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Neuroprotective action of *Smilax china* ethanolic bark extract in treatment of a prominent aging disorder: Parkinson's disease induced by rotenone

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Abstract

Background Tremors, psychological difficulties, mental health issues, depression, impulsive acts, and other behavioral abnormalities are all symptoms of Parkinson's disease, a neurodegenerative disorder of the central nervous system. *Smilax china* ethanolic extract was tested for its anti-Parkinson's activity using a Wistar rat model of rotenone-induced Parkinson's disease. Spectroscopic, acute toxicity and pharmacognostic analyses were performed.

Result Brownish, the bark of *Smilax china* included vascular bundles and fibers upon microscopic inspection and alkaloids, carbohydrates, and phenolic substances upon phytochemical analysis. Acute toxicity testing as per Organization for Economic Corporation and Development 423 (OECD 423) on male Wistar rats revealed no harmful effects. The biochemical analysis of rotenone-induced groups revealed a disproportion. Improved body weight, mobility, coordination, and a lower incidence of catalepsy were seen in animals treated with *Smilax china* ethanolic extract (100 and 200 mg/kg). *Smilax china* 200 mg/kg extract substantially lowered motor defects determined by catalepsy score using bar test 17.061.74/s against rotenone-induced group 67.593.27/s. It also prevented the brain from oxidative stress by enhancing superoxide dismutase (SOD) levels to 5.440.01 units/mg protein compared to 2.050.104 units/mg protein in the rotenone-induced group. The vagus nerve, substantia nigra, and basal ganglia of the treated groups indicated a reduction in inflammation and alpha-synuclein destruction.

Conclusion Based on our research, an ethanolic extract of *Smilax china* bark provides an effective antioxidant with promising neuroprotective properties in male Wistar rats induced with Parkinson's disease.

Keywords Biochemical, Brain, CAT, GSH, Histopathology, MDA, Parkinsonism, *Smilax china*, SOD, Spectroscopy, Substantia nigra, TLC, Toxicity, Vagus nerve

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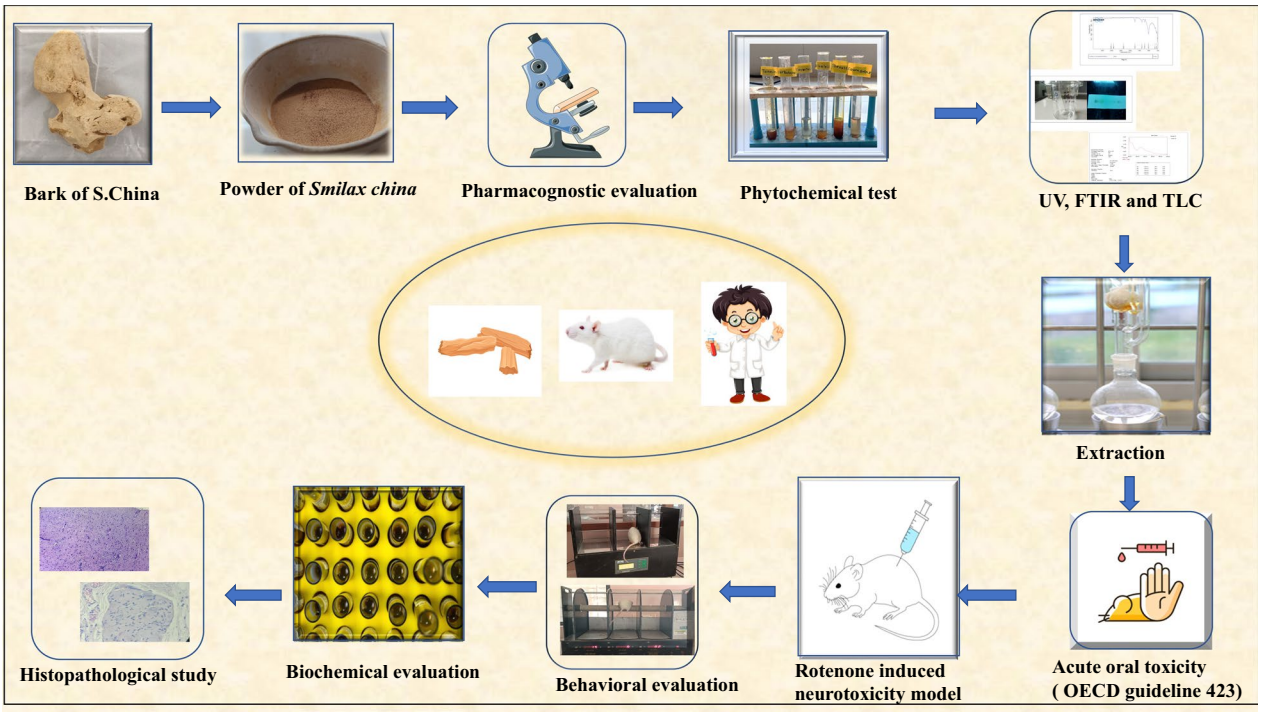
Graphical abstract



Background

Stiffness, slowness, and tremors of movement are common symptoms of Parkinson's disease, a neurological degenerative disease. Furthermore, the substantia nigra, as well as other areas of the brain, become impacted, which results in slow neurodegeneration. When the condition is developed, non-motor signs including dementia and dysautonomia commonly appear. Parkinson's disease is developed or triggered because of the reduction of mitochondrial complex-1, various cell-damaging mechanisms which include protein aggregation, apoptosis, calcium homeostasis, inflammation, excitotoxicity, atypical energy metabolism, and associations between environmental interactions and genetics very well seen [1, 2]. Oxidative stress, which interacts with dopamine metabolism, is the main cause of Parkinson's disease. This oxidative damage results in the generation of reactive oxygen species (ROS), resulting in neuronal death. This was shown by a reduction in spontaneous antioxidant molecule levels. According to these results, it is now essential to use antioxidants as an effective treatment for Parkinson's disease in addition to other preventative treatments [3].

The ethanolic extract of *Smilax china* was targeted on reducing the oxidative stress in the substantia nigra. The ethanolic extract of *Smilax china* shows the anti-oxidant property because Smilax china bark contains flavonoid is bioactive against oxidative stress and shows anti-oxidant action. The mechanism of Parkinson's disease is due to dopamine auto-oxidation in dopaminergic neurons that is a significant source of reactive oxygen species that causes neuronal oxidative stress. Microglia and astrocytes can cause neuroinflammation, which can speed the course of pathogenic damage to the substantia nigra. The mechanism of Parkinson's disease that causes tremors, rigidity, and bradykinesia is a decrease in striatal dopamine Parkinson's disease is a neurodegenerative disorder of the central nervous system that is one of the mental disorders that cause tremors, rigidity, and bradykinesia. Many factors determine the development of disease. Majorly neurodegeneration of dopaminergic neurons in the substantia nigra is due to an increase in oxidative stress [4].

Plants have been used as a source of medicine since prehistoric times. Plants have a crucial role in maintaining the life cycle and enhancing human well-being. Flavorings, cosmetic compounds in medicines, colors,

and beverages are all ways in which they have benefited humankind. Recent years have seen a surge in interest in plant science, and vast data sets have been tested to demonstrate the enormous potential of medicinal herbs used in a wide range of alternative medical processes. *Smilax china* bark is a woody climber native to the Western Ghats in southern India (Table 1). The roots of *Smilax china* have been used to cure boils, swellings, gonorrhea, syphilis, and abscesses [5]. Traditional medicines are the plants to cure a wide range of conditions, including skin conditions, colic, leucorrhoea, dysuria, fever, and dysentery [6]. It was additionally used to treat lower-limb rheumatism, swellings, wounds, pain, and abscesses. *Smilax china* is a valuable Ayurvedic drugs are used to treat a wide range of diseases, ranging from disorders of the neurological system, epilepsy, psychosis, rejuvenator, hemiplegia, polyuria, Parkinson's disease, urinary problems, blood purifier, congenital diseases, and leprosy. The primary benefit of *Smilax China* is that it has been linked to antiepileptic effects [7]. The glycoside diosgenin (steroidal saponin) is found in the roots of *Smilax china* [8]. However, there have been no known studies carried out on the plant's anti-Parkinson activities. In the western tribal region of India using *Smilax china* bark for treating neuronal disorders is a common practice; therefore, it intrigued me to conduct scientific experimentation for the identification of probable bioactive compounds in *Smilax china* bark. Also, Haixing Feng 2020 has stated the anti-inflammatory action of *Smilax china* bark in his research study which might be fruitful in treating Parkinson's and other brain-related disorders [9].

The objective of the current study was to find out if *Smilax china* bark might protect male Wistar rats from the neurotoxicity that is rotenone-induced Parkinson's disease.

Table 1 The common names of *Smilax china* and its taxonomic classification [10, 11]

S. No	Characteristics	Features
1	Name	Chopchine
2	Botanical Name	*Smilax china (L.)*
3	Kingdom	*Plantae*
4	Subkingdom	*Tracheobionta*
5	Super division	*Spermatophyta*
6	Division	*Magnoliophyta*
7	Class	*Liliopsida*
8	Order	Liliales
9	Family	Smilacaceae
10	Genus	*Smilax*
11	Species	*China*
12	Ayurvedic Names	Dwipantaravacha
13	Vernacular Names	English: *China root*
		Bengali: *Kumarika*
		Marathi: *Ghotvel*
		Oriya: *Mootrilata*
		Tamil: *Ayadi*
		Hindi: *Chopchini, Chobchini, Toupchini*
		Kannada: *Kaadu hambu*
		Malayalam: *Kaltamara*
		Mizoram: Kaitha
		Telugu: *Kondadantena*

Methods

Herbal plants collection

Smilax china plants were purchased from the herbal market and verified as genuine by our research institution's Department of Pharmacognosy.

The dried barks of *Smilax china* were collected from Ramana Koppa village, Taluka Kundagolai, Karnataka, India. It was authenticated by a registered plant taxonomist in our institute with Ref No: JOR/ DCM 08/2022. The herbarium sheet has been submitted to the taxonomist, and the authentication number provided for the same is Ref No: JOR/ DCM 08/2022.

Bark extract preparation

The bark of the plant *Smilax china* was used in this research study (Fig. 1). The bark was dried and then finely powdered before undergoing continuous hot percolation with ethanol in a Soxhlet apparatus between 65 and 70 degrees Celsius (Fig. 2). A rota flash evaporator (Fig. 3) was used to remove the solvent from the extract at low pressure. The obtained orally administered extract was suspended in 1% v/v Tween 80 [12]. The picture of the extract is shown in Fig. 4 [13].

Fig. 1 The bark of *Smilax china* used in the research study

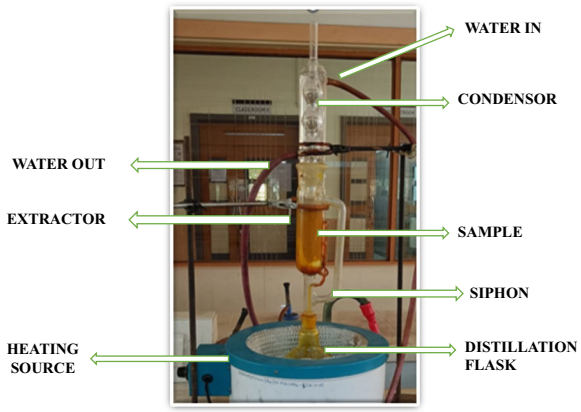


Fig. 2 The *Smilax china* bark was dried and finely powdered before continuous hot percolation with ethanol in a Soxhlet apparatus between 65 and 70ºC

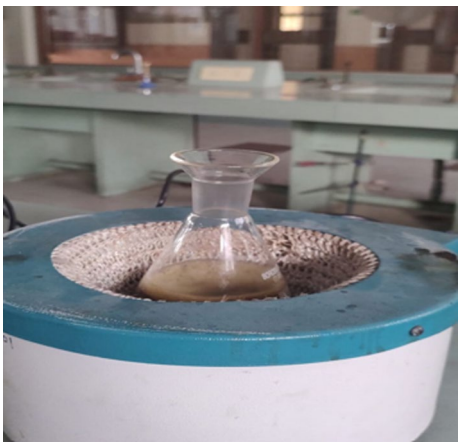


Fig. 3 Rota flash evaporator used for the removal of the solvent from the extract by evaporation

$$\%\ \text{of extractive yield}\ (w/w) = \frac{\text{Weight of dried extract}}{\text{Weight of dried bark powder}} \times 100 \quad (1)$$

Macroscopic evaluation of the bark of Smilax china

Drug testing relies only on objective, visible symptoms. Form, size, fracture, surface, texture, external and internal, scent, color, and taste are just a few of the features that were noticed [14].

Fig. 4 The ethanolic extract of *Smilax china* bark

Microscopic examination of the bark of Smilax china

Every plant has special characteristics that may be examined with an appropriate staining method that begins with cells, tissues, and the way they are organized. A standard rate of formalin-aceto-alcohol (FAA) storage (Formalin 5 ml + Acetic acid 5 ml + 70% ethyl alcohol 90 ml) is used to keep the collected component for longer than 48 h. Next, eosin staining was performed on thin transverse sections of the sample. The Nikon Eclipse E 200 trinocular microscope and a digital camera were used to capture the bright field images [15].

Powder microscopy of the Smilax china

A small amount of powder was mounted and colored with glycerine to analyze the characteristics of the powder form of the crude herbal drug [16].

Physical–chemical analysis to determine variables

The factors identified were responsible for the Loss on Drying (LOD), total ash value, acid insoluble ash, water-soluble extractive value, and saponin value [17].

Loss on drying (LOD)

Based on the test of loss on drying, the water content of the ethanolic extract of Smilax china was calculated. In a petri dish that had already been dried and tarred, around 2 g of the extract was weighed. The sample has been dried to a consistent weight in an oven at 105 °C, cooled down, and weighed. Three times the experiment was carried out, and the outcome was obtained as a percent reduction of weight and calculated percentage of loss on drying by using the following formula [18].

$$\%\ \text{Loss on drying} = \frac{\text{Initial weight of sample} - \text{weight of sample after drying}}{\text{Initial weight of sample}} \times 100 \quad (2)$$

Total ash value

Add precisely 2 g of dried Smilax china bark powder to a crucible that was previously heated and tested. Until the material ignited and became white, the temperature was gradually increasing to 600 °C. After cooling in a desiccator and being weighed, the crucible was heated once again to retain weight. The proportion of total ash has been calculated as milligrams of ash per gram of air-dried sample. The experiment was carried out three times, and the following formula was used to compute the percentage of the total ash value [19].

$$\%\ \text{Total ash value} = \frac{\text{Weight of the ash}}{\text{Weight of the original sample}} \times 100 \quad (3)$$

Acid insoluble ash

The crucible containing all the ash was then filled with 25 ml of hydrochloric acid (HCl), closed with a watch glass, and warmed for 5 min. Using an ashless filter paper, the insoluble material was collected, and the filtrate was heated until it became neutral. After that, the filter was added back to the original crucible and burned to maintain its weight. The crucible was weighed after cooling in a desiccator. The total amount of acid-insoluble ash was measured in mg/g of air-dried material and expressed as a percentage. The test was performed thrice, and the percentage of acid-insoluble ash value was calculated using the following formula [20].

$$\%\ \text{Acid insoluble ash} = \frac{\text{Acid insoluble ash weight}}{\text{weight of sample}} \times 100 \quad (4)$$

Water-soluble extractive value

The crucible holding whole ash was filled with twenty-five milliliters of water, and it was boiled for five minutes. An ashless filter paper was used for filtering the insoluble material. Hot water was used to wash the filter paper before it was burned in a crucible for 15 min at a temperature of no more than 450 °C. After that, the crucible was weighed to a set weight. By calculating the remaining weight in mg from the weight of all ash and quantifying the result in %, water-soluble ash was computed. The test was performed twice and calculated the percentage of water-soluble extractive value by using the following formula [21].

$$\%\ \text{Water soluble extractive value} = \frac{\text{Initial mass} - \text{mass of water extractive residue}}{\text{Initial mass}} \times 100 \quad (5)$$

Saponin percentage

0.30 gm of extract was mixed with 30 cm^3 distilled water and then boiled for 10 min in the water bath and used Whatman filter paper number 42 (125 mm) for filtration. Filtrate 10 cm^3 and distilled water 5 cm^3 mixture was heated strongly for the stable persistence of froth. Calculate the percentage of saponin by using the following formula [22].

$$\%\ \text{Saponin} = \frac{\text{Weight of Saponin}}{\text{Weight of Sample}} \times 100 \quad (6)$$

Phytochemical screening of ethanolic extract of *Smilax china*

The presence of phytochemicals in an ethanolic extract of *Smilax china* was analyzed as part of a phytochemical screening. All the procedures were carried out in proper accordance with the standard procedures [23].

Anti-oxidant activity

2,2-Diphenyl-1-picrylhydrazyl (DPPH) radical scavenging activity of the Smilax china

The DPPH radical scavenging activity of Smilax china was determined using the technique invented by You et al. [24]. After diluting the *Smilax china* with DMSO, 0.1 mL of each Smilax china was mixed with 0.9 mL of 0.041 mM DPPH in ethanol for 30 min. The absorbance of the sample was then measured at 517 nm using a spectrophotometer (UV-1601; Shimadzu, Kyoto, Japan). The percentage of radical scavenging activity was calculated as follows [25]:

$$\%\ \text{DPPH radical scavenging activity} = 1 - \frac{\text{A sample}}{\text{A control}} \times 100 \quad (7)$$

ABTS radical scavenging activity

The radical scavenging activity of ABTS was determined using Muller's technique [26]. Each diluted Smilax china (0.1 mL), potassium phosphate buffer (0.1 mL, 0.1 M, pH 5.0), and hydrogen peroxide (20 L, 10 mM) were mixed and incubated at 37oC for 5 min. After preincubation, ABTS (30 L, 1.25 mM, in 0.05 M phosphate-citrate buffer, pH 5.0) and peroxidase (30 L,

1 unit/mL) were added to the mixture, which was then incubated at 37°C for 10 min. At 405 nm, the absorbance level was measured using a multiple reader (Sunrise; Tecan, Manne Dorf, Switzerland) [27].

Spectroscopy and chromatographic analysis of an ethanolic herbal extract of Smilax china

Spectroscopic analysis of an ethanolic herbal extract of *Smilax china* was performed on the solid residue that remained after extraction and evaporation. Thin-layer chromatography (TLC), ultraviolet-fluorescence analysis, Fourier transform infrared (FTIR) spectroscopy, and ultraviolet (UV) radiation [28] are used.

Ultraviolet–visible spectrum analysis of ethanolic extract of Smilax china

UV 1900 Series Spectrophotometer from SHIMADZU was used from DYPCOP, Akurdi laboratory facility for performing UV spectroscopy. The solid residue of ethanolic bark extract of *Smilax china* was collected after isolation and evaporation of extract was used for UV–visible spectrum analysis. The solid residue was spun at 3000 revolutions per minute for 10 min, this is an ethanolic extract of *Smilax china* bark. Whatman (No. 1) filter paper was used to purify the water. Next, ethanol (1:10) was added to the sample, diluting it to the desired concentration, and scanning was performed at 200-400 nm [29].

Fourier transform infrared (FTIR) analysis of ethanolic extract of Smilax china

FTIR-Spectrum from Bruker (USA) was used from DYPCOP, Akurdi laboratory facility for performing FTIR spectroscopy, while dried powder of ethanolic bark extract of *Smilax china* was used for FTIR analysis. One milligram of the dried ethanolic powder extract was encapsulated in a 10 mg potassium bromide pellet (10 mg) to make sample discs, using ethanol as a solvent, the sample was introduced into the pellet of an FTIR spectroscope. Scanning was performed at a resolution of 4 cm^{-1} over a range of 400–4000 cm^{-1} [30].

High-performance liquid chromatography (HPLC)

Using HPLC equipment, a quantitative evaluation of the flavonoid content of the sample was performed. On a computer-controlled high-pressure gradient La Chrom-HPLC system (Merck-Hitachi), analytical HPLC was performed. This system involved an Interface L-7000, a diode array detector L-7450, two pumps of L-7100 (one required for each eluent), a high-pressure gradient mixer, an autosampler with 100 µL sample loop, Rheodyne injection valve 7725i, 20 µL sample loop, and solvent degasser L-7612; administration device, data recording and data recording analysis were performed by using La Chrom Software version 4.0. The column was an isocratic mode Thermo ODS Hypersil C18 (250 mm × 4.6 mm, 5 µm). Glacial acetic acid (15: 85: 1, v/v/v) was used as the mobile phase, phosphoric acid was used to adjust the pH to 4.0 at a flow rate of 1.0 mL/min, and acetonitrile, 0.1 M phosphate buffer. Utilizing UV detection with a wavelength of 300 nm, the effluent was seen before use, the mobile phase was filtered with a 0.45 µm nylon filter [31]. Percentage content of flavonoids in the bark of *Smilax china* was calculated.

Thin-layer chromatographic (TLC) evaluation of ethanolic herbal extract

This thin-layer chromatography (TLC) used silica gel-G as the stationary phase and a toluene/ethyl acetate/acetic acid (5:4:1) as the mobile phase. This was run on a TLC plate. Run on TLC where ethanol was used as a developing phase and silica gel-G as a stationary phase. The spraying reagent used iodine vapors. These solvents were selected to identify the presence of flavonoids that have potential antioxidant action in the treatment of Parkinson's disease. After that, the retention factor (R_f) is calculated, established, and tallied. To ascertain how effectively a molecule is preserved, the retention factor (R_f) was calculated by using the below formula [32, 33].

$$\text{Retention factor} = \frac{\text{Distance traveled by compound}}{\text{Distance traveled by solvent}} \quad (8)$$

Fluorescence analysis for identifying the drug when it is in powder form

Fluorescence analysis was used from DYPCOP, Akurdi laboratory facility for performing fluorescence analysis. Take about 0.5 gm of plant powder into clean and dried test tubes. To each tube, 5 ml of different organic solvents like distilled water, acetone, ethanol, benzene, chloroform, diethyl ether, methanol, glacial acetic acid, sulfuric acid, nitric acid, hydrochloric acid, 5% FeCl3, 5% I2, picric acid, 1N NaOH and 1N NaOH + methanol was added separately. Then, all the tubes were shaken and they were allowed to stand for about 20–25 min. When exposed to UV light, 254 nm, and 365 nm while being treated with different substances, a powdered pharmaceutical emits a spectrum of colored radiations that can be used to identify the drug (Table 7) [34].

Pharmacological study

Animal

This research study utilized 8–10-week-old Wistar male rats weighing 150–170 g. A 25.2 °C environment with a 12-h dark/12-h light photoperiod was maintained in the animal housing. The animals housed in plastic cages were separated into different sections of the animal house. Regular pellets and plenty of water were provided. In compliance with the Committee for Control and Supervision of Experiments on Animals (CPCSEA) regulations (DYPCOP/IAEC/2022/12/09), the Institutional Animal Ethical Committee (IAEC) approved the project for research and education purposes. Institutional Animal Ethics Committee on Animal Research guidelines were followed in designing the research.

Acute oral toxicity studies conducted on male Wistar rats for estimation of toxicity of ethanolic extract of Smilax china as per Organization for Economic Corporation and Development guidelines 423 (OECD 423)

The acute oral toxicity research followed the protocols laid out in Organization for Economic Corporation and Development 423 (OECD guideline 423). Three male Wistar rats (weighing between 150 and 170 g) were used for each phase of testing. Normal saline was administered orally at 1000 and 2000 mg per kilogram of body weight (Table 2). Oral administration of the extract to fasting rats, who had access to water before dosing, yielded a lethal dose (LD)-50 value. Before and after surgery, the rat's weight was recorded. Behavior, mobility, mortality, and convulsions were tracked in male Wistar rats for 14 days to look for symptoms of toxicity and danger [35] (Fig. 5).

There were nine rats in total, so randomly divided them into three groups of three. The toxic effects, including death, restlessness, skin color, clutching, writhing, water intake, and food intake, were monitored continuously for 14 days. Animals were sacrificed with cervical dislocation process after the trial was over and no anesthesia was used as per the institutional guidelines.

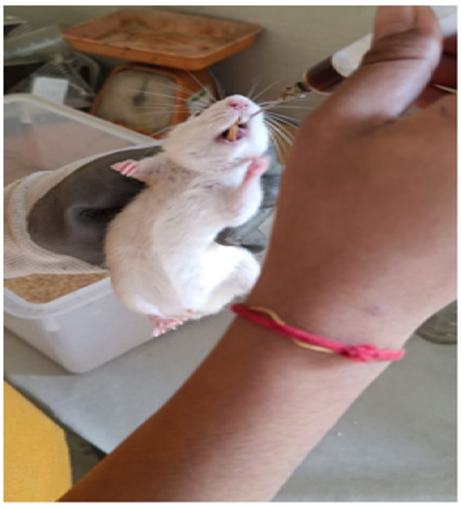


Fig. 5 Acute oral toxicity studies conducted on male Wistar rats for the evaluation of toxicity of ethanolic extract of Smilax china as per Organization for Economic Corporation and Development guidelines 423 (OECD 423)

Anti-Parkinson's action of ethanolic extract of Smilax china bark against the rotenone-induced model of Parkinson's disease

Thirty rodents were evenly dispersed among five groups of six. Group-1 (Control) was treated with 1% dimethyl sulfoxide (DMSO); 0.1 mL/100 g; S.C. every other day, and Tween 80 (10% v/v) daily, for a total of 21 days. In Group-2 (Induction), 1.5 mg/kg of rotenone dissolved in 1% dimethyl sulfoxide (DMSO) was injected subcutaneously every other day for 21 days. DMSO offers unique polar, aprotic, and amphiphilic features, which makes it an ideal solvent for a wide variety of both polar and nonpolar molecules. Furthermore, DMSO in combination with Tween 80 is often used as a cryoprotectant for cell-based research and is mostly used for inducing Parkinson's disease. Here the disease is successfully induced when rotenone is administered with these solvents and

Table 2 Dosing of acute oral toxicity of ethanolic extract of *Smilax china* (as per OECD guidelines 423)

S. No	Group	Dosing	Observation period	No. of Animals required (*n*)
1	Control	Vehicle	4 h, 72 h, 7 days, 14 days	3
2	Treatment-I	Drug extract 1000 mg/kg	4 h, 72 h, 7 days, 14 days	3
3	Treatment-II	Drug extract 2000 mg/kg	4 h, 72 h, 7 days, 14 days	3
			Total	9

causes neurotoxic effects which is a crucial parameter to induce the disease in animals successfully.

The solvent system selected used for the study was 1% dimethyl sulfoxide (DMSO) and Tween 80 (10% v/v) under established protocols considered as a standard for the study [36].

A combination dose (Levodopa and carbidopa combination) available in the market as a standard treatment for Parkinson's disease was used as the standard group. It included Levodopa and carbidopa in respective concentrations (30/1 mg/kg, I.P. once a day for a week) were administered to Group-3 (Standard). Group-4 (Treatment-I) and Group-5 (Treatment-II) rats were given an ethanolic extract of *Smilax china* suspended in Tween 80 and distilled water (10% v/v) once daily for 21 days. Rotenone was administered on alternate days in the study duration of 21 days for inducing Parkinson's disease. Both 100 and 200 mg per kilogram were administered. Each rat's weight was recorded on days 7th, 15th, and 21st of the study. The animals were put through a series of behavioral tests just before they were killed with the cervical dislocation process after their final rotenone injection. Immediate biochemical analysis was performed on substantia nigra from both cerebral hemispheres after they were removed from the brain, homogenized in ice-cold saline (10% w/v), and frozen. When analyzing the results of a histological examination, these parameters [37] (Fig. 6).

Rotenone-induced neurotoxicity model for Parkinson's disease

Preparation of dose for the experimental work

1% dimethyl sulfoxide (DMSO; 0.1 mL/100 g; S.C.) and Tween 80 were used to make the two dosages of the ethanolic extract of *Smilax china*, which were 100 mg/kg and 200 mg/kg in distilled water (10% v/v). A mixture of Tween 80 (10% v/v) and 1% dimethyl sulfoxide (DMSO; 0.1 mL/100 g; S.C.) every other day. Before administration, every solution was freshly formulated every day [38].

Study design for the rotenone-induced model of Parkinson's disease

Dosing schedule for the administration of ethanolic extract of Smilax china

Rats were given an ethanolic extract of *Smilax china* suspended in distilled water with 10% v/v Tween 80 daily for 21 days (1 h before the day of the treatment's injection of rotenone). Behavioral and biochemical parameters were examined to assess the neurotoxicity on the 1st, 7th, 15th, and 21st days following the administration of a rotenone injection (Fig. 7).

Fig. 6 Anti-Parkinson's experimental activity of ethanolic extract of *Smilax china* bark against the rotenone-induced model of Parkinson's disease

Fig. 7 Evaluation of the behavioral parameters and body weight for Parkinson's disease

Fig. 8 Centrifuge used for the biochemical evaluation of all groups of animals

Evaluation of the behavioral parameters and body weight for Parkinson's disease

Body weight variations during and after treatment

In the estimation of body weight as a crucial parameter for the estimation of Parkinson's disease, animals were divided into five groups as mentioned in experimental design for these six animals in each group.

A digital balance was used for recording the body weight of each animal from all groups on the 7th, 15th, and 21st days of the trial for 21 days.

Each rat's weight was carefully measured following the last doses of rotenone and *Smilax china* on the 7th, 15th, and 21st days of the trial. Weight was also noted one day after the final injection of rotenone [39].

Assessment of locomotor activity by using an actophotometer

Six animals in each groups were divided into five groups as per the experimental design for assessment of the locomotor activity by using an actophotometer (Fig. 8).

On days 7th, 15th, and 21st of the experiment, an actophotometer to constantly monitor each animal's locomotor activity for a total of 21 days. The animals were housed in this actophotometer (30 × 30 × 30 cm) that was closed off and had photocells on the outside wall. The digital counter noted the beam interruptions [40].

Locomotor activity can be described as movement from one location to another. The main feature of Parkinson's disease is a decrease in movement or locomotor activity. An actophotometer was used to assess locomotor activity. An actophotometer, also known as an activity cage, is made up of six photocells and a digital counter. A light beam misses a photocell as an animal moves, which causes the count to be digitally recorded and displayed. The overall amount of locomotor activity of an animal is determined using this method. Each animal was individually placed on the actophotometer five minutes before the cognitive test, and its overall activity

was recorded by counting the number of times the photo beam was interrupted. Here, the results of an experimental investigation are reported in which locomotor activity was measured on days 7th, 15th, and 21st [41].

Measurement of catalepsy for evaluation of the limb's stiffness

Six animals in each group were divided into five groups as per the experimental design for the measurement of catalepsy for the evaluation of limb stiffness.

Rotenone-induced catalepsy has been generated and measured on a common bar test every 30 min to 180 min. Animals were placed such that their forelimbs rest on a horizontal bar with a 1 cm diameter that was 6–9 cm above the bench and their hindquarters were on the bench to test for catalepsy. Animals were timed using a stopwatch (mean of three consecutive trials; interval: 1 min) to determine how long they remained in this position. If they remained in this position for 30 min or longer, animals would consider the judge to be cataleptic [42].

The limbs indicate the most severe muscular rigidity in catalepsy. Animals with stiffness are unable to move or maintain their posture, regardless of where it is. A cataleptic's conduct was evaluated using a bar test and a blocking technique. These tests took place on days 7th, 15th, and 21st [43].

Bar test for the evaluation of cataleptic score

Six animals in each group were divided into five groups as per the experimental design for the evaluation of the cataleptic score by using a bar test.

Used bar test apparatus for recording the cataleptic score of each animal from all groups on the 7th, 15th, and 21st days of the trial for 21 days continuously. The Wistar rat's front right and left paws were placed throughout a horizontal, 1 cm-diameter metal bar that was raised 9 cm above the table top for this test. The Wistar rat's forepaws were brought to the table surface at the time of the cut-off period for catalepsy which is 180 s [44].

Block method for evaluation of cataleptic score

Six animals in each group were divided into five groups as per the experimental design for the evaluation of the cataleptic score by using the block method.

Used block method for the evaluation of the cataleptic score of each animal from all groups on the 7th, 15th, and 21st days of the trial for 21 days continuously. The process of scoring is split down into three phases (Table 3).

Step 1: First, the Wistar rat was taken out of its cage and placed on a table. If a Wistar rat did not respond to soft probing or stroking, it received a score of 0.5.

Step 2: Rats had their front paws placed randomly on a 3-cm-high block. If a Wistar rat remained in the same position for 15 s, the first step score was decreased by 0.5.

Step 3: The scores from stages I and II have been raised by one point for each paw if the Wistar rat was not changing its posture after 15 s. The Wistar rat's front paws are alternately placed on a 9 cm high block. Thus, 3.5 (cut-off score), indicating total cataplexy, was the highest possible result for any animal [45].

Test of motor coordination with a rotarod to measure grip strength

Six animals in each group were divided into five groups as per the experimental design for the evaluation grip strength of animals by the motor coordination test.

Rotarod was used to measure the grip strength of each animal from all groups on the 7th, 15th, and 21st days of the trial for 21 days continuously. Animals were observed for coordinated motor balance using a rotarod apparatus (90 cm height, 3 cm diameter, and 25 rpm). Before the investigation, the rats were acclimatized on the rotarod via 3 training sessions of 5 min

Table 3 Experimental design of rotenone-induced neurotoxicity model

Sr. no	Group	Treatment	Drug administered	Concentration	No. of animals required (*n*)
1	Group-1	Control	Tween 80 and 1% dimethyl sulfoxide (DMSO)	1 ml/kg; S.C	6
2	Group-2	Induction	Rotenone	1.5 mg/kg; S.C	6
3	Group-3	Standard	Levodopa + carbidopa	100 + 25 mg/kg; I.P	6
4	Group-4	Treatment-I	Rotenone + *Smilax china* extract	1.5 mg/kg; S.C. + 100 mg/kg; P.O	6
5	Group-5	Treatment-II	Rotenone + *Smilax china* extract	1.5 mg/kg; S.C. + 200 mg/kg; P.O	6
					Total: 30

The male Wistar rats utilized in this research were separated into 5 groups, each including 6 animals (Table 3)

each for 3 days. After finishing the open field test, the rats were assessed for a period of 5 min and the latency to fall off was documented [46].

On days 7th, 15th, and 21st, the male Wistar rats in all groups had their grip strength measured using the rotarod to track their neuromuscular coordination. The rotarod, at a speed of 25 revolutions per minute, was used to house the rats. In the first five minutes of the race, each group's rate of decline was recorded [47].

Biochemical evaluation of all groups of animals

Six animals in each group were divided into five groups as per the experimental design for evaluation the evaluation of biochemical parameters.

A biochemical evaluation of each animal of all groups was performed on the 21st day, the very last day of the experiment, a biochemical analysis was performed on every group of animals. For the evaluation of the level of oxidative parameters in brain tissue homogenate of malondialdehyde (MDA), reduced glutathione (GSH), superoxide dismutase (SOD), and catalase (CAT) activity of enzymes was performed using the described method on brain tissue homogenate [48].

Malondialdehyde (MDA) level assessment

The 6 animals from each group were taken to evaluate the level of malondialdehyde content which was calculated by exploiting the reaction between thiobarbituric acid (TBA), a marker of lipid peroxidation, and malondialdehyde. To prepare 3 mL of the TBA reagent, 1 mL of supernatant aliquots was mixed with 0.25 M hydrochloric acid (HCl) and 15% trichloroacetic acid. The cocktail was chilled in an ice bath after being shaken for 15 min. After the solution had cooled, it was spun in a centrifuge for 10 min at 3500 g. The top layer was sampled, and its spectral characteristics were examined at 532 nm. Each result was evaluated three times. The outcomes were published [49] in units of nanomoles per milligram of protein. Malondialdehyde (MDA) concentration was determined by the following formula.

$$\text{Conc.of MDA} = \frac{\text{Abs}_{523} \times 100 \times V_{\text{T}}}{(1.56 \times 105) \times W_{\text{T}} \times V_{\text{U}}} \tag{9}$$

In this equation, absorbance (Abs_{532}), total volume (V_{T}) (4 mL), molar extinction coefficient (1.66) (105), dissected brain weight (W_{T}) (1 g), and aliquot volume (V_{U}) (1 mL) all play important roles [50].

Level of superoxide dismutase (SOD)

Six animals from each group were taken to evaluate the superoxide dismutase (SOD) level to collect the serum and centrifuge to evaluate the level of superoxide dismutase (SOD). Following the procedure specified by the study's authors, superoxide dismutase (SOD) activity was determined. One milliliter of epinephrine was mixed with 0.5 ml of carbonate buffer (pH 9.7), 0.1 ml of EDTA (1×10^{-4} M) mixed with 0.1 mL of supernatant to achieve a 1 mM concentration. After letting the adrenochrome sit for three minutes at 480 nm, the spectrophotometer read its optical density. The enzyme's activity was measured in units per minute per milligram. The concentration at which chromogen production falls by half in one minute under typical conditions is equal to one unit of enzyme activity [51].

Level of catalase (CAT) enzyme activity

The 6 animals from each group were taken and evaluated for the level of catalase (CAT). Collect the serum and centrifuge for the evaluation. The supernatant from 10% tissue homogenate should be combined with 1.95 mL of 50 mM phosphate buffer (pH 7.0) in a 3 mL cuvette. Injecting 1 mL of 30 mM hydrogen peroxide (H_2O_2) led to 15-s measurements of absorbance at 240 nm over 30 s. Catalase activity was expressed as the amount of hydrogen peroxide oxidized in micromoles per minute per milligram of protein, which was determined using the millimolar extinction coefficient of hydrogen peroxide (0.071 mmol cm^{-1}) [52].

$$\text{CAT activity} = \frac{\delta \text{ O.D.}}{E \times \text{Vol. of Sample (mL)} \times \text{mg of protein}} \tag{10}$$

where E represents hydrogen peroxide extinction coefficient (0.071 mmol cm1) and δ O.D. denotes the variance in absorbance per minute [53].

Reduced glutathione (GSH) level

The reduced glutathione (GSH) level was evaluated by the selection of 6 animals from each group collecting serum and centrifuging them and following procedure steps to evaluate the level. There are decreased levels of the antioxidant glutathione (GSH). One milliliter of the supernatants was analyzed for reduced glutathione levels using 10% tris (hydroxymethyl) amino acid. After combining a supernatant sample with 4 mL of phosphate solution and 0.5 mL of 5,5-dithiobis-(2-nitrobenzoic acid) (DTNB) reagent, the absorbance at 412 nm was measured. Reduced glutathione levels were reported in units of nM per mg of protein [54].

$$\text{GSH level} = \frac{Y - 0.00314}{0.0314} \times \frac{D_{\text{F}}}{B_{\text{T}} \times V_{\text{U}}} \tag{11}$$

where B_T: brain tissue (1 mL) homogenate, D_F: dilution factor (1), and V_U is aliquot volume, Y: Abs_{412} of (1 mL) brain tissue homogenate [55].

Isolation of vagus nerve from the male Wistar rats after treatment with ethanolic extract of *Smilax china*

Vagus nerve stimulation improves learning and neuronal dopamine release, according to recent studies in rats [56]. All the male Wistar rats had their vagus nerves severed on the final day of the experiment. On the last day, the Wistar rat was killed by severing the nerve from the spinal cord by tugging on the neck with the left hand and the base of the tail with the right hand. Surgical silk thread was used to restrict the bilateral carotid arteries after they were cut at the vagus nerve. After making an incision in the neck, the vagus nerve was cut and then severed. A 6.9 pH buffered; 4% formaldehyde solution was used to treat the vagus nerve. Frozen specimens were analyzed by histopathologists after a 24-h chilling period. Once the parts were set in resin, a very sharp blade was used to cut them apart. Eosin solution was used to stain the specimens. The 22nd day ended with the killing of the animals [57].

Table 4 Organoleptic features of the bark *Smilax china*

S. No	Features	Results of *smilax china*
1	Length	5–7 cm
2	Thickness	5 cm
3	Color	Brownish
4	Odor	Characteristic
5	Taste	Slightly bitter

Histopathological analysis of the Wistar rat

Dissection of animals from each group isolation of the brain and paraffin wax was used to preserve the brains of both the control and trial groups before they were fixed in 10% formalin and sliced longitudinally at 5 mm thickness. On day 22 of the experiment, brain tissue from all groups was extracted, including the vagus nerve, substantia nigra, and basal ganglia. Hematoxylin and eosin dyes were used to stain the sections before histological investigation [58, 59].

Statistical analysis

A total of nine animals were used ($n=3$ in each group) for the experimental design of the Acute toxicity study (as per OECD guideline 423), and a total of thirty animals were used ($n=6$ in each group) for the experimental design of anti-Parkinson's study. The quantitative data were analyzed using a one-way ANOVA by SPSS (29), and then, the Tukey–Kramer test was used for post hoc comparisons. The results were considered statistically significant when $p<0.05$.

Results

A summary of the spectroscopic, phytochemical, and pharmacological findings is provided below for your convenience.

Macroscopic evaluation of the ethanolic extract of *Smilax china*

The macroscopic data show that the bark is 5–7 cm in length and 5 cm in thickness; it is also woody; it is brownish to blackish on the outside and yellowish on the inside; it is rough; it gives rise to several roots at various points; it fractures fibrously; it has a distinctive odor and a slightly bitter taste; and it has all these characteristics (Table 4).

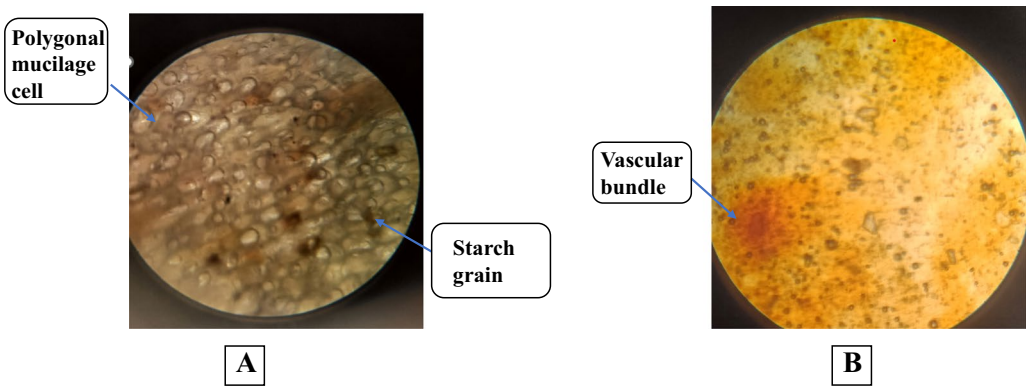


Fig. 9 Microscopic cross sections of *Smilax china* bark shown in T.S. **A** Starch grains and polygonal mucilage cells and **B** vascular bundles were visible

Microscopic examination of the bark of *Smilax china*

Cortex can be found in the bark's undulating transverse part. Cortex cells are mucilaginous parenchyma that are polygonal in shape and extended in length. There are an outside layer, a middle layer, and an inner layer that make up the cortex. The inner cortex is composed of polygonal cells without mucilage, while the outer cortex is composed of elongated parenchyma cells rich in mucilage. There may be both simple and complicated starch grains of varied sizes in the inner cortical cells, which have a central hilum. There is healthy tissue and a network of blood arteries in the inner cortical region. Long and aseptate describe the fibers. Some cells have calcium oxalate crystals that look like raphides. T.S. microscopic bark slices are included in Fig. 9.

Powder microscopy of *Smilax china*

Images acquired by microscopy with *Smilax china* powder were analyzed using a compound microscope. Under a compound microscope, both the fibers and the tannins could be seen. Figure 10 depicts their campus's architectural layout. In the quest for *Smilax china* fruit standardization, this is of great assistance.

Physicochemical and phytochemical analysis of herbal drug extract

The ash value, extractive value, loss on drying (LOD), and foaming index were measured as part of the physicochemical analysis of *Smilax china* bark powder and are presented in Table 5; the results of the phytochemical analysis are presented in Table 6 and Fig. 11, which show the presence of flavonoid, tannin, alkaloids, etc. The powder has a wide spectrum of colors, from brown to red to green.

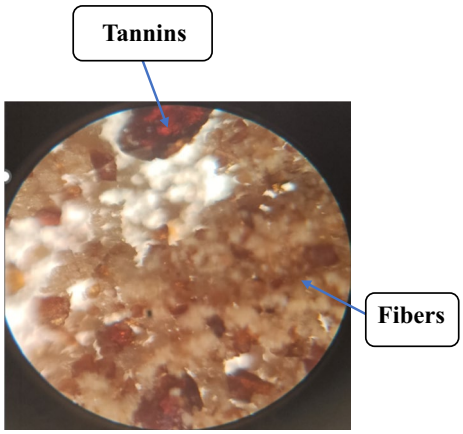


Fig. 10 Powder microscopy of *Smilax china* bark shows the presence of tannins and fibers

Table 5 Physicochemical analysis of ethanolic extract of *Smilax china* bark

S. no	Parameters	Result
1	% Yield of ethanolic extract of *Smilax china*	10%
2	Loss on drying (LOD)	6.90 (% w/w)
3	Total Ash Value	3.24 (% w/w)
4	Acid Insoluble Ash	0.17 (% w/w)
5	Water-soluble extractive value	34.20 (% w/w)
6	Saponin value (Weight of saponin = 0.0236 and weight of sample = 0.3)	7.88 (%w/w)

Table 6 The results for the phytochemical analysis of ethanolic and aqueous extract of *Smilax china* (where + present and − absent)

S. No	Phytoconstituents	Aqueous extract	Ethanol extract
1	**Test for Alkaloids**		
	a. Dragendorff's test	+	+
	b. Hager's test	+	+
	c. Wagner's test	−	+
	d. Mayer's test	+	+
2	**Test for Carbohydrates**		
	a. Benedict's test	+	+
	b. Fehling's test (free reducing sugars)	+	+
	c. Barfoed test (monosaccharides)	+	+
3	**Test for Flavonoids**		
	a. Ferric chloride test	+	+
	b. Lead ethanoate test	+	−
	c. Alkaline reagent test	+	+
4	**Test for Tannins**		
	a. Lead acetate test	+	+
	b. Ferric chloride test	+	+
5	**Test for Phenolic compounds**		
	a. Lead acetate test	+	+
	b. Ferric chloride test	+	+
6	**Test for Coumarins**	+	+

Anti-oxidant activity

DPPH radical scavenging activity of the Smilax china

The DPPH radical scavenging activity of the *Smilax china* extract compared with L-ascorbic acid (a positive control) is expressed in Table 7. The *Smilax china* extract potential to scavenge DPPH radicals significantly improved when extraction concentration was increased. Ethanol, acetone, and methanol extracts for *Smilax china* extract had relatively higher DPPH radical scavenging activity (89.40, 94.76, and 90.86, respectively) at 1,000 g/mL concentration, whereas water extracts did not exhibit

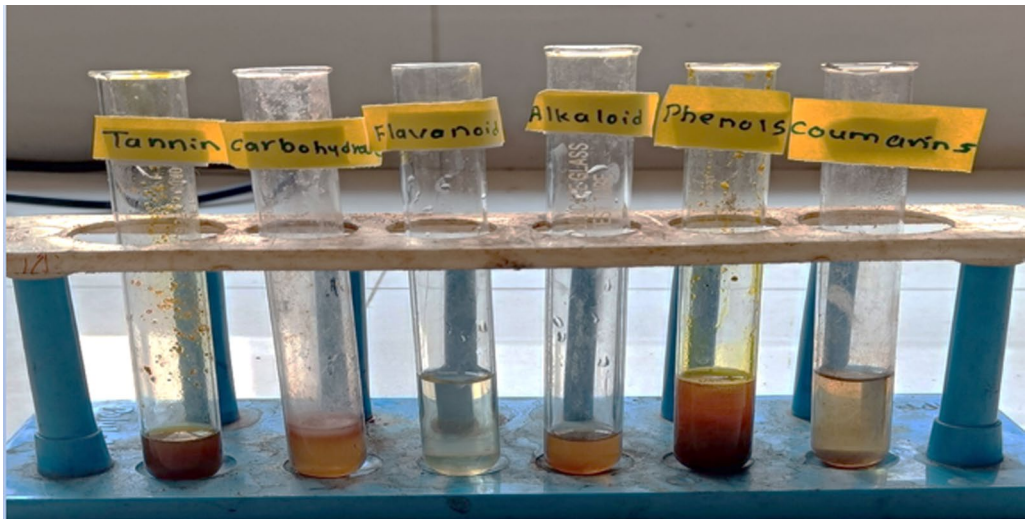


Fig. 11 Phytochemical analysis of an ethanolic extract of *Smilax china* shows the presence of tannins, carbohydrates, flavonoids, phenols, and coumarins

significant DPPH radical scavenging activity (47.24%) at the same concentration. L-ascorbic acid (IC_{50} = 29.56 g/mL), which was used as the positive control in this investigation, was compared to the IC_{50} value of each extract. The *Smilax china* ethanolic extract had the highest DPPH radical scavenging activity (IC_{50} = 48.93 g/mL), whereas the water extract had the lowest DPPH radical scavenging activity (IC_{50} = 1,007.62 g/mL). These results indicated that most *Smilax china* solvents have significant effects in scavenging free radicals.

ABTS radical scavenging activity

Antioxidants depend on the electron-/hydrogen-donation process to scavenge ABTS radicals, and the efficacy of this process is measured through observation of the decline in 405 nm absorption. The ABTS radical

Table 7 DPPH radical scavenging activity of *Smilax china* bark extract from 4 solvents (Unit: %)

µg/mL	Water	Acetone	Ethanol	Methanol	L-Ascorbic acid
50	1.80 ± 0.10^{dw}	33.38 ± 0.60^{cw}	54.02 ± 0.84^{aw}	40.50 ± 0.77^{bw}	–
100	6.08 ± 0.15^{dx}	50.80 ± 0.30^{bx}	72.73 ± 0.02^{ax}	52.52 ± 1.78^{cx}	–
500	29.40 ± 0.20^{cy}	83.84 ± 0.45^{by}	86.73 ± 0.79^{ay}	77.04 ± 0.18^{by}	–
1000	47.24 ± 0.27^{dz}	94.76 ± 0.14^{cz}	89.40 ± 0.11^{az}	90.86 ± 0.50^{az}	–
IC_{50}	$1{,}007.62 \pm 5.29^{d}$	82.76 ± 0.38^{b}	48.93 ± 1.89^{a}	98.40 ± 8.10^{c}	29.56 ± 0.22

1. All measurements were taken in triplicate, and the average of three replications was used to calculate the values. Different values within a different row (a–d) and a column (w–z) are significantly different ($p < 0.05$), $n = 3$

2. IC_{50} (mg/mL) is the concentration for scavenging 50% of DPPH radicals

Table 8 ABTS radical scavenging activity of *Smilax china* bark extract from 4 solvents (Unit: %)

µg/mL	Water	Acetone	Ethanol	Methanol	L-Ascorbic acid
50	8.50 ± 1.80^{cw}	60.30 ± 1.41^{bw}	65.27 ± 3.57^{abx}	64.45 ± 1.50^{aw}	–
100	20.74 ± 7.90^{cx}	76.36 ± 4.50^{bx}	72.30 ± 5.16^{by}	92.52 ± 0.47^{ax}	–
500	57.91 ± 2.30^{cy}	90.28 ± 0.60^{bz}	92.23 ± 0.29^{bz}	94.97 ± 0.02^{ay}	–
1000	67.14 ± 0.80^{dz}	87.65 ± 1.30^{bz}	86.68 ± 0.75^{bz}	92.15 ± 0.30^{az}	–
IC_{50}	408.6 ± 20.39^{b}	26.87 ± 2.66^{a}	34.89 ± 2.78^{a}	35.32 ± 1.65^{a}	9.64 ± 0.72

1) All measurements were taken in triplicate, and the average of three replications was used to calculate the values. Different values within a different row (a–d) and a column (w–z) are significantly different ($n = 3$), $^{*}p < 0.05$ in comparison with the control

2) IC_{50} (mg/mL) is the concentration for scavenging 50% of DPPH radicals

scavenging activity of L-ascorbic acid and four *Smilax china* extracts (Table 8). The ethanol extract (86.68%) had the greatest ABTS radical scavenging activity, followed by the methanol extract of *Smilax china* (92.15%), water extract (67.14%), and acetone extract (87.65%) at a concentration of 1,000 g/mL. L-ascorbic acid was used as positive control in this investigation, and the IC_{50} value of each extract was compared with that substance. L-ascorbic acid had an ABTS radical scavenging activity of 9.64 g/mL (IC_{50}), which was greater than the greatest value found in the acetone extract of *Smilax china* (IC_{50} = 26.87 g/mL). *Smilax china* contains important antioxidant activity when compared with L-ascorbic acid, which was a pure single antioxidant component, and the extracts, which were made up of many different chemicals.

Spectroscopic and chromatographic studies and fluorescence analysis

Ultra-violet (UV) analysis of the 5 peaks spectrum was recorded as shown in Fig. 12, and Fourier transform infrared (FTIR) analysis of 9 peaks was recorded, shown in Fig. 13. The High-Performance Liquid Chromatographic (HPLC) analysis was performed and were observed five peaks in Fig. 14. The chromatographic analysis of thin-layer chromatography (TLC) was performed for four different extracts. Hence, the presence of multiple phytoconstituents in the ethanolic extract of *Smilax china* at 254 nm, 365 nm, and visible daylight is shown in Fig. 15. Ultra-violet (UV)–Fluorescence analysis shows the brown and greenish-brown color of powder shown in Table 9.

Acute oral toxicity studies conducted on male Wistar rats for estimation of toxicity of ethanolic extract of *Smilax china* (as per OECD guidelines 423)

There were no fatalities or cases of toxicity from chronic or acute exposure, hence it was determined that *Smilax china* extract is safe for herbal use. Table 10 displays the behavioral and physiological reactions. Table 11 displays the impact of *Smilax china* ethanolic extract at normal control, 1000 mg/kg, and 2000 mg/kg on food and water intake over 14 days. Macroscopic changes were checked in isolated vital organs in case of acute poisoning, as shown in Fig. 16.

Anti-Parkinson's activity against the rotenone-induced model of Parkinson's disease

Smilax china ethanolic extract, when administered in doses of 100 and 200 mg/kg to groups of rats in a rotenone-induced scenario, showed a strong anti-Parkinson effect. Furthermore, the extract's efficacy in treating

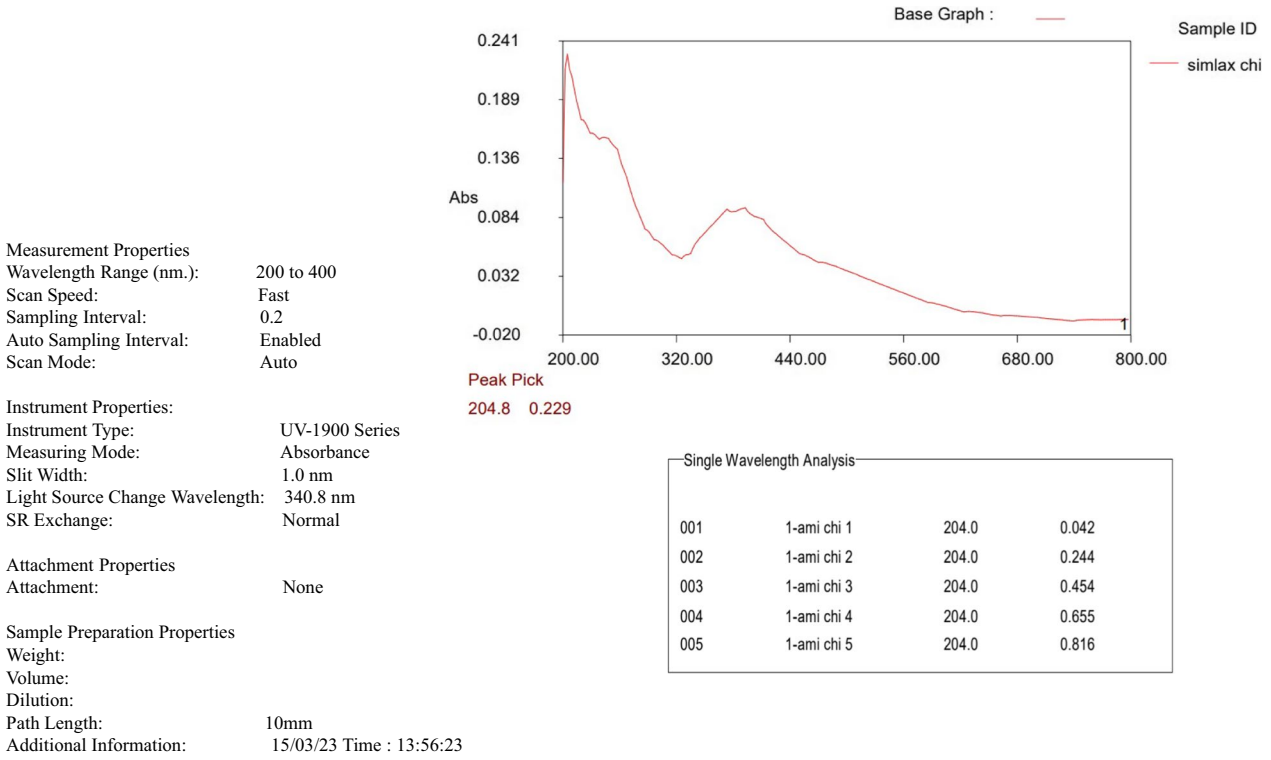


Fig. 12 The above spectra show 5 peaks by Ultraviolet (UV)–spectroscopy for ethanolic extract of *Smilax china* bark

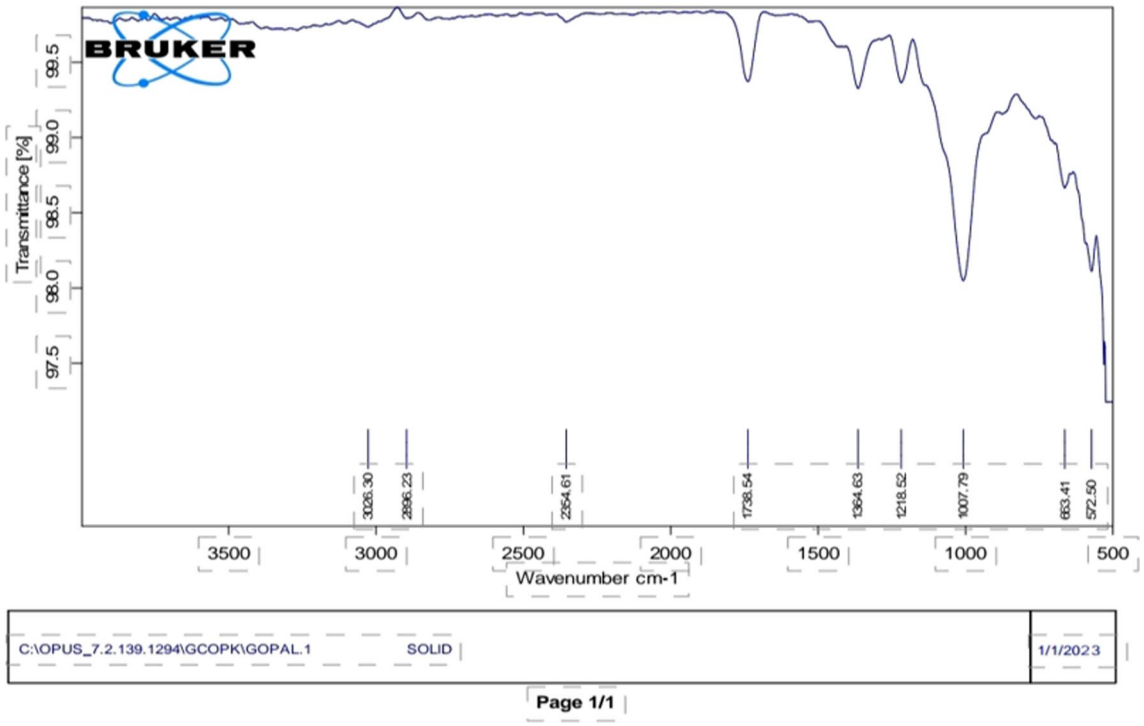


Fig. 13 The above Fourier transform infrared (FTIR) analysis spectra show 9 peaks by Fourier transform infrared (FTIR) analysis spectroscopy for ethanolic extract of bark of *Smilax china*

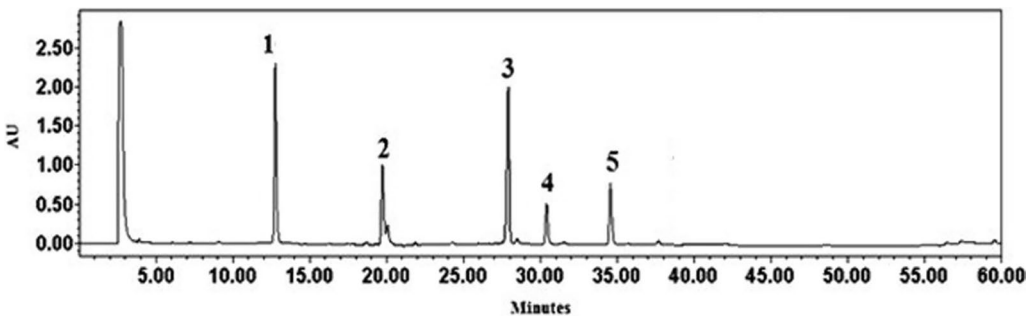


Fig. 14 HPLC chromatogram of ethanolic extract of *Smilax china* was observed 5 peaks. (1) 2.3 AU, (2) 1.1 AU, (3) 2.0 AU, (4) 0.4 AU, (5) 0.8 AU

neurotoxicity was further demonstrated by the fact that rats given 200 mg/kg benefited more than those given 100 mg/kg.

Evaluation of the physical parameters for Parkinson's disease: body weight variations during and after treatment

They gained body weight compared from day 7th to 21st of treatment group I and II male Wistar rats. Weight loss is a symptom of Parkinson's disease, and the treatment group shows the gain of body weight in male Wistar rats. Hence, improvement in disease within the treatment groups where the average body weight was recorded as 177.8 ± 3.25and 181.6 ± 2.45 gm of treatment group I and II respectively, while the average body weight of 146.2 ± 1.35 gm of Group-2 (Induction) was recorded. As compared to the results of Group-2 (Induction) from the 21st-day, the results are significant. The results are presented in Table 12.

Assessment of locomotor activity by using an actophotometer

The overall amount of time spent moving around in 5 min was significantly lower in Group-2 (Rotenone) than in

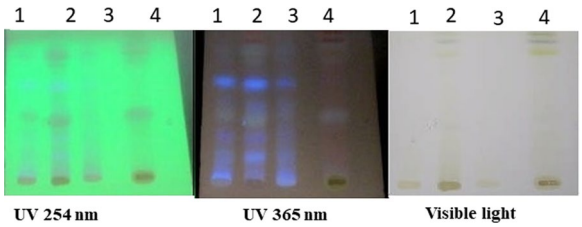


Fig. 15 Thin-layer chromatography (TLC) is used for the chromatographic analysis. To pinpoint the origin of the two discolorations, iodine vapor was sprayed into the area. Scanning at 254 nm, 365 nm, and visible light. Toluene/ethyl acetate/acetic acid (5:4:1) used as the mobile phase. Extracts used during TLC were 1—ethanol extract, 2—water fraction, 3—ethyl acetate fraction, and 4—*N*-hexane fraction which were viewed under Ultraviolet 254 nm, Ultraviolet 365 nm, and visible light. Multiple spots were seen in a thin layer of prepared silica gel-G on a chromatographic plate

Group-1 (Control) throughout all days of testing. Locomotor activity was seen to improve across the board after treatment with levodopa + carbidopa (100 + 25 mg/kg) and *Smilax china* (100, 200 mg/kg). The 21st day saw an increase in locomotor activity (count/5 min) in both treatment groups I and II. On the 21st day, it was recorded at 69.72 ± 3.21 and 74.90 ± 3.03, which are both significantly higher than Group-2's (Induction) 39.75 ± 2.64. You can see the outcomes in Table 13.

Measurement of catalepsy for evaluation of the limb's stiffness

Bar test for the evaluation of cataleptic score

Tables 14, 15, and 16 detail the cataleptic scores on the bar test on days 7th, 15th, and 21st. On every day of observation, catalepsy ratings were significantly higher in Group- 2 (Induction). Group-5 (Treatment-II) and Group-4 (Treatment-I) both demonstrated substantial reductions in catalepsy scores compared to Group-1 (Control) on days 15th and 21st, but not on day 7th. Effects were observed to be dose- and time-dependent in treated groups. Starting on day 7th, Group-5 (Treatment-II) showed effects that were comparable to Group-3 (Standard), and starting on day 15th, a decrease in catalepsy score was observed compared to the catalepsy score of day 7th and day 15th of the same group, which was significantly higher than the control and all treated groups. On day 21st, the catalepsy scores for Groups-4 (Treatment group-I) and 5 (Treatment group-II) were considerably lower than those of Group-2 (Induction), 24.92 ± 2.17 and 17.0 ± 61.74 respectively.

Block method for evaluation of cataleptic score

In Tables 17, 18, and 19, you can see the results of the block method's cataleptic scoring. On every day of observation, the rotenone-treated group had significantly higher catalepsy scores. On all days of evaluation, the catalepsy scores of patients in Groups-3 (Standard) and 5 (Treatment-II) were significantly lower than those in Group-1 (Control). On days 15th and 21st, catalepsy ratings significantly decreased in Group-4 (Treatment-I). The catalepsy score decreased in a dose- and time-dependent manner in the treated groups. Results for Group-5 (Treatment-II) were like those shown in Group-3 (Standard). On day 21st of treatment-I and treatment-II, a substantial decrease in the second time was observed. Compared to Group-2 (Induction), where it was 3.69 ± 0.07 after the 21st day, both groups' averages were 0.80 ± 0.07 (0.47 ± 0.17).

Rotarod test (motor coordination) to evaluate grip strength

On day 21 of the experiment, the Wistar rats in Groups-4 (Treatment-I) and 5 (Treatment-II) showed greater improvements in motor coordination and grip strength than those in Group 2 (Induction), which showed a 10.4 ± 5.62 decrease. One-way ANOVA followed by Tukey–Kramer multiple comparison tests revealed a

Table 9 The ultra-violet (UV)–fluorescence analysis of ethanolic extract of *Smilax china*

S. No	Reagent	Color		
		Daylight	Short wavelength 254 nm	Long wavelength 365 nm
1	Powder	Light brown	Greenish brown	Brown
2	Powder + water	Creamy white	Lime yellow	Pale yellow
2	Powder + Conc. HCL	Pinkish red	Mild yellow fluorescent	Yellowish orange
3	Powder + NAOH	Pale pink	Pale yellow	Crimson red
4	Powder + $FeCl_3$	Greenish brown	Fluorescent green	Orange
7	Powder + Acetic acid	Light brown	Brown	Dark brown
8	Powder + Conc. H_2SO_4	Greenish brown	Greenish brown	Crimson red
9	Powder + Conc. HNO_3	Yellow	Fluorescent yellow	Crimson brown
10	Powder + Glacial acetic acid	Turbid white	Lime yellow	Pale yellow

Table 10 Effect of ethanolic extract of *Smilax china* during acute toxicity study

S. No	Response	Control	Dose per kg/ body weight 2000 mg/kg
1	Restlessness	−Ve	−Ve
2	Alertness	Usual	Usual
3	Righting reflux	Usual	Usual
4	Tremors	−Ve	−Ve
5	Grooming	Usual	Usual
6	Pupil	Usual	Usual
7	Pain response	−Ve	−Ve
8	Water intake	Usual	Usual
9	Food intake	Usual	Usual
10	Salivation	Usual	Usual
11	Writhing	Usual	Usual
12	Urination	Usual	Usual
13	Torch response	−Ve	−Ve
14	Skin color	−Ve	−Ve
15	Gripping	Usual	Usual
16	Fur shedding/density	−Ve	−Ve
17	Convulsion	−Ve	−Ve
18	Mortality	Usual	Usual
19	Touch response	Usual	Usual

Table 11 Effect of ethanolic extract of *Smilax china* normal control, Treatment-I (1000 mg/kg b. wt.) Treatment-II (2000 mg/kg b. wt.) on food and water consumption for 14 days

S. No	Groups	Consumption	
		Water intake (ml/day)	Food intake (gm/day)
1	Control	13.51 ± 2.15	18.10 ± 1.30
2	Treatment-I	14.77 ± 2.88	20.09 ± 0.95
3	Treatment-II	15.68 ± 2.65	21.10 ± 0.84

Data indicates mean ± SEM, where $n = 3$. ($p > 0.05$) no significant differences were observed between the control and treated groups in terms of food and water consumption

statistically significant difference ($^{*}p < 0.001$) between Groups-4 (Treatment-I) and 5 (Treatment-II) when compared to Group-2 (Induction). Therefore, *Smilax china* protects nerve cells (Table 20).

Biochemical evaluation of all groups of animals

Smilax china ethanolic extract's influence on rotenone reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) all show signs of Parkinson's disease after being artificially stimulated. Significant changes in biochemical markers were seen after rotenone administration compared to a control group of male Wistar rats. Higher malondialdehyde (MDA) levels and lower superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) levels in the brain were indicators of oxidative stress in the Group-2 (Induction) male Wistar rats compared to Group-1 (Control). Malondialdehyde (MDA) levels in Group-5 (Treatment-II) male Wistar rats were significantly ($^{*}p < 0.001$) higher than in Group-2 (Induction) male Wistar rats (3,978 ± 0.018). 6.486 ± 0.040. Furthermore, daily treatment with *Smilax china* (200 mg/kg) Group-5 (Treatment-II) increased the level of superoxide dismutase (SOD) 5.44 ± 0.01 and the level of catalase (CAT) 6.23 ± 5.23 compared with Group-II (Induction) for the level of superoxide dismutase (SOD) 2.05 ± 0.104 and level of catalase (CAT) 3.08 ± 0.23. Hence, a decrease in the level of Group-II (Induction) of superoxide dismutase (SOD) and chloramphenicol catalase (CAT) as compared to Group-5 (Treatment-II). Increase in reduced glutathione (GSH) level in Group-5 (Treatment-II) 6.456 ± 0.0381 as compared to Group-II (Induction) 3.886 ± 0.021 in the brain (Table 21).

Isolation of the vagus nerve from near the lower back brain

In, the vagus nerve removed from its usual location in the neck (near the lower back brain) and the bilateral carotid arteries separated by a blunt incision and tightened with surgical silk thread shown in Fig. 17. The results of the microscopic examination of nerve slices that were colored and mounted after being frozen in formalin solution following nerve isolation from each group shown in Fig. 18.

Vagus nerve analysis (histopathology)

Histological sections from male Wistar rats treated with *Smilax china* ethanolic extract for 21 days were analyzed to determine its effects. After 21 days, there are no symptoms of inflammation or degenerative changes in the vagus nerves of those in Group-1 (control). The hallmarks of Parkinson's disease are inflammation, cell death, and reduced intrastromal accumulation of alpha-synuclein. Group-1 (control) showed no signs of inflammation or degeneration, whereas Group-3 (standard) showed minimal inflammation and degenerative alterations (healing effect). Photos of Groups-4 and 5 (treatments I and II) indicate their progress toward recovery. Because of this, there are curative and anti-Parkinson's effects in the ethanolic extract of *Smilax china*. Figure 18 displays the recorded parts.

Histopathological study of substantia nigra and basal ganglia of the Wistar rat

The neuroprotective effect of *Smilax china* is validated by histopathological investigation. Histopathological

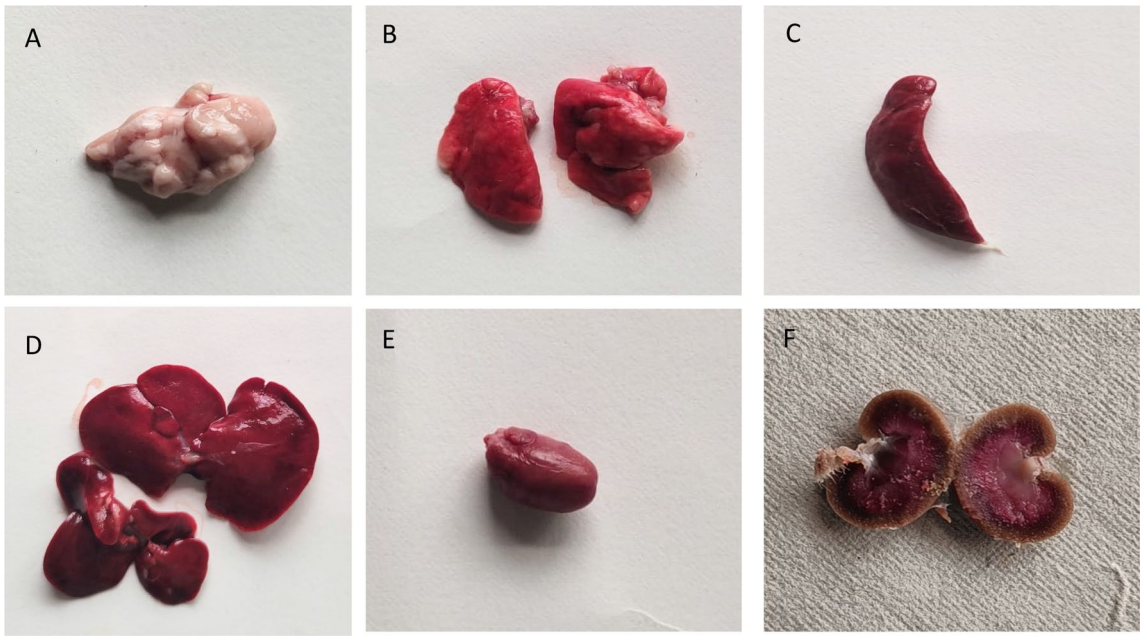


Fig. 16 Vital organs were isolated to check toxicity to the concentration 2000 mg/kg **A**. Brain, **B**. Lungs, **C**. Spleen, **D**. Liver, **E**. Heart, **F**. Kidney

Table 12 The 7th, 15th, and 21st day body weights of male Wistar rats were recorded after neurotoxicity was induced to produce Parkinson's disease

	Body weight (gm)		
Group	**7th day**	**15th day**	**21st day**
Group-1 (Control)	152 ± 2.04	170 ± 2.22	202 ± 2.05
Group-2 (Induction)	161.8 ± 2.39[a]	153.4 ± 1.33[a]	146.2 ± 1.35[a]
Group-3 (Standard)	156.8 ± 1.25*	161.8 ± 0.36*	163.2 ± 1.40*
Group-4 (Treatment-I)	158.6 ± 1.33*	168.2 ± 2.25*	177.8 ± 3.25*
Group-5 (Treatment-II)	165.8 ± 1.34*	168.4 ± 2.15*	181.6 ± 2.45*

Mean standard error of the mean, $n = 6$. The use of one-way ANOVA: Tukey–Kramer test for multiple comparisons: [a]$p < 0.001$ in comparison with normal control; *$p < 0.001$ in comparison with the induction group. Body weight was significantly improved in comparison with the induction group where a significant reduction in average body weight was noticed which is a prominent feature of Parkinson's

Table 13 All groups of male Wistar rats were tested on days 7th, 15th, and 21st using an Actophotometer for their high levels of locomotor activity during the Exploratory Test

	Total locomotor count/5 min		
Group	**7th day**	**15th day**	**21st day**
Group-1 (Control)	81.73 ± 3.15	77.42 ± 3.71	78.05 ± 2.62
Group-2 (Induction)	40.86 ± 3.75[a]	27.02 ± 2.27[a]	39.75 ± 2.64[a]
Group-3 (Standard)	74.65 ± 3.92*	66.77 ± 3.02*	71.26 ± 2.56*
Group-4 (Treatment-I)	62.11 ± 2.45*	64.26 ± 3.08*	69.72 ± 3.21*
Group-5 (Treatment-II)	68.37 ± 2.88*	69.03 ± 2.47*	74.90 ± 3.03*

Average standard error of the mean; sample size = 6. The use of one-way ANOVA: Multiple comparisons using the Tukey–Kramer method: [a]$p < 0.001$ in comparison with normal control; *$p < 0.001$ in comparison with the Induction group. Locomotor activity in the actophotometer was significantly reduced in the induction group which was improved in the treatment groups, hence denoting good locomotor functions in the treatment group and effectivity of treatment

observation of substantia nigra and basal ganglia of the brain of male Wistar rats of each group revealed mild neuronal degeneration, minimal infiltration of neutrophils, and vacuolated neutrophils in the induction of the group of Parkinson's disease when compared with Group-1 (Control). There was a significant reversal of neuronal damage in treated group-I and II with ethanolic extract of *Smilax china* (100 and 200 mg/kg) in comparison with control and Group-3 (Standard) in substantia nigra and basal ganglia of Wistar rat shown in Figs. 19 and 20, respectively.

Discussion

From the phytochemical analysis, flavonoid is the secondary metabolite which is responsible for the decrease in oxidative stress and that is responsible for the anti-Parkinson's action [60].

Smilax china has been shown to have a neuroprotective effect against Wistar rat models of rotenone-induced neurotoxicity, a major consequence, and symptom of Parkinson's disease. Neurotoxicity-induced animal models of Parkinson's disease such as rotenone-lesioned rodents, show a greater neurotoxic effect in male Wistar rats than

Table 14 After 7 days of inducing Parkinson's disease in male Wistar rats, the catalepsy score was recorded using a bar test

Time interval (min)	Catalepsy score in seconds				
	Group-1 (Control)	Group-2 (Induction)	Group-3 (Standard)	Group-4 (Treatment-I)	Group-5 (Treatment- II)
0	2.75 ± 0.21	5.73 ± 0.21	5.74 ± 0.16	5.76 ± 0.17	5.79 ± 0.14
30	2.73 ± 0.20	67.34 ± 0.62[a]	13.37 ± 1.28*	33.92 ± 1.07*	28.35 ± 0.65*
60	2.59 ± 0.14	139.07 ± 5.82[a]	16.12 ± 0.97*	42.68 ± 4.90*	34.67 ± 1.42*
120	2.76 ± 0.16	176.41 ± 4.01[a]	18.61 ± 1.43*	69.75 ± 4.79*	43.91 ± 3.01*
180	2.65 ± 0.15	178.80 ± 0.02[a]	18.27 ± 1.20*	65.19 ± 5.27*	45.88 ± 2.33*

Average standard error of the mean; sample size = 6. The use of one-way ANOVA: Multiple comparisons using the Tukey–Kramer method: [a]$p < 0.001$ versus the control group; *$p < 0.001$ versus the induction group. Catalepsy scores were significantly increased in induction group stating the induction of Parkinson's disease

Table 15 The catalepsy score was recorded after 15th days after PD was induced in male Wistar rats using a bar test for catalepsy

Time interval (min)	Catalepsy score in seconds				
	Group-1 (Control)	Group-2 (Induction)	Group-3 (Standard)	Group-4 (Treatment-I)	Group-5 (Treatment-II)
0	2.75 ± 0.24	6.38 ± 0.37	4.46 ± 0.21	5.32 ± 0.28	5.43 ± 0.33
30	2.82 ± 0.21	31.28 ± 4.91[a]	23.43 ± 4.12*	24.26 ± 2.67*	20.8 ± 2.97*
60	2.66 ± 0.16	131.68 ± 5.08[a]	32.07 ± 4.78*	19.39 ± 2.08*	19.01 ± 2.38*
120	2.75 ± 0.15	96.26 ± 6.07[a]	32.60 ± 3.62*	20.38 ± 1.40*	20.45 ± 4.07*
180	2.86 ± 0.13	73.45 ± 5.04[a]	27.30 ± 3.24*	21.11 ± 1.38*	17.58 ± 4.23*

Average standard error of the mean; sample size = 6. The use of one-way ANOVA: Multiple comparisons using the Tukey–Kramer method: [a]$p < 0.005$ in comparison with normal control; *$p < 0.005$ in comparison with the Induction group. A significant improvement in the catalepsy score was seen in the treatment group after 15 days of treatment when compared with the induction and control group

Table 16 On day 21st after PD induction in male Wistar rats, the catalepsy score was recorded using a bar test

Time interval (min)	Catalepsy score in seconds				
	Group-1 (Control)	Group-2 (Induction)	Group-3 (Standard)	Group-4 (Treatment-I)	Group-5 (Treatment-II)
0	2.55 ± 0.12	6.06 ± 0.31	4.16 ± 0.28	4.83 ± 0.18	4.87 ± 0.43
30	2.74 ± 0.17	35.48 ± 3.28[a]	22.37 ± 3.15*	24.63 ± 2.92*	12.27 ± 0.61*
60	2.88 ± 0.12	84.30 ± 6.02[a]	18.36 ± 2.53*	27.83 ± 1.24*	20.40 ± 2.13*
120	2.85 ± 0.14	96.68 ± 4.55[a]	19.66 ± 1.92*	29.08 ± 2.22*	18.48 ± 1.42*
180	2.76 ± 0.17	67.59 ± 3.27[a]	21.96 ± 3.21*	24.92 ± 2.17*	17.06 ± 1.74*

Average standard error of the mean; sample size = 6. The use of one-way ANOVA: Multiple comparisons using the Tukey–Kramer method: [a]$p < 0.005$ in comparison with normal control; *$p < 0.005$ in comparison with the Induction group

in female Wistar rats. As observed Parkinson's disease tends to affect men more than women [61]. Hence male Wistar rats were selected for this research study. In the development of rotenone-induced Parkinson's disease, oxidative stress in the mitochondria has been shown as a primary contributor to this problem. Psychosis, epilepsy, urinary problems, hemiplegia, polyuria, leprosy, rejuvenator, congenital illnesses, and blood purification have all been treated using *Smilax china*. Researchers have shown that it inhibits lipid peroxidation and has antioxidant properties. Therefore, there is evidence that this plant offers therapeutic benefits [62].

Evidenced by a substantial increase in the average duration spent on the block compared to the control group, catalepsy was found in the male Wistar rats with Parkinson's disease in the current experiment. *Smilax china* treatment effectively attenuated male Wistar rats' development of catalepsy in response to rotenone. *Smilax china* ethanolic extract rescued dopaminergic neurotransmission in the striatum at doses of 100 and 200 mg/

Table 17 The block test was used to record the catalepsy score 7th days after Parkinson's disease was induced in male Wistar rats

Time interval (min)	Catalepsy score in seconds				
	Group-1 (Control)	Group-2 (Induction)	Group-3 (Standard)	Group-4 (Treatment-I)	Group-5 (Treatment-II)
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
30	0.0 ± 0.0	1.93 ± 0.22[a]	1.03 ± 0.12*	1.37 ± 0.11*	0.75 ± 0.12*
60	0.0 ± 0.0	3.15 ± 0.23[a]	1.30 ± 0.22*	1.89 ± 0.13*	0.84 ± 0.12*
120	0.0 ± 0.0	3.39 ± 0.11[a]	1.21 ± 0.17*	2.40 ± 0.16*	1.31 ± 0.17*
180	0.0 ± 0.0	3.54 ± 0.00[a]	1.36 ± 0.12*	2.05 ± 0.12*	1.65 ± 0.07*

Average standard error of the mean; sample size = 6. The use of one-way ANOVA: Multiple comparisons using the Tukey–Kramer method: [a]$p < 0.001$ in comparison with normal control; *$p < 0.001$ in comparison with the Induction group. In the block test, a statistically significant improvement was noticed in treatment groups for catalepsy scores in comparison with the induction and control groups

Table 18 The catalepsy score was recorded 15th days after Parkinson's disease was induced in male Wistar rats using a block test for catalepsy

Time interval (min)	Catalepsy score in seconds				
	Group-1 (Control)	Group-2 (Induction)	Group-3 (Standard)	Group-4 (Treatment-I)	Group-5 (Treatment-II)
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
30	0.0 ± 0.0	1.47 ± 0.21[a]	0.61 ± 0.30*	0.89 ± 0.12*	0.67 ± 0.12*
60	0.0 ± 0.0	2.62 ± 0.34[a]	1.08 ± 0.15*	1.02 ± 0.15*	0.88 ± 0.13*
120	0.0 ± 0.0	3.36 ± 0.17[a]	0.82 ± 0.13*	1.04 ± 0.18*	0.79 ± 0.14*
180	0.0 ± 0.0	3.05 ± 0.18[a]	0.70 ± 0.14*	0.82 ± 0.12*	0.75 ± 0.08*

Average standard error of the mean; sample size = 6. The use of one-way ANOVA: Multiple comparisons using the Tukey–Kramer method: [a]$p < 0.001$ in comparison with normal control; *$p < 0.001$ in comparison with the Induction group

Table 19 The block test was used to record the catalepsy score 21st days after Parkinson's disease was induced in male Wistar rats

Time interval (min)	Catalepsy score in seconds				
	Group-1 (Control)	Group-2 (Induction)	Group-3 (Standard)	Group-4 (Treatment-I)	Group-5 (Treatment-II)
0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0	0.0 ± 0.0
30	0.0 ± 0.0	2.58 ± 0.28[a]	0.50 ± 0.13*	0.80 ± 0.26*	0.74 ± 0.08*
60	0.0 ± 0.0	3.48 ± 0.00[a]	0.58 ± 0.13*	1.06 ± 0.18*	0.78 ± 0.17*
120	0.0 ± 0.0	3.54 ± 0.08[a]	0.65 ± 0.11*	0.87 ± 0.16*	0.58 ± 0.15*
180	0.0 ± 0.0	3.69 ± 0.07[a]	0.60 ± 0.09*	0.80 ± 0.07*	0.47 ± 0.17*

Average standard error of the mean; sample size = 6. The use of one-way ANOVA: Tukey–Kramer test for multiple comparisons: [a]$p < 0.001$ in comparison with normal control; *$p < 0.001$ in comparison with the Induction group. Post 21 days of regular treatment the catalepsy score from the block test showed statistically significant improvement in treated groups

kg against rotenone-induced neurotoxicity. Locomotor and muscular activity were observed to be significantly reduced in the rotenone-induced group of male Wistar rats in the present study. The inability to maintain a normal posture for one's limbs and issues with motor coordination have both been linked to Parkinson's disease. Our results indicate that male Wistar rats exposed to rotenone exhibited behavioral changes characteristic of Parkinson's disease, as well as degeneration of dopaminergic neurons. Treatment with *Smilax china* at doses of 100 and 200 mg/kg significantly reduced locomotor activity and increased muscle activity in male Wistar rats, indicating an influence on the central nervous system (CNS). The breakdown of mitochondrial complex I, which results in oxidative stress, is a crucial factor in the development of Parkinson's disease. Catalase, malonaldehyde, superoxide dismutase, and reduced glutathione levels were measured to assess oxidative stress in brain tissue [63].

Table 20 Using the Rota rod apparatus, measured the levels of neuromuscular coordination and grip strength (Motor coordination) in all three groups of male Wistar rats on days 7th, 15th, and 22nd

Treatment group	Rota Rod Test (Counts)		
Group	7th day	15th day	21st day
Group-1 (Control)	106.8 ± 1.12	108.4 ± 1.13	123.8 ± 1.03
Group-2 (Induction)	27.8 ± 2.10[a]	17.8 ± 5.60[a]	10.4 ± 5.62[a]
Group-3 (Standard)	39 ± 6.15*	61.6 ± 1.12*	66.6 ± 5.32*
Group-4 (Treatment-I)	36.8 ± 6.12*	53.2 ± 6.90*	64.2 ± 6.30*
Group-5 (Treatment-II)	27.9 ± 2.10*	55.2 ± 6.10*	68.2 ± 8.30*

Average standard error of the mean; sample size = 6. The use of one-way ANOVA: Multiple comparison using the Tukey–Kramer method: [a]$p < 0.005$ in comparison with normal control; *$p < 0.005$ in comparison with the Induction group

These reactive oxygen species are generated in a non-enzymatic manner due to the inhibitory actions of complexes I and IV in the mitochondrial electron transport chain [64]. Characteristics of Parkinson's disease include increased glutathione levels, protein denaturation, and lipid peroxidation [65].

Thio barbituric acid concentrations are measured by measuring lipid peroxidation, a sensitive biomarker of oxidative stress. When an unpaired electron from a free radical combine with a double bond in an unsaturated fatty acid or arachidonic acid, lipid peroxyl radicals are formed. Reduced fluidity, compromised structural integrity, and inactivation of several enzymes are all problems caused by lipid peroxidation, the oxidative destruction of polyunsaturated fatty acids, in biological membranes. Lipid peroxidation product was found in high concentrations in the substantia nigra of Parkinson's disease patients. The present investigation found similar outcomes when analyzing brain homogenates from rotenone-treated male Wistar rats [66].

An antioxidant enzyme, catalase (CAT) neutralizes hydrogen peroxide. Hydrogen peroxide can be safely decomposed by catalase, an enzyme, into oxygen and water. Oxidative stress causes a decrease in catalase activity. The decrease in catalase levels in the

Table 21 Effect of *Smilax china* on reduced glutathione (GSH), malondialdehyde (MDA), catalase (CAT), and superoxide dismutase (SOD) levels in the rat brain

Treatment groups	GSH (nM/mg of protein)	CAT (μmoles of H_2O_2 used/min/mg protein)	SOD (units/mg protein)	MDA (nM/mg of protein))
Group-1 (Control)	4.391 ± 0.0781	5.38 ± 0.31	3.182 ± 0.194	4.375 ± 0.065
Group-2 (Induction)	3.886 ± 0.021[a]	3.08 ± 0.23[a]	2.05 ± 0.104[a]	3.978 ± 0.018[a]
Group-3 (Standard)	7.124 ± 0.7114*	6.25 ± 0.31*	8.42 ± 0.820*	7.025 ± 0.701*
Group-4 (Treatment-I)	5.681 ± 0.1412*	4.75 ± 0.24*	4.43 ± 0.01*	5.606 ± 0.142*
Group-5 (Treatment-II)	6.456 ± 0.0381*	6.23 ± 5.23*	5.44 ± 0.01*	6.486 ± 0.040*

Values are expressed as mean ± SEM; $n = 6$. One-way ANOVA; Tukey–Kramer multiple comparison tests: [a]$p < 0.001$ in comparison with normal control; *$p < 0.001$ in comparison with the Induction group. Biochemical markers were significantly reduced in the induction group whereas were found to be statistically improved in treated groups

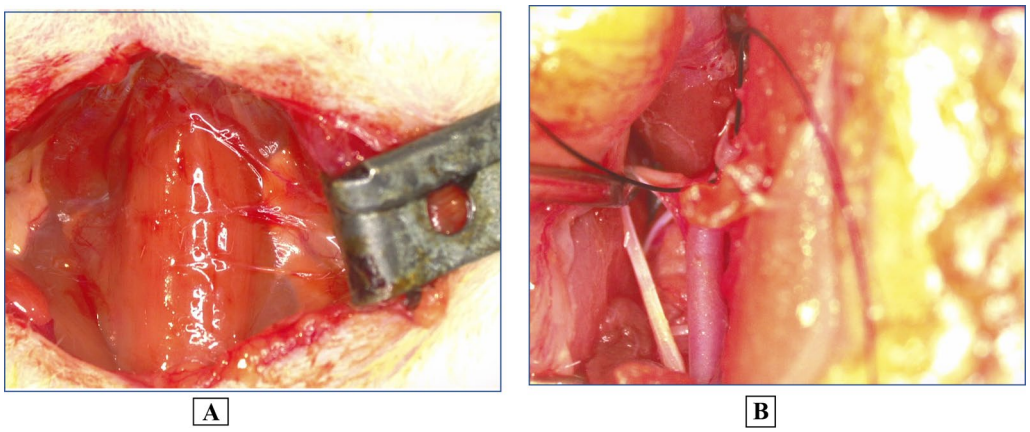


Fig. 17 The vagus nerve is cut off at the base of the skull in the neck. **A** The vagus nerve was constricted with surgical silk thread. **B** To prevent the vagus nerve from abruptly separating the bilateral carotid arteries

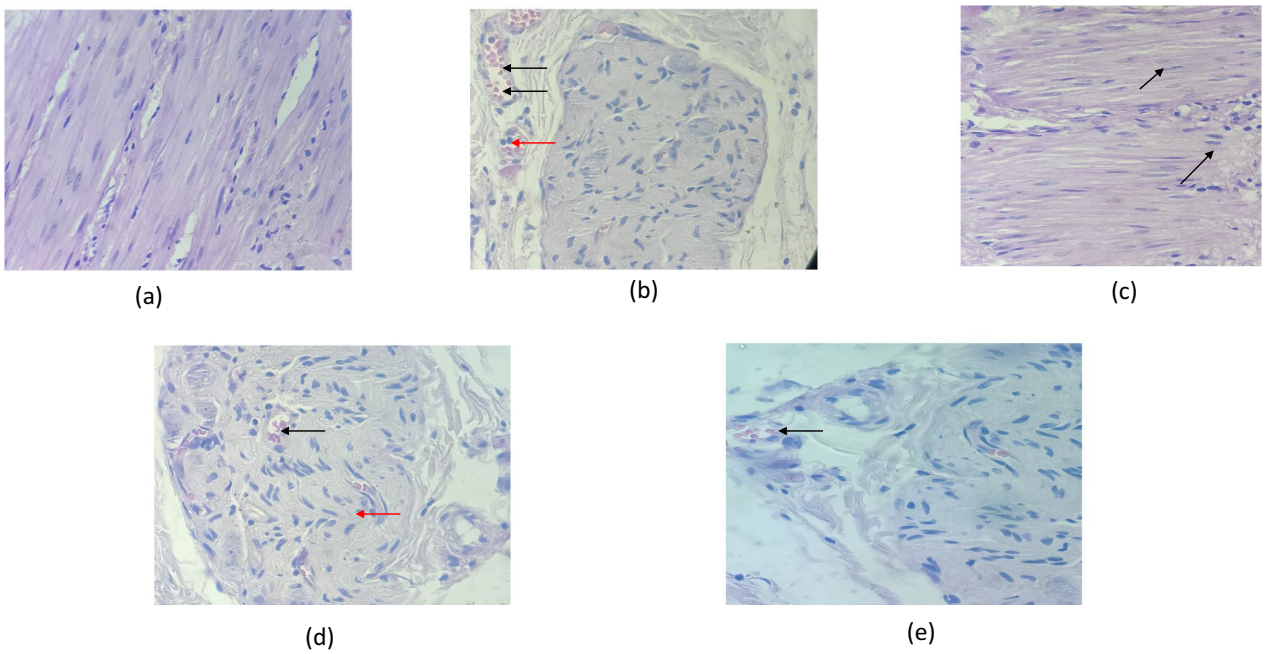


Fig. 18 Stained images of vagus nerve longitudinal section isolated from respective groups **a** Group-1 (Control): Normal nerve tissue which is not showing any sign of axonal inflammation and degenerative changes **b** Group-2 (Induction): Lipid degenerated axons showing a peripheral axonal loss**,** inflammation and degeneration (black arrow) and alpha-synuclein accumulation (red arrow) **c** Group-3 (Standard): No axonal degeneration, inflammation, and there is no alpha-synuclein accumulation **d** Group-4 (Treatment-I): minimum axonal degeneration, inflammation(black arrow) and minimum alpha-synuclein accumulation (red arrow). **e** Group-5 (Treatment-II): Major recovery of the axonal degeneration and inflammation H & E, (hematoxylin and eosin) 10X

rotenone-exposed Wistar rat group demonstrates the occurrence of oxidative damage [67].

The enzyme superoxide dismutase (SOD) catalyzes the transformation of superoxide into innocuous oxygen species and hydrogen peroxide. As a result, it is an essential part of the antioxidant defense mechanisms used practically all cells when exposed to oxygen. Superoxide dismutase is made to neutralize free radicals and protect cells from their damage. Oxidative stress may have begun in the rotenone-induced group, as evidenced by reduced superoxide dismutase (SOD) levels in the brain [68].

Reduced glutathione (GSH), a potent enzyme, has a role in the progression of Parkinson's disease. Neuronal death in Parkinson's disease has been linked to glutathione depletion in the substantia nigra. Lower levels of glutathione have been associated with more cell death in the brain. Reduced glutathione levels would impair neurons' detoxification of hydrogen peroxide and raise the danger of free radical generation and lipid peroxidation. A decrease in glutathione (GSH) levels was seen in male Wistar rats that were administered rotenone [69].

As a result, thiobarbituric acid (a marker of the amount of lipid peroxidation) was much higher in the rotenone-induced group of male Wistar rats compared to the control group, while reduced glutathione (GSH) and superoxide dismutase (SOD) were significantly lower. These variables explain why male Wistar rats exposed to rotenone have increased oxidative stress in their brains. Malondialdehyde (MDA) was reduced and superoxide dismutase (SOD), catalase (CAT), and reduced glutathione (GSH) were enhanced in the brains of rotenone-treated rats at a higher dose (200 mg/kg) of ethanolic extract of *Smilax china* [70].

Histopathological examination of the brains of male Wistar rats treated with an ethanolic extract of *Smilax china* revealed less neutrophil infiltration, restoration of neurodegeneration, attenuation of alpha-synuclein aggregation, restoration of normal brain architecture, and little necrosis. Plants with medicinal properties are common in many parts of the world and are used to cure a wide range of diseases. Most people still rely on Western pharmaceuticals, Ayurvedic treatments (such as churn and ghritas), herbal combinations, and home-made herbal cures to combat disease [71]

Conclusion

Based on the evidence provided, the ethanolic extract of the Smilax china plant can be helpful in showing neuroprotective action toward male Wistar rats with Parkinson's disease. Based on the evidence provided, an

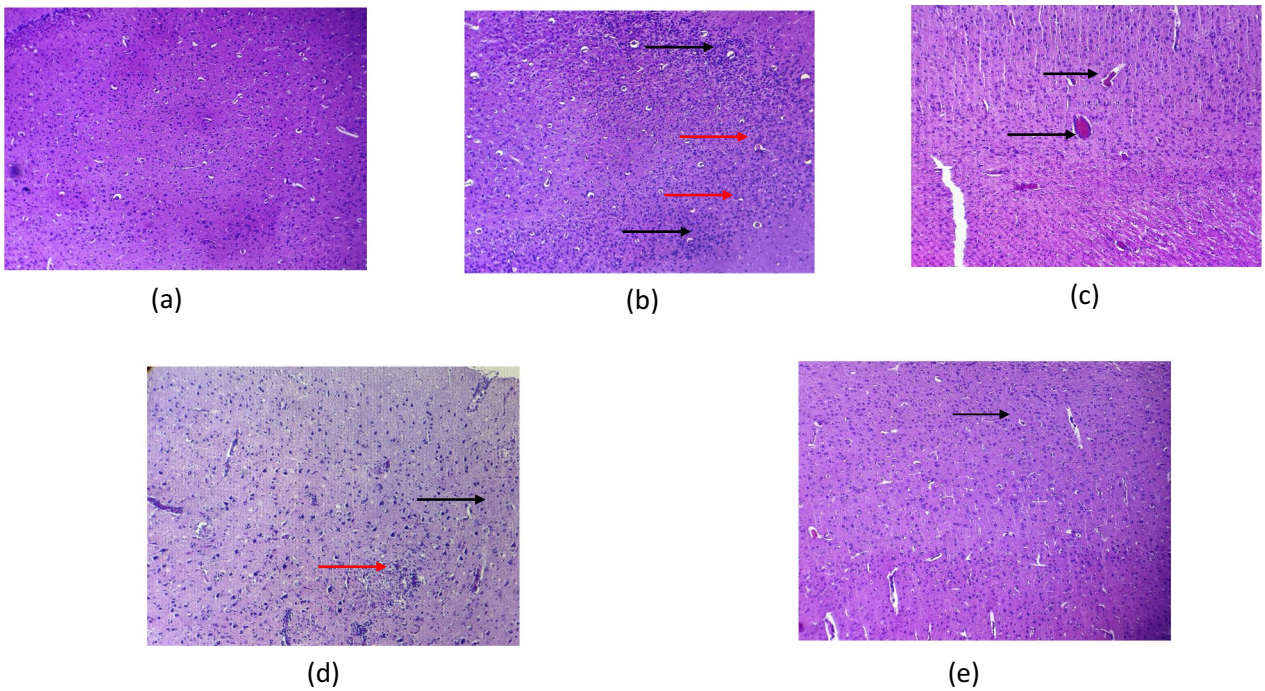


Fig. 19 Histopathological examination of substantia nigra: **a** Group-1 (Control): Normal neuronal density and normal brain architecture no abnormality detected. **b** Group-2 (Induction): Major degradation of neurons (red arrow) and infiltration of vacuolated neutrophils (Black arrow). **c** Group-3 (Standard): Minimal neurofibrillary tangles (black arrow). **d** Group-4 (Treatment-I): Minimal degradation of neurons (Red arrow) and infiltration of vacuolated neutrophils (Black arrow). **e** Group-5 (Treatment-II): Minimal infiltration of vacuolated neutrophils (Black arrow) and reduced necrosis H & E, (hematoxylin and eosin) 10X

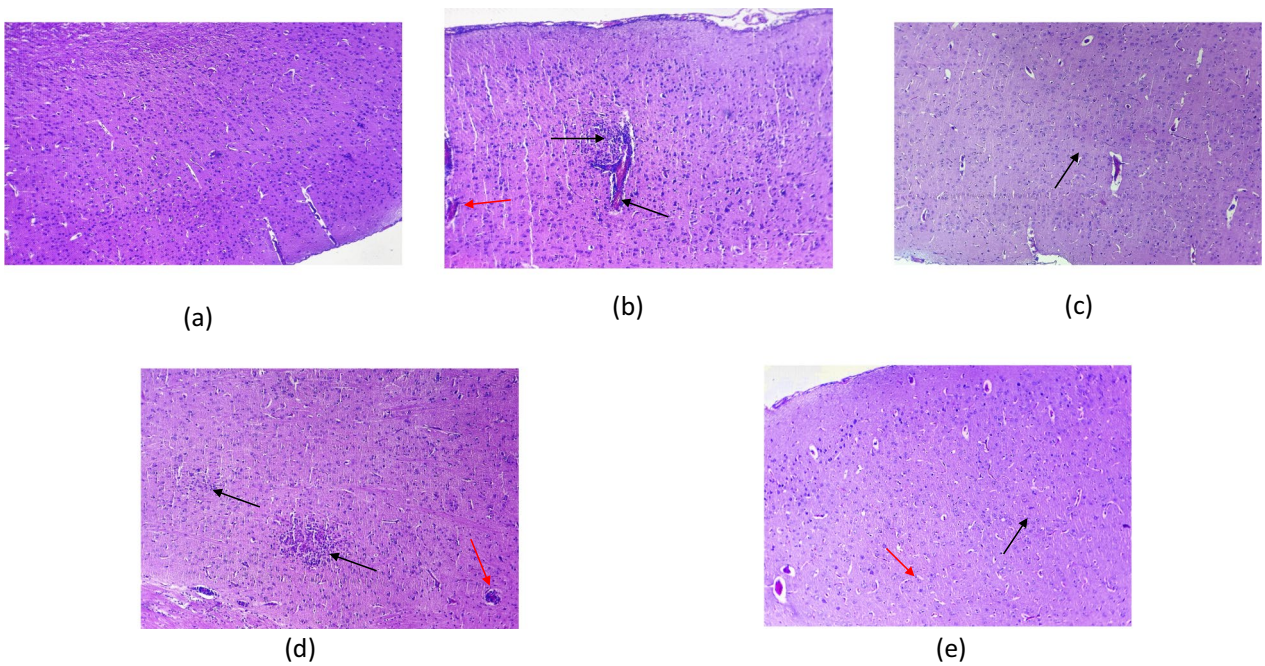


Fig. 20 Stained histopathological images of basal ganglia isolated from respective groups **a** Group-1 (Control): Shown normal brain architecture of basal ganglia **b** Group-2 (Induction): Degeneration of neuron(black arrow) and cell integrity (red arrow) **c** Group-3 (Standard): Shown improvement in neuronal cell integrity (red arrow) and architecture as compared to control group **d** Group-4 (Treatment-I): Mild decrease in neurons (black arrow) and cellular hypertrophy (red arrow) **e** Group-5 (Treatment-II): Decrease in neurons (black arrow) and cellular hypertrophy(red arrow). H & E, (hematoxylin and eosin) 10X

ethanolic extract of the *Smilax china* plant can help male Wistar rats with Parkinson's disease. A detailed research study on the isolation of potential bioactive compounds responsible for neuroprotective action must be conducted in future studies. A better understanding of cellular pathways involved in the treatment of Parkinson's disease by the bioactive compound flavonoid from *Smilax china* will bring more insight into our research.

Current and future aspects

This study will <>establish baseline data for standardization purposes and provide a contribution to plant monographs. More study is required to identify the bioactive compounds in this plant that may be responsible for its anti-Parkinson's effects. The herb has been shown to have anti-inflammatory capabilities, and tests on rats have shown no ill effects. Therefore, more research is required on higher animals, and if it is safe, people might participate as well.

Abbreviations

OECD	Organization for Economic Corporation and Development
SOD	Superoxide dismutase
CAT	Catalase
MDA	Malondialdehyde
GSH	Reduced glutathione
TLC	Thin-layer chromatography
UV	Ultraviolet
FTIR	Fourier transform infrared
FAA	Formalin-aceto-alcohol
LOD	Loss on drying
R_f	Retention factor
CPCSEA	Committee for the Purpose of Control and Supervision of Experiments on Animals
(LD)-50	Lethal dose
DMSO	Dimethyl sulfoxide
I.P.	Intraperitoneal route
S.C.	Subcutaneous route
P.O.	Oral route
rpm	Rotation per minute
TBA	Thio barbituric acid
HCL	Hydrochloric acid
Abs	Absorbance
V_T	Total mixture volume
W_T	Dissected brain weight
V_U	Is the aliquot volume
EDTA	Ethylenediaminetetraacetic acid
H_2O_2	Hydrogen peroxide
E	Hydrogen peroxide extinction coefficient
δ O.D.	Variance in absorbance per minute
B_T	Brain tissue
D_F	Dilution factor
Y	Abs 412 of Brain Tissue homogenate
pH	Potential of hydrogen
ANOVA	Analysis of variance
T.S.	Transverse section
SEM	Standard error of mean
n	Number
mg	Milligram
kg	Kilogram
nM	Nanometer
H & E	Hematoxylin and eosin

Author contributions
AS complied the paper with through interpretations, NS worked on the induction of Parkinson's disease, performing animal studies, NV worked on designing the experimental design, protocols, guidelines for study and AK conducted the pharmacognostic, spectroscopic studies.

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Availability of data and materials
https://pubmed.ncbi.nlm.nih.gov/.

Declarations

Ethics approval and consent to participate
Yes, (IAEC) as per CPCSEA guidelines approved the study for research and education purposes (DYPCOP/IAEC/2022/12/09).

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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