≤0.05 when compared with negative control.

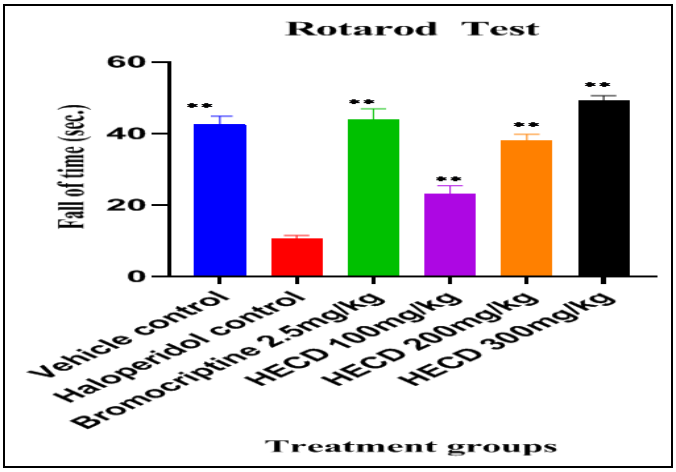

FIG. 2: EFFECT OF BROMOCRIPTINE AND HECD ON MOTOR COORDINATION TEST USING ROTAROD

Test for Locomotor Activity: Spontaneous motor activity was significantly decreased in haloperidol treated group as compared to the vehicle control group. Bromocriptine 2.5 mg/kg and HECD 300 mg/kg, significantly increased the locomotor activity as compared to haloperidol treated animals.

TABLE 4: EFFECT OF BROMOCRIPTINE AND HECD ON LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER

Treatment Groups	Ambulations Counts/10 min Mean ± SEM
Vehicle Control	241.7±4.77****
Haloperidol Control	1.677±0.21
Bromocriptine 2.5mg/Kg	15.83±0.60***
HECD 100 mg/kg	8.83±0.94
HECD 200 mg/kg	11.67±1.28*
HECD 300 mg/kg	12.17±1.27**

All values are expressed in Mean ± SEM (n = 6). Significance: ****indicated P≤0.0001,***indicated P ≤0.001, ** indicated P≤0.01,*indicates P≤0.05 when compared with negative control.

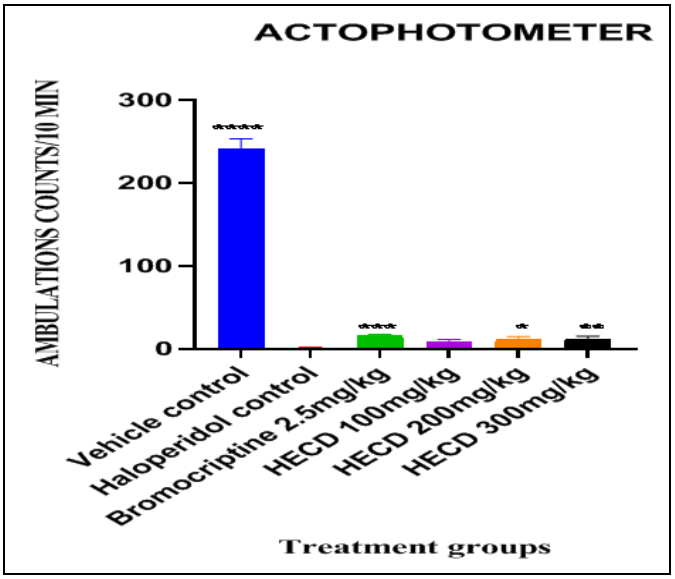

FIG. 3: EFFECT OF BROMOCRIPTINE AND HECD ON LOCOMOTOR ACTIVITY USING ACTOPHOTOMETER

C. Biochemical Parameters:

Determination of dopamine by HPLC: In bar test **Table 5**, haloperidol control group significantly decrease in dopamine level as compared to the vehicle control group. Bromocriptine 2.5 mg/kg and HECD 300 mg/kg showed a significant increase in dopamine levels.

TABLE 5: EFFECT OF BROMOCRIPTINE AND HECD ON DOPAMINE LEVEL USING HPLC

Treatment Groups	Dopamine (ng/mg) of tissue Mean ± SEM
Vehicle Control	26.86±0.83**
Haloperidol Control	18.83±0.30
Bromocriptine 2.5mg/Kg	33.50±0.22**
HECD 100 mg/kg	25.50±0.22**
HECD 200 mg/kg	27.00±0.36**
HECD 300 mg/kg	31.73±0.67**

All values are expressed in Mean ± SEM (n = 6). Significance: ****indicated p≤0.0001, ***indicated P ≤0.001, **indicated P≤0.01, *indicates P≤0.05 when compared with negative control.

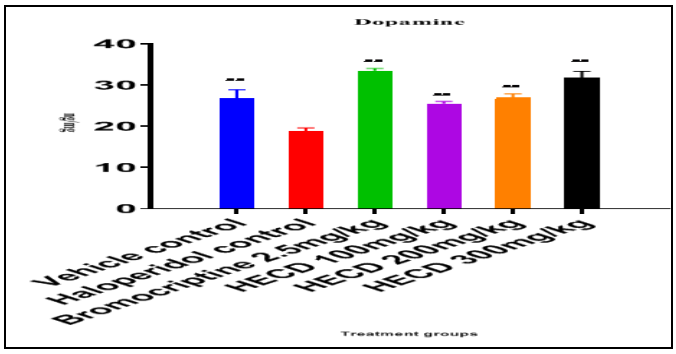

FIG. 4: EFFECT OF BROMOCRIPTINE AND HECD ON DOPAMINE LEVEL USING HPLC

Determination of Catalase by UV: In this test **Table 6**, haloperidol control group significantly decrease in catalase level as compared to the vehicle control group. Bromocriptine 2.5 mg/kg and HECD 300 mg/kg showed a significant increase in catalase level.

TABLE 6: EFFECT OF BROMOCRIPTINE AND HECD ON CATALASE LEVEL USING UV

Treatment Groups	UNIT/mg Mean ± SEM
Vehicle Control	30.71±0.23**
Haloperidol Control	22.29±1.93
Bromocriptine 2.5mg/Kg	43.21±0.23**
HECD 100 mg/kg	32.89±0.55**
HECD 200 mg/kg	33.36±0.47**
HECD 300 mg/kg	40.43±0.19**

All values are expressed in Mean ± SEM (n = 6). Significance: ****indicated p≤0.0001, ***indicated P ≤0.001, **indicated P≤0.01,*indicates P≤0.05 when compared with negative control.

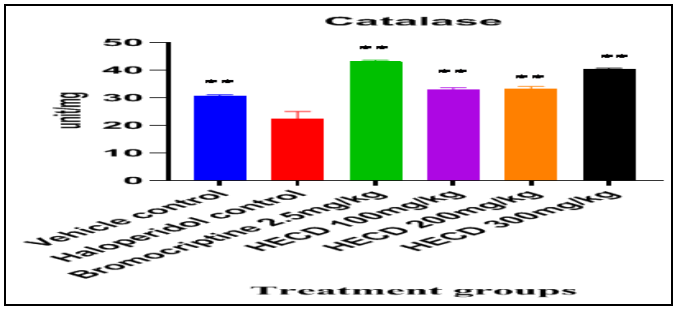

FIG. 5: EFFECT OF BROMOCRIPTINE AND HECD ON CATALASE LEVEL USING UV

D. Evaluation of Antioxidant Activity by DPPH Radical Scavenging Method: Absorbance of *Capparis decidua (stem)* with standard ascorbic acid at 517 nm by UV visible spectrophotometer (DPPH scavenging assay method) **Table 7.**

TABLE 7: ABSORBANCE OF *CAPPARIS DECIDUA* (STEM) WITH STANDARD ASCORBIC ACID

Concentration [µg/ml]	Extract (abs)	Ascorbic Acid (abs)
5	0.294	0.028
10	0.257	0.018
20	0.195	0.016
40	0.126	0.010

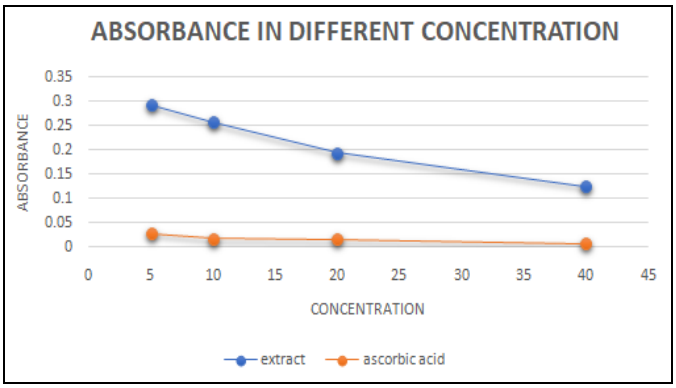

FIG. 6: ABSORBANCE AT DIFFERENT CONCENTRATION OF EXTRACT AND ASCORBIC ACID

In **Table 8**, DPPH scavenging effect (%) or Percent inhibition = $A_0 - A_1 / A_0 \times 100$.

Where A_0 was the Absorbance of control reaction and A_1 was the Absorbance in presence of test or standard sample.

TABLE 8: % INHIBITION OF *CAPPARIS DECIDUA* (STEM) WITH ASCORBIC ACID

Concentration [µg/ml]	Extract (% Inhibition)	Ascorbic Acid (% Inhibition)
5	86.01	98.66
10	87.74	99.14
20	90.72	99.23
40	93.98	99.52

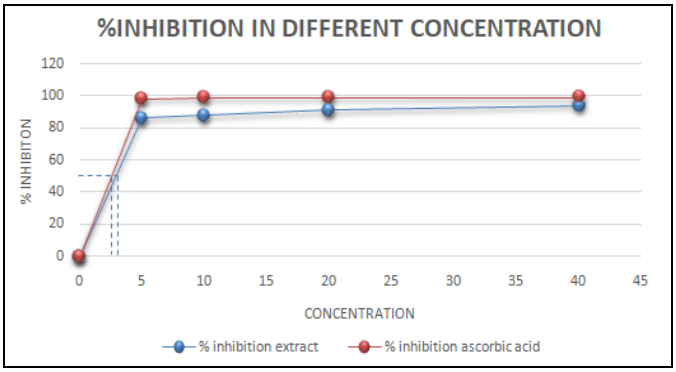

FIG. 7: %INHIBITION OF EXTRACT AND ASCORBIC ACID AT DIFFERENT CONCENTRATION

DISCUSSION:

Haloperidol Induced Catalepsy in Mice: Catalepsy (rigidity in movements), akinesia (slowing of movement), tremors, and memory loss are some of the major symptoms of PD. Amongst this catalepsy is one of the major symptoms which make the life of PD patient uneasy.

Bromocriptine is well-known dopamine (D2) receptor agonist and is commonly used to improve the symptoms related to rigidity. Hence, this drug was used as a standard in the present study to compare the efficiency of the models (mice).

Catalepsy was induced in mice by intraperitoneal (i.p.) administration of haloperidol (1 mg/kg). This cataleptic behaviour induced by haloperidol and the protective effect of standard (bromocriptine) and HECD used was evaluated by using bar test, rotarod apparatus and actophotometer.

Bar Test: This test gives the idea about the extent of catalepsy induced in an animal. In a present study, Bromocriptine 2.5 mg/kg and HECD 300 mg/kg reversed the effects of haloperidol in a bar test by decreasing cataleptic score significantly.

Motor Co-ordination Test by Rotarod: Imbalance is one of the symptoms of PD, to evaluate it, this test was carried out. The test consists of a rotating rod upon which the animal balances. Haloperidol treated mice, subjected to the rotarod test, exhibited a significant loss of muscular coordination, it could be due to loss of muscular strength. Bromocriptine 2.5mg/kg, HECD 200 and 300 mg/kg prevented the motor impairment significantly, which was altered by haloperidol. It indicates that HECD may have active constituents with CNS stimulant activity.

Locomotor Activity by Actophotometer: Due to the catalepsy; movement restrictions or sometimes freezing of the movements is exhibited by PD patient. Hence, the drug which improves the locomotor activity can modify the condition of PD patient. The results indicated that haloperidol caused significantly decreased locomotor counts in actophotometer. Bromocriptine 2.5 mg/kg and HECD 300 mg/kg, significantly increased the locomotor activity as compared to haloperidol treated animals. Daily treatment with HECD significantly reversed the decrease in locomotor activity as assessed on day 14.

Biochemicals Parameters:

Determination of Dopamine by HPLC: The turnover of dopamine in nigral cells plays a major role in controlling motor function. In the present study, HECD 300 mg/kg caused a pronounced increase in in dopamine levels in mid-brain regions of haloperidol-induced mice. It could be a result of the protection of dopaminergic neurons by these drugs. The beneficial roles of in HECD retaining dopamine levels demonstrated the protection of nigral neurons by test drugs.

Determination of Catalase by UV: Catalase is an antioxidant that helps in neutralizing the toxic effects of hydrogen peroxide. Hydrogen peroxide is converted by the catalase enzyme to form water and non-reactive oxygen species, thus preventing the accumulation of precursor from freeing radical biosynthesis. Oxidative stress results in a decrease in catalase level. Bromocriptine 2.5 mg/kg and HECD 300 mg/kg, significantly increased the catalase level as compared to haloperidol treated animals.

Evaluation of Antioxidant Activity by DPPH Radical Scavenging Method: This study determined that hydroalcoholic extract of stem of *Capparis decidua* plant species showed better antioxidant potential by DPPH radical scavenging method when compared to standard ascorbic acid and IC_{50} value found to be as 2.0 and 2.5 µg/ml for ascorbic acid and hydroalcoholic extract respectively. So, we can say that this plant may have antioxidant activity.

CONCLUSION: *Capparis decidua* exhibited significant antiparkinsonian activity in a haloperidol mouse model. It appears to be the most promising plant due to its potential antioxidant activity. The predictable mode of action of this plant may be due to antioxidant activity and its presence of flavonoids and polyphenols. These findings provide evidence for its use as antiparkinsonian medication, including prevention of PD, improvement of PD symptoms. Future studies are required to investigate the phytoconstituents responsible for the activity and establish the exact mode of action.

ACKNOWLEDGEMENT: We are thankful for their guidance and help to our Principal Dr. (Mrs.) Sudha Rathod, Mr. Imtiyaz Ansari, and the Department of Pharmacology, Oriental College of Pharmacy, Navi Mumbai.

AUTHORS' CONTRIBUTIONS: Mr. Imtiyaz Ansari guided with designing the study, making protocol, and managing the work done. Shaheen Khan performed the literature searches, performed the biochemical test and DPPH, models, phytochemical screening, and completed the manuscript writing.

CONFLICTS OF INTEREST: We announce we do not have conflicting interests.

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How to cite this article:
Khan S and Ansari I: Antiparkinsonian activity of hydroalcoholic extract of the stems of *Capparis decidua* (Forssk.) Edgew. Int J Pharm Sci & Res 2021; 12(10): 5388-95. doi: 10.13040/IJPSR.0975-8232.12(10).5388-95.