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Egyptian Journal of Neurosurgery (2025) 40:75
https://doi.org/10.1186/s41984-025-00419-4

Egyptian Journal of Neurosurgery

RESEARCH Open Access

Evaluating the Antiparkinsonian activity of *Tamarix gallica* extract in a reserpine-induced rat model of Parkinsonism: behavioral and neurochemical assessments

Savankumar Chhatrola[1*] and Pravin Tirgar[1]

Abstract

Background Parkinson's disease (PD) is the second most common neurodegenerative disorder in the world and it is a challenge for doctors. This study investigates the effect of *Tamarix gallica (TG)* on the behavioral, neurochemical and tissue changes induced by reserpine, a chemical that causes PD.

Methods Female Wistar rats were divided into different groups (n = 6). Eight groups each for reserpine induced PD model to mimic Parkinson-like conditions and treated with different doses of extract of TG and nanosuspension of extract of *TG*. 24 h after the last dose, animals were subjected to behavioral, biochemical and histopathological evaluations. The data were analyzed by one-way ANOVA and Dunnett's comparison test.

Results Reserpine decreased locomotor activity and motor coordination ability. Reserpine also decreases catalase, superoxide dismutase (SOD), and increases lipid peroxidation (LPO) levels as compared to normal control group animals. Reserpine also showed the presence of vacuolated cytoplasm. Levodopa and extract of *TG* and nanosuspension of extract of TG as well as in combination normalized the behavioral, biochemical and histopathological complications.

Conclusion Nanosuspension of extract of TG and alone extract of TG showed neuroprotection by antioxidant activity as well as improved reserpine-induced behavioral defects. Extracts of TG have active potential and can be used clinically with some further investigations.

Keywords Tamarix gallica, Parkinson's disease, Reserpine, Ellagic acid

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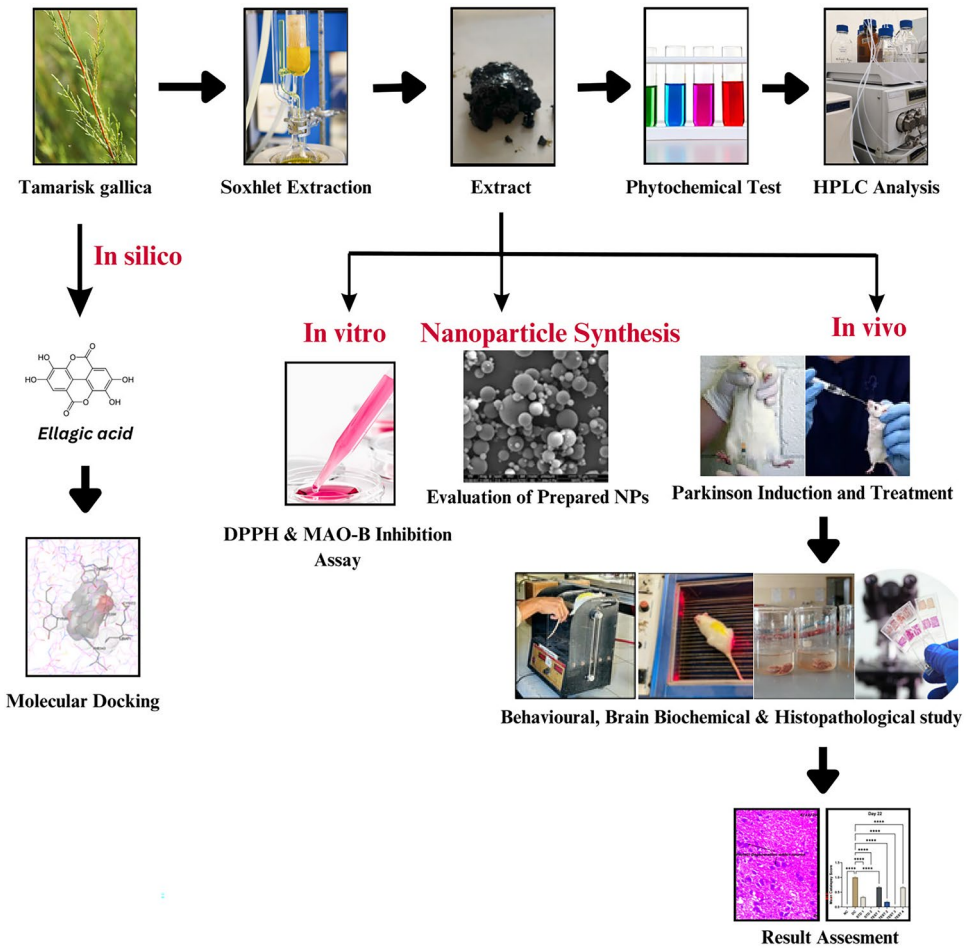
Graphical abstract



Introduction

Parkinson's disease (PD) is a progressive neurodegenerative condition initially identified as shaking palsy by James Parkinson. It is characterized by pathological changes in the brain, including the loss of dopaminergic neurons in the substantia nigra and the accumulation of protein alpha-synuclein within neurons to form Lewy bodies [1]. Clinically, PD is characterized by various motor symptoms like slow movement (bradykinesia), akinesia, resting tremors and balance problems, along with non-motor symptoms such as sleep disturbances, pain, fatigue and gastrointestinal issues [2, 3]. The specific mechanisms responsible for the significant and irreversible degeneration of dopaminergic neurons remain unknown. However, several cellular processes are thought to be involved in this disease, including neuroinflammation, oxidative stress and mitochondrial dysfunction [4–6].

Current therapies for Parkinson's disease (PD primarily focus on improving motor symptoms by addressing dopamine deficiency. Although dopamine itself cannot cross the blood–brain barrier, its precursor, levodopa, is actively transported into the central nervous system where it is converted to dopamine [7]. Monoamine oxidase type B (MAO-B) inhibitors are drugs used to treat Parkinson's disease (PD). They prevent the enzyme MAO-B from absorbing dopamine that the brain is not using, making more dopamine available to treat symptoms. MAO-B inhibitors can be used as monotherapy in early-stage PD or as an add-on to other drugs in the advanced stages. They can improve motor and non-motor symptoms, can reduce "OFF" time and may potentially prevent or delay disease progression [8].

Medicinal plants are commonly used as alternative therapeutic methods to prevent or treat a range of diseases globally. Tamarix gallica (TG), belonging to the

Tamaricaceae family, contains ellagic acid as its primary constituent, along with compounds such as gallic acid, rhamnetin, naringenin, quercetin, kaempferol and tamarixetin. In vitro studies have shown that ellagic acid has beneficial effects, and including inhibiting monoamine oxidase B and TG also shows beneficial activity against Alzheimer's disease [9–11].

With advancements in the medical field, novel nanotechnology-based approaches have emerged for drug delivery systems capable of crossing the blood–brain barrier [12]. Molecules and drugs of interest can be attached to these delivery systems, allowing for targeted delivery to specific damaged tissues or organs. The primary objective of employing nanosized drug delivery systems in Parkinson's disease (PD) is to enhance drug availability in the brain without increasing the administered dose, thereby reducing drug-related adverse effects [13]. The objective of this study is to evaluate the therapeutic efficacy of the plant extract of Tamarix gallica as well as its nanosuspension in a reserpine-induced rat model of Parkinson's disease.

Material and methodology

Plant collection and identification

Tamarix gallica was purchased from the e-commerce site Lalit Enterprise, and it was authenticated by the Faculty of Science, RK University (Fig. 1).

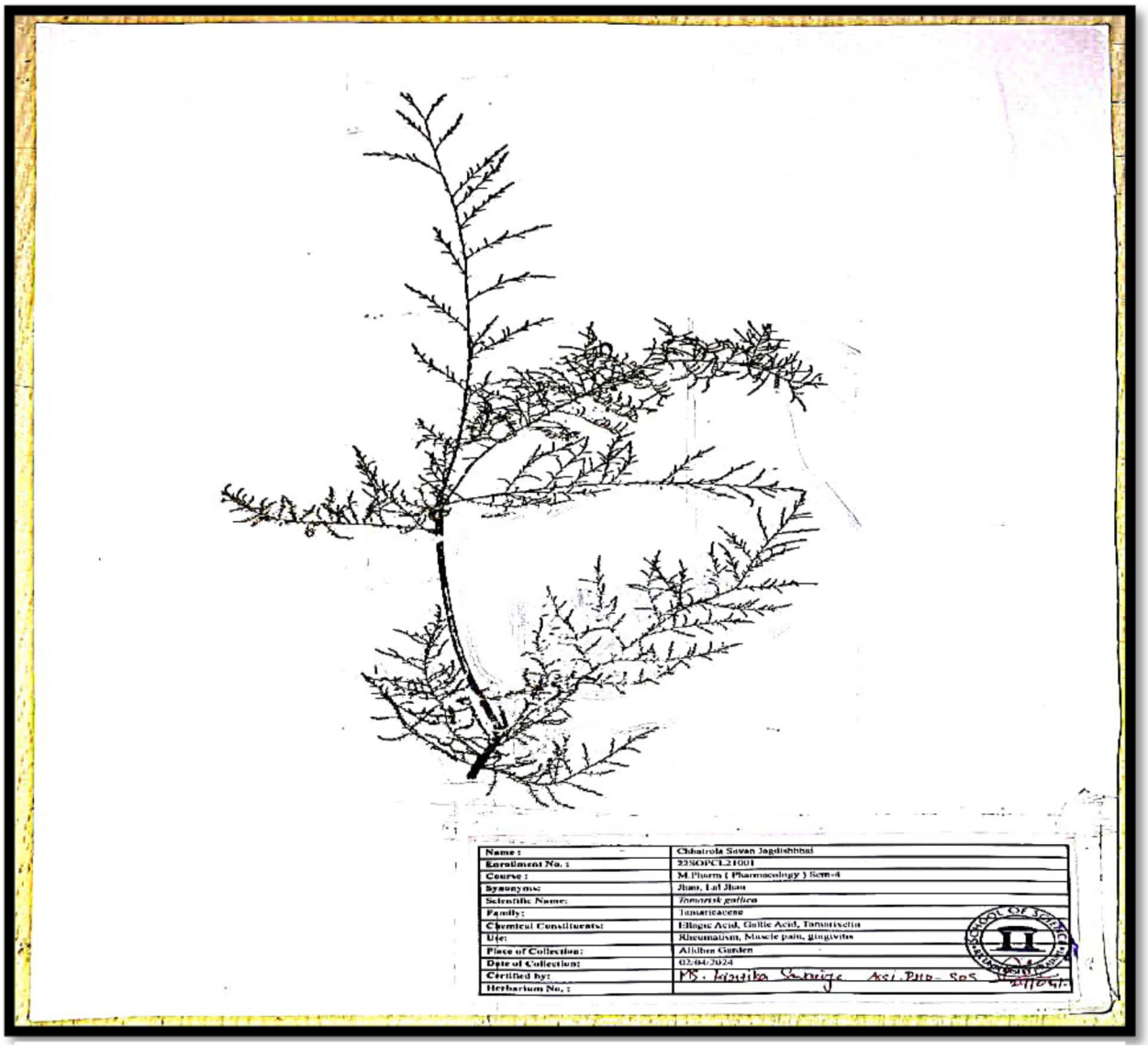


Fig. 1 Herbarium of Tamarix gallica

Preparation of methanolic extract of *Tamarix gallica*

Aerial parts of *Tamarix gallica* were collected, washed, dried under shade and extracted by using the Soxhlet extraction method (Fig. 2). In this method, the crushed sample was placed inside the thimble chamber of the Soxhlet apparatus. Methanol, which was 80% pure, was used as the extraction solvent. The solvent was heated in the bottom flask, vaporized into the sample thimble, condensed in the condenser and then dripped back into the bottom flask. This continuous cycle allowed for efficient extraction of the desired compounds from the sample.

About 100 g of dried sample powder was weighed, and the extraction process was carried out using 250 ml of 80% pure methanol in the Soxhlet apparatus for 12 h. The resulting extract was concentrated by evaporation at 70 °C for 4 h and then dried. The concentrated extract was then transformed into a gel form and stored at room temperature before phytochemical screening.

Preliminary phytochemical screening

The crude methanol extract was analyzed for secondary metabolites including phenols, tannins, flavonoids, saponins, terpenoids, triterpenes, diterpenes, glycosides, anthraquinones, alkaloids and steroids using the standard method as referenced in sources [14–16](Fig. 3, Table 1).

In vitro DPPH assay

The antioxidant capacity of *Tamarix gallica* (Tamarix) extract was evaluated using the DPPH (2,2-diphenyl-1-picrylhydrazyl) method [17]. The experiment involved preparing *Tamarix gallica* extract solutions directly at concentrations of 20, 40, 60, 80 and 100 µg/mL. Freshly prepared DPPH solution (1.3 mg/mL in methanol) was added to each test tube containing the extract solution, followed by incubation in the dark for 30 min. During this incubation period, the interaction between the extract and DPPH led to the scavenging of DPPH radicals by the antioxidant compounds present in the *Tamarix gallica* extract. After incubation, the absorbance of each solution was measured at 517 nm using a spectrophotometer (UV–UV–visible spectrophotometer). The decrease in absorbance at 517 nm indicated the extent of DPPH radical scavenging by the *Tamarix gallica* extract. Measured percentage inhibition values were obtained from the absorbance readings. This method allowed for the quantitative assessment of the antioxidant activity of *Tamarix gallica* extract, providing valuable insights into its potential health-promoting properties related to free radical scavenging and oxidative stress mitigation.

A control sample was prepared with the same volume of solvent but without any extract, and a reference containing ascorbic acid was included. A 95% methanol solution was used as a blank. The percentage of DPPH free radical scavenging was calculated using the formula (Table 2):

$$\%\ \text{inhibition} = \{(\text{A control} - \text{A sample})/(\text{A control})\} \times 10$$

$$\text{Control} = \text{absorbance of DPPH alone}$$

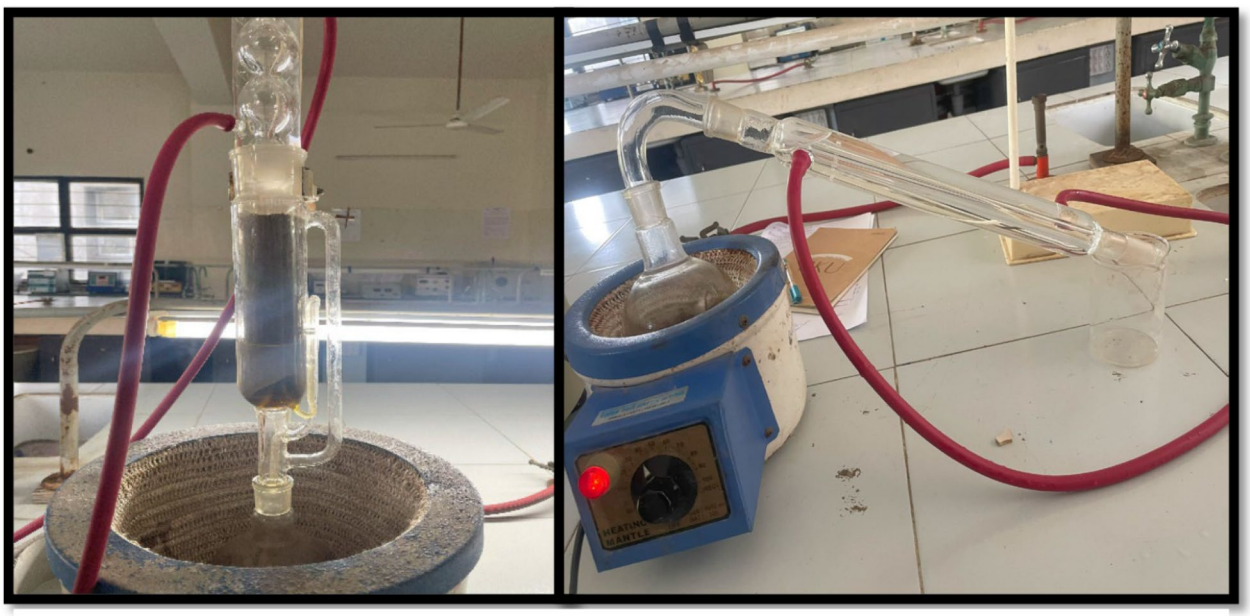


Fig. 2 Soxhlet extraction of *Tamarix gallica*

Sample = absorbance of DPPH along with different concentrations of extract.

Experimental animals

In this study, we followed the CCSEA (Committee of Control And Supervision of Experiments on Animals) guidelines for animal handling. Approval for all animal procedures was granted by the institutional Ethics Committee at the School of Pharmacy, RK University, with reference no. RKCP/COL/RE/23/135, on December 30, 2023. The experiments followed authorized protocols and the Guide for Care and Use of Laboratory Animals as per CCSEA guidelines. Forty-eight female albino Wistar rats, aged three months old, were used in total for the experiments. The rats were housed in standardized cages, with 2–3 rats per cage, in a room maintained at a controlled temperature (25 ± 3 °C), humidity (30–70%) and light (12:12 light/dark cycle, lights on at 6:30 a.m.), with ad libitum access to food and water. The experimental rats were randomly assigned to eight groups, each containing six animals.

Acute oral toxicity test

Methanolic extracts of *Tamarix gallica* showed no death or mortality in acute oral toxicity studies up to the dose of 3000 mg/kg in albino Wistar rats [9, 18].

Induction of Parkinson's disease

Parkinson's disease was induced in rats by administering reserpine intraperitoneally (i.p) on days 1, 3 and 5 at a dose of 1 mg/kg body weight each day. After 7 days from the administration of reserpine, behavioral parameters were evaluated to assess the development of Parkinsonian symptoms in the rats [19, 20].

Treatment design

The rats were divided into 8 groups with 6 animals (n = 6) each as below.

Group I—Normal control (received ml normal saline, *p.o.*)

Group II—Disease control untreated (received distilled water 10 ml*/kg*, *p.o.*) [21]

Group III—Treated with standard drug L-DOPA (22.5 mg/kg oral (P.O.), daily) for 22 days [22]

Group IV—Treated with L-DOPA (22.5 mg/kg oral (P.O.), daily) and selegiline (10 mg/kg i.p, daily) for 22 days [23, 24]

Group V—Treated with L-DOPA (22.5 mg/kg oral (P.O.), daily) and methanolic extract of *Tamarix gallica* (METG) (100 mg/kg oral (P.O.), daily) for 22 days [24, 25]

Group VI – Treated with L-DOPA (22.5 mg/kg oral (P.O.), daily) and METG (200 mg/kg oral (P.O.), daily) for 22 days.

Group VII – Treated with METG nanosuspension (drug equivalent to 50 mg/ml/day/kg oral (P.O.), daily) for 22 days.

Group VIII – Treated with METG (200 mg/kg oral (P.O.), daily) for 22 days.

For 22 days, behavioral parameter was measured on the 1st, 7th, 14th and 22nd days of the study. Finally on day 22, animals were deeply anesthetized using 2 mg of pentobarbital. The brains were surgically removed for further processing.

Evaluation of behavioral parameter

Rotarod

The rotarod test is a widely used method for evaluating motor coordination and balance in rodents, particularly mice and rats. The test apparatus consists of a rotating rod or cylinder, often textured to provide grip, which rotates at a constant speed or accelerates gradually. Animals are placed individually on the rotating rod, and their ability to maintain balance and coordination is assessed as the rod rotates (Fig. 4). The duration that each animal remains on the rod before falling off is recorded as a measure of motor performance. Animals are typically tested multiple times at different speeds or durations, and the results are averaged to obtain a measure of motor coordination. The rotarod test is commonly used in preclinical research to assess the effects of pharmacological compounds on motor function, particularly in neurological and neurodegenerative disease models [26].

Photoactometer

The photoactometer is a valuable tool used for assessing motor function and locomotor activity in animal models of Parkinson's disease. This device operates by emitting a beam of light within a designated arena, and when the animal moves and interrupts this beam, the interruptions are recorded and quantified as a measure of movement and activity levels (Fig. 5). In Parkinson's disease research, the photoactometer plays a crucial role in evaluating motor deficits characteristic of the disease, such as bradykinesia (slowed movement) and hypokinesia (reduced spontaneous movement) [27].

Estimation of various components in rat brain

Brain homogenate preparation

After completing the experiment, rats were killed, and their brains were dissected (Fig. 6). The brain tissue was

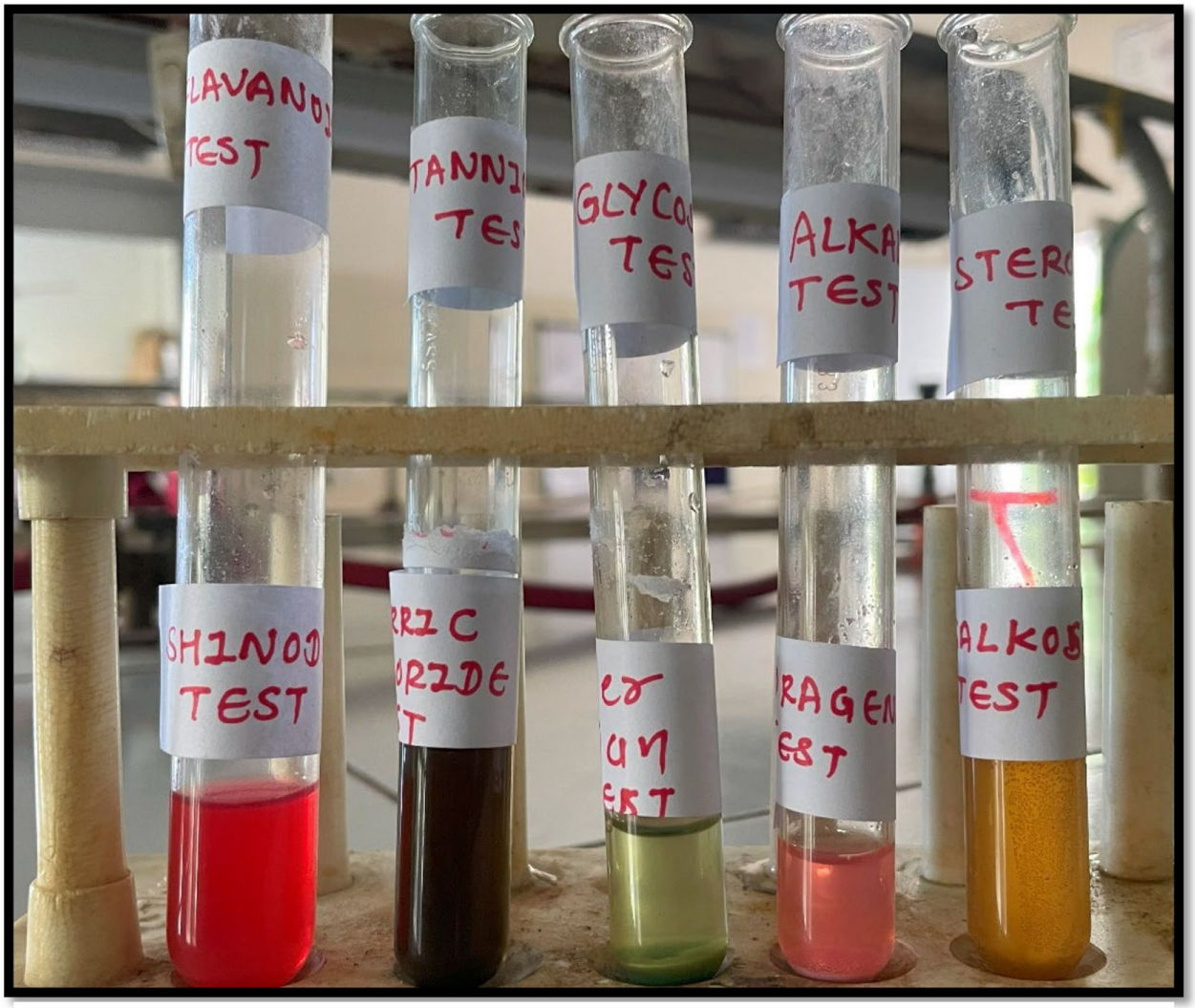


Fig. 3 Phytoconstituent test

Table 1 Phytoconstituent test

Sr. no	Metabolite	Tests	Result
1	Tannins	Ferric chloride test	+
2	Flavonoids	Shinoda test	+
3	Alkaloids	Dragendorff test	+
4	Steroids	Salkowski test	+
5	Glycosides	Keller–Kiliani test	+
6	Saponin	Frothing test	+

Table 2 %Inhibition—DPPH assay

Concentration (µg/ml)	% Inhibition
50	31.25
100	40.24
200	49.06
300	56.16
400	61.25
500	74.34

homogenized in 5 mL of HCl–butanol using a motor-driven Teflon-coated homogenizer for approximately 1 min. The resulting sample was then centrifuged for 10 min at 2000–3000 rpm. The supernatant phase was carefully removed, and a centrifuge tube containing heptane (3 mL) and 0.1 M HCl (0.30 mL) was added. After shaking the tube for at least 10 min, it was centrifuged again to separate the two phases. The aqueous phase was subsequently used for other enzyme assays.

Fig. 4 Rotarod test

Estimation of protein

This method uses a mix of the Folin–Ciocalteau and Biuret reactions to measure protein levels in a sample. First, proteins bind with copper in a basic solution, turning the copper into Cu^{++} ions. Next, these Cu^{++} ions help oxidize aromatic amino acids, changing phosphomolybdotungstate into heteropolymolybdanum, which creates a blue color. The intensity of this color is measured at 640 nm to determine the protein amount in the sample. This method offers a simple way to gauge protein levels based on color changes, which is useful for protein analysis in different research.

0.1 ml of the homogenate, 0.9 ml of water and 4.5 ml of alkaline copper sulfate reagent were added and allowed to stand at room temperature for 10 min. To this, 0.5 ml of Folin's reagent was added. After 20 min, the color developed was measured at 640 nm. The level of protein present was expressed as mg/g/ tissue or mg/dl [28].

Estimation of dopamine

To estimate dopamine (DA) levels in rat brains, a mixture was prepared consisting of 1 ml of homogenized supernatant, 1 ml of ferric chloride solution (1.5×10^{-2} M) and 1 ml of potassium ferricyanide solution (1.5×10^{-2} M) in a total volume of 25 ml of distilled water. This mixture was allowed to react for 30 min. The resulting color change was then measured using a UV–visible double-beam spectrophotometer at a wavelength of 735 nm [29].

Estimation of catalase

Catalase activity was measured by using following the method described by Luck [30]. The breakdown of hydrogen peroxide (H2O2) was measured at 240 nm. The

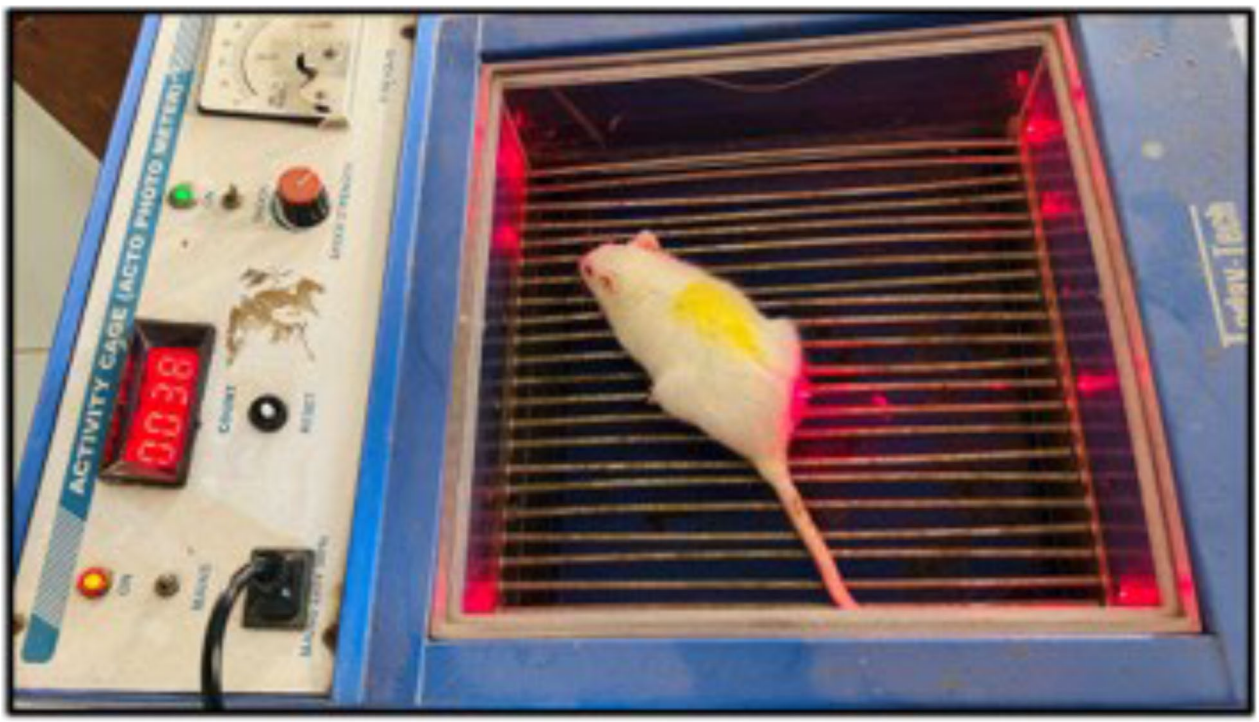


Fig. 5 Actophotometer test

Fig. 6 Isolated brain

assay mixture contained 3 mL of 0.01 M H2O2–phosphate buffer (pH 7) and 0.05 mL of the supernatant from tissue homogenate (10%). The change in absorbance was recorded after 1 min at 240 nm. Enzyme activity was calculated using the millimolar extinction coefficient of H2O2 (0.071). The results were expressed as micromoles of H2O2 decomposed per minute per milligram of protein.

Estimation of superoxide dismutase

SOD levels in Wistar rat brains were estimated using the method described by Weydert and Cullen [30]. A prepared mixture containing 0.1 mL of supernatant, 0.1 mL of EDTA (1×10^{-4} M), 0.5 mL of carbonate buffer (pH 9.7) and 1 mL of epinephrine (1 mM) was subjected to spectrophotometric analysis. The optical density of the resulting adrenochrome was measured at 480 nm over 3 min. Enzyme activity was quantified based on the amount required to inhibit chromogen production by 50% within one minute under the specified assay conditions.

Estimation of brain malondialdehyde level

The MDA content was determined by using the TBA (thiobarbituric acid) method. 1 mL aliquots of the supernatant was mixed with 3 mL of TBA reagent, which consisted of 0.38% (w/w) TBA, 0.25 M hydrochloric acid and 15% trichloroacetic acid (TCA). After 15 min of reaction time, the mixture was cooled in an ice bath. Following centrifugation at 3500 RPM for 10 min, the upper layer was collected, and its absorbance at 532 nm was measured using a spectrophotometer. The results were shown as nanomoles of MDA per milligram of protein.

Statistical analysis

Collected data were analyzed by using one-way ANOVA (analysis of variance) followed by Dunnett's test for multiple comparisons. Results are expressed as mean ± SEM, and a p-value of less than 0.05 was considered statistically significant.

Result and discussion

Phytoconstituent test

In vitro DPPH assay

The antioxidant activity of the extract of TG is given in Table 2. It was found that the antioxidant activities of TG extract increase with the increased concentration of extract. Maximum antioxidant activity was observed at a concentration of 500 µg/ml (Fig. 7).

Evaluation of behavior parameters

Actophotometer test

The results of the study on the effects of various treatments on the cutoff value/min are given in Table 3. The study evaluated the effects of various treatments on the cutoff value/min in different groups over 22 days. The normal control group maintained stable values, while the disease control group showed a significant decrease. Treatment with reserpine and levodopa (STD 1) initially mirrored the disease control but improved by day 22. Adding selegiline (STD 2) resulted in significant improvements, remain normal values by the end of the study. Test groups with METG and levodopa showed dose-dependent improvements, with higher doses giving better outcomes. The combination of reserpine, levodopa and NPMETG gives the most improvement, like a potent therapeutic effect (Fig. 8).

Rotarod test

The results of the study on the "time for falling" measure across different treatment groups over 22 days showed significant variations (Table 4). The normal control group exhibited stable times throughout the study period, indicating consistent motor performance. Conversely, the disease control group experienced a drastic reduction in falling time, particularly noticeable by day 7, and maintained low levels through to day 22. The group treated with reserpine and levodopa (STD 1) also showed initial deterioration, similar to the disease control, but demonstrated gradual improvement by day 22. The addition of selegiline (STD 2) to the reserpine and levodopa treatment significantly enhanced the performance, with near-normal times by the end of the study. The test groups treated with varying doses of METG and levodopa showed dose-dependent improvements, with higher doses (200 mg/kg) performing better. The combination of reserpine, levodopa and NPMETG (test 3) resulted in marked improvement, with times approaching those of the normal control group by day 22. The group treated with reserpine and METG alone (test 4) showed moderate improvement. Overall, combination therapies,

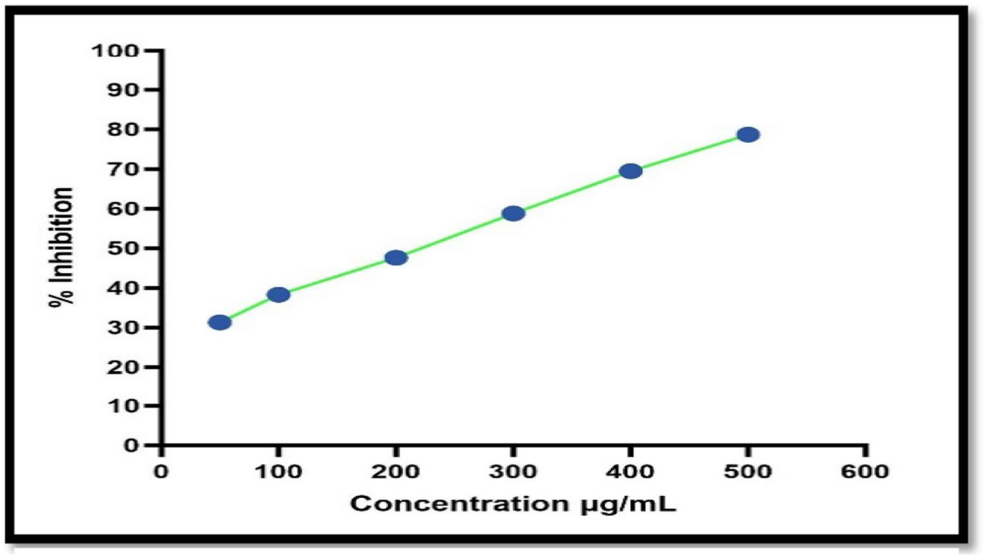


Fig. 7 In vitro DPPH assay

particularly those including selegiline or NPMETG, were more effective in restoring motor function compared to lower doses of METG (Fig. 9).

Evaluation of biochemical parameter

In this analysis, we examine the levels of endogenous antioxidant enzymes in the brains of rats, an important parameter for assessing antioxidant activity in Parkinson's disease. We prepared brain tissue homogenate and conducted the following tests to evaluate these enzymes (Table 5).

Brain total protein level

Total protein levels in brain tissue are indicative of overall cellular health and function, and they can reflect the neurodegenerative processes occurring in diseases such as Parkinson's disease. Assessing total protein content helps in understanding the extent of neurodegeneration and the efficacy of different treatments.

In our study, we measured the total protein levels in brain tissue homogenate across various groups. The normal control group displayed the highest total protein level at 6.42 ± 0.15 mg/100 mg of tissue, indicating healthy brain tissue. The disease control group exhibited a significantly lower total protein level of 2.36 ± 0.102 mg/100 mg of tissue, reflecting extensive neurodegeneration.

Treatment with reserpine and levodopa increased the total protein level to 3.21 ± 0. mg/100 mg of tissue. Combining this treatment with selegiline further improved the total protein level to 4.41 ± 0.064 mg/100 mg of tissue, showing a significant protective effect.

The groups treated with reserpine, levodopa and METG at different dosages showed varying total protein levels. The reserpine + levodopa + METG (100 mg/kg) group had a total protein level of 3.38 ± 0.033 mg/100 mg of tissue, while the reserpine + levodopa + METG (200 mg/kg) group exhibited a higher level at 3.65 ± 0.163 mg/100 mg of tissue. The reserpine + levodopa + NPMETG (200 mg/kg) group demonstrated the highest total protein level among the treatment groups at 4.46 ± 0.10 mg/100 mg of tissue.

Interestingly, the group treated with reserpine and METG (200 mg/kg) alone had a lower total protein level of 2.83 ± 0.072 mg/100 mg of tissue, underscoring the importance of combined treatments for better neuroprotection (Fig. 10).

Statistical comparison: In each group (n = 6), each value represents Mean ± SEM. One-way ANOVA followed by Dunnett's test was performed. ns denotes nonsignificant *denotes $P < 0.05$ **denotes $P < 0.01$, ***denotes $P < 0.001$ and **** denotes $P < 0.0001$.

Brain dopamine level

Dopamine is a crucial neurotransmitter involved in various brain functions, including motor control and reward mechanisms. Measuring dopamine levels can provide insights into the neurochemical changes associated with neurological disorders such as Parkinson's disease.

In our study, we assessed the dopamine levels in brain tissue homogenate across different treatment groups. The normal control group exhibited the highest dopamine level at 0.5237 ± 0.013 µg/ml,

Table 3 Activity score in actophotometer method

Group	Cutoff Value/Min			
	Day 1	Day 7	Day 14	Day 22
Normal control	89.66 ± 1.52	86 ± 4	86.66 ± 4.16	86.66 ± 4.16
Disease control	93 ± 2	30.33 ± 1.52	36 ± 3	33.33 ± 3.05
Reserpine + levodopa (STD 1)	91.66 ± 4.61	26 ± 3	45.66 ± 4.04	52.66 ± 4.04
Reserpine + levodopa + selegiline (STD 2)	82 ± 4.58	26 ± 3.60	50.33 ± 1.52	77 ± 6.24
Reserpine + levodopa + METG (100 mg/kg) (test 1)	90.66 ± 4.16	34.33 ± 3.78	41 ± 2.64	67 ± 5.29
Reserpine + levodopa + METG (200 mg/kg) (test 2)	87 ± 2.64	29.66 ± 2.51	54 ± 3.60	69 ± 4
Reserpine + levodopa + NPMETG (200 mg/kg) (test 3)	92 ± 7.81	28.66 ± 6.50	58.33 ± 5.03	73.33 ± 5.13
Reserpine + METG (200 mg/kg) (test 4)	85.66 ± 6.42	31 ± 5.19	36.33 ± 3.05	47.33 ± 6.65

indicating healthy dopaminergic function. The disease control group had a significantly lower dopamine level of 0.1922 ± 0.01200 µg/ml, reflecting dopaminergic deficiency.

Treatment with reserpine and levodopa (STD 1) resulted in a moderate increase in dopamine levels to 0.2860 ± 0.01300 µg/ml. Combining this treatment with selegiline (STD 2) significantly improved dopamine levels to 0.4982 ± 0.012 µg/ml, nearing normal control levels.

The groups treated with reserpine, levodopa and METG at different dosages showed varying dopamine levels. The reserpine + levodopa + METG (100 mg/kg) group (test 1) had a dopamine level of 0.3325 ± 0.0099 µg/ml, while the reserpine + levodopa + METG (200 mg/kg) group (test 2) exhibited a higher level at 0.3970 ± 0.013 µg/ml. The reserpine + levodopa + NPMETG (200 mg/kg) group (test 3) demonstrated the highest dopamine level among the treatment groups at 0.4676 ± 0.0125 µg/ml.

Interestingly, the group treated with reserpine and METG (200 mg/kg) alone (test 4) had a lower dopamine level of 0.2682 ± 0.01250 µg/ml, highlighting the importance of combined treatments for better dopaminergic support (Fig. 11).

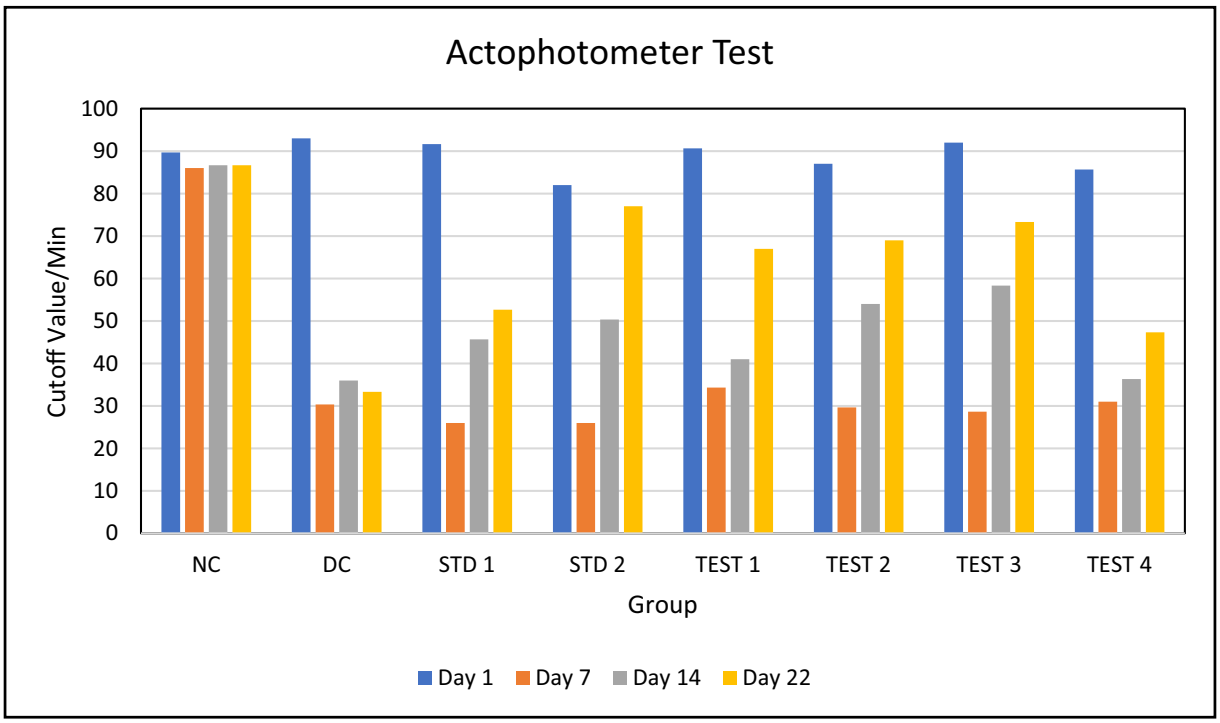


Fig. 8 Result of actophotometer test

Table 4 Mean falloff time using the rotarod method

Group	Time for falling			
	Day 1	Day 7	Day 14	Day 22
Normal control	115.67 ± 5.13	113.33 ± 8.02	109.67 ± 7.57	112.33 ± 12.04
Disease control	139.67 ± 2.08	44.66 ± 2.51	40.66 ± 2.88	46 ± 4.35
Reserpine + levodopa (STD 1)	139.33 ± 2.51	47 ± 2	59.33 ± 1.52	77.33 ± 1..52
Reserpine + levodopa + selegiline (STD 2)	141.33 ± 2.51	41.66 ± 3.05	90.66 ± 2.08	123.33 ± 1.52
Reserpine + levodopa + METG (100 mg/kg) (test 1)	140.66 ± 4.72	42 ± 3.60	75.33 ± 3.51	95.66 ± 2.51
Reserpine + levodopa + METG (200 mg/kg) (test 2)	142.66 ± 0.57	41.33 ± 2.51	81.33 ± 2.51	103.33 ± 2.08
Reserpine + levodopa + NPMETG (200 mg/kg) (test 3)	136.66 ± 4.50	40.33 ± 1.52	88.33 ± 4.04	114.66 ± 3.05
Reserpine + METG (200 mg/kg) (test 4)	141.66 ± 4.50	42.33 ± 3.51	54.33 ± 2.51	71.33 ± 2.51

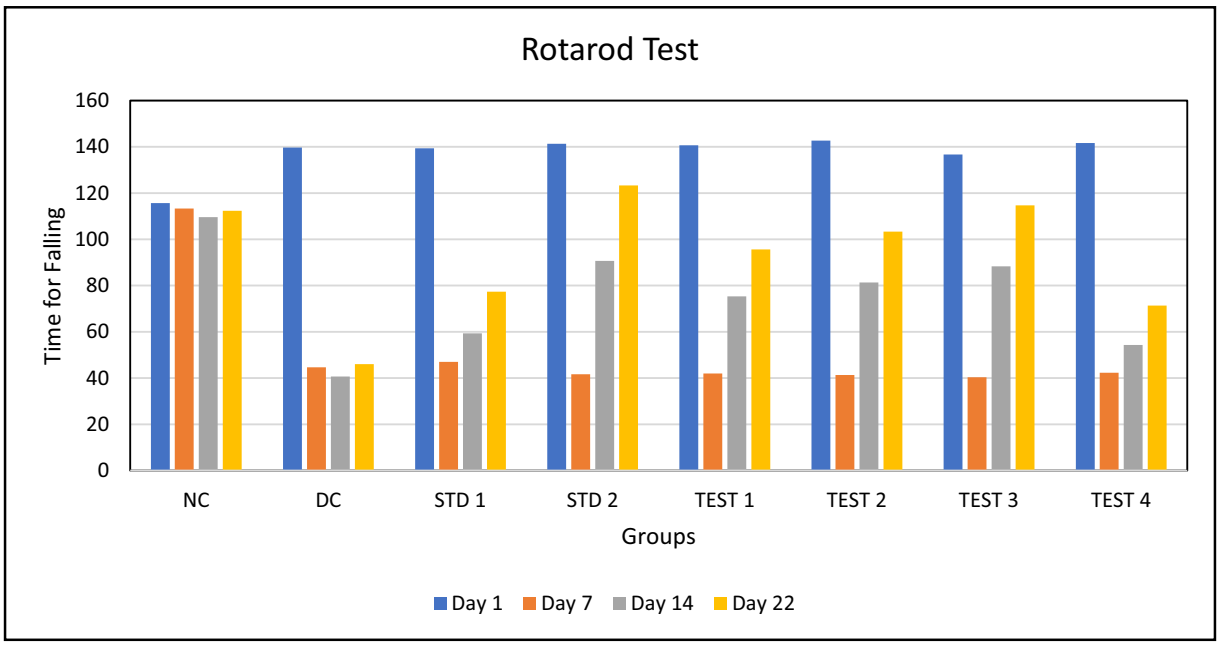


Fig. 9 Result of rotarod test

These findings indicate that combined treatments, especially those including levodopa and selegiline, significantly enhance dopamine levels in the brain, potentially offering better therapeutic outcomes for conditions associated with dopaminergic deficits.

Brain catalase level

Catalase is a crucial antioxidant enzyme that decomposes hydrogen peroxide into water and oxygen, protecting cells from oxidative damage. Evaluating catalase activity helps understand the antioxidant defense mechanisms in the brain.

In our study, we measured catalase activity in brain tissue homogenate across different groups. The normal control group displayed the highest catalase activity at 8.165 ± 0.110 µMole of H2O2 decomposed/min/mg protein, indicating strong antioxidant defense. The disease control group had significantly reduced catalase activity of 4.160 ± 0.1094 µMole of H2O2 decomposed/min/mg protein, reflecting increased oxidative stress.

Treatment with reserpine and levodopa increased catalase activity to 6.011 ± 0.190 µMole of H2O2 decomposed/min/mg protein. Combining this treatment with selegiline further improved catalase activity to 7.290 ± 0.180 µMole of H2O2 decomposed/min/mg protein, nearing the normal control levels.

The groups treated with reserpine, levodopa and METG at different dosages showed varying catalase activities. The reserpine + levodopa + METG (100 mg/kg) group had a catalase activity of 6.368 ± 0.2606 µMole

Table 5 Biochemical parameters of the brain

Group	Total Protein (mg/100 mg of tissue)	Dopamine level (µgm/ml)	Catalase (µMole of H2O2 decomposed/min/mg/ protein)	MDA (nmol of MDA/mg protein)	SOD (U/mg protein)
Normal control	6.42 ± 0.15	0.5237 ± 0.0130	8.165 ± 0.110	6.321 ± 0.67	9.34 ± 0.15
Disease control	2.36 ± 0.10	0.1922 ± 0.0120	4.160 ± 0.109	22.10 ± 1.12	3.56 ± 0.70
Reserpine + levodopa (STD 1)	3.21 ± 0.15	0.2860 ± 0.0130	6.011 ± 0.190	12.27 ± 0.25	6.87 ± 0.34
Reserpine + levodopa + selegiline (STD 2)	4.41 ± 0.06	0.4982 ± 0.0122	7.290 ± 0.180	8.50 ± 0.48	8.45 ± 0.30
Reserpine + levodopa + METG (100 mg/kg) (test 1)	3.38 ± 0.03	0.3325 ± 0.0099	6.368 ± 0.260	10.60 ± 0.50	6.87 ± 0.24
Reserpine + levodopa + METG (200 mg/kg) (test 2)	3.65 ± 0.16	0.3970 ± 0.0134	6.650 ± 0.210	9.98 ± 0.30	7.34 ± 0.21
Reserpine + levodopa + NPMETG (200 mg/kg) (test 3)	4.46 ± 0.10	0.4676 ± 0.0125	6.670 ± 0.174	8.91 ± 0.40	7.89 ± 0.72
Reserpine + METG (200 mg/kg) (test 4)	2.83 ± 0.07	0.2682 ± 0.0125	5.560 ± 0.176	16.04 ± 0.68	5.88 ± 0.32

of H2O2 decomposed/min/mg protein, while the reserpine + levodopa + METG (200 mg/kg) group exhibited slightly higher activity at 6.650 ± 0.210 µMole of H2O2 decomposed/min/mg protein. The reserpine + levodopa + NPMETG (200 mg/kg) group demonstrated catalase activity of 6.670 ± 0.174 µMole of H2O2 decomposed/min/mg protein.

Interestingly, the group treated with reserpine and METG (200 mg/kg) alone had a lower catalase activity of 5.560 ± 0.176 µMole of H2O2 decomposed/min/mg protein, underscoring the importance of combined treatments for better antioxidant defense (Fig. 11).

These results indicate that combined treatments, especially those including levodopa, enhance the antioxidant defense in the brain by increasing catalase activity, thereby potentially mitigating oxidative stress.

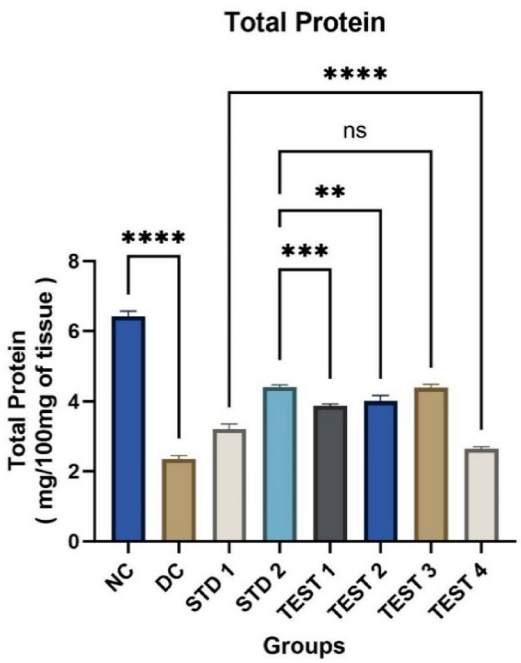


Fig. 10 Total protein estimation

Brain superoxide dismutase (SOD) level

Superoxide dismutase (SOD) is an important antioxidant enzyme that helps protect cells from oxidative damage by catalyzing the conversion of superoxide radicals into oxygen and hydrogen peroxide. Measuring SOD activity provides insight into the oxidative stress status within the brain.

In our study, we evaluated the SOD activity in the brain tissue homogenate of various groups. The normal control group exhibited the highest SOD activity at 9.34 ± 0.15 U/mg protein. In contrast, the disease control group had a significantly lower SOD activity of 3.56 ± 0.70 U/mg protein, indicating increased oxidative stress.

Treatment with reserpine and levodopa resulted in a moderate increase in SOD activity to 6.87 ± 0.34 U/mg protein. Further combination treatments showed different levels of efficacy in restoring SOD activity. The group treated with reserpine, levodopa and selegiline had a SOD activity of 8.45 ± 0.30 U/mg protein, which is close to the normal control group.

The reserpine + levodopa + METG (100 mg/kg) group had a SOD activity of 6.87 ± 0.246 U/mg protein, while the reserpine + levodopa + METG (200 mg/kg) group showed a slight improvement with 7.34 ± 0.21 U/mg protein. The reserpine + levodopa + NPMETG (200 mg/kg) group demonstrated a SOD activity of 7.89 ± 0.72 U/mg protein.

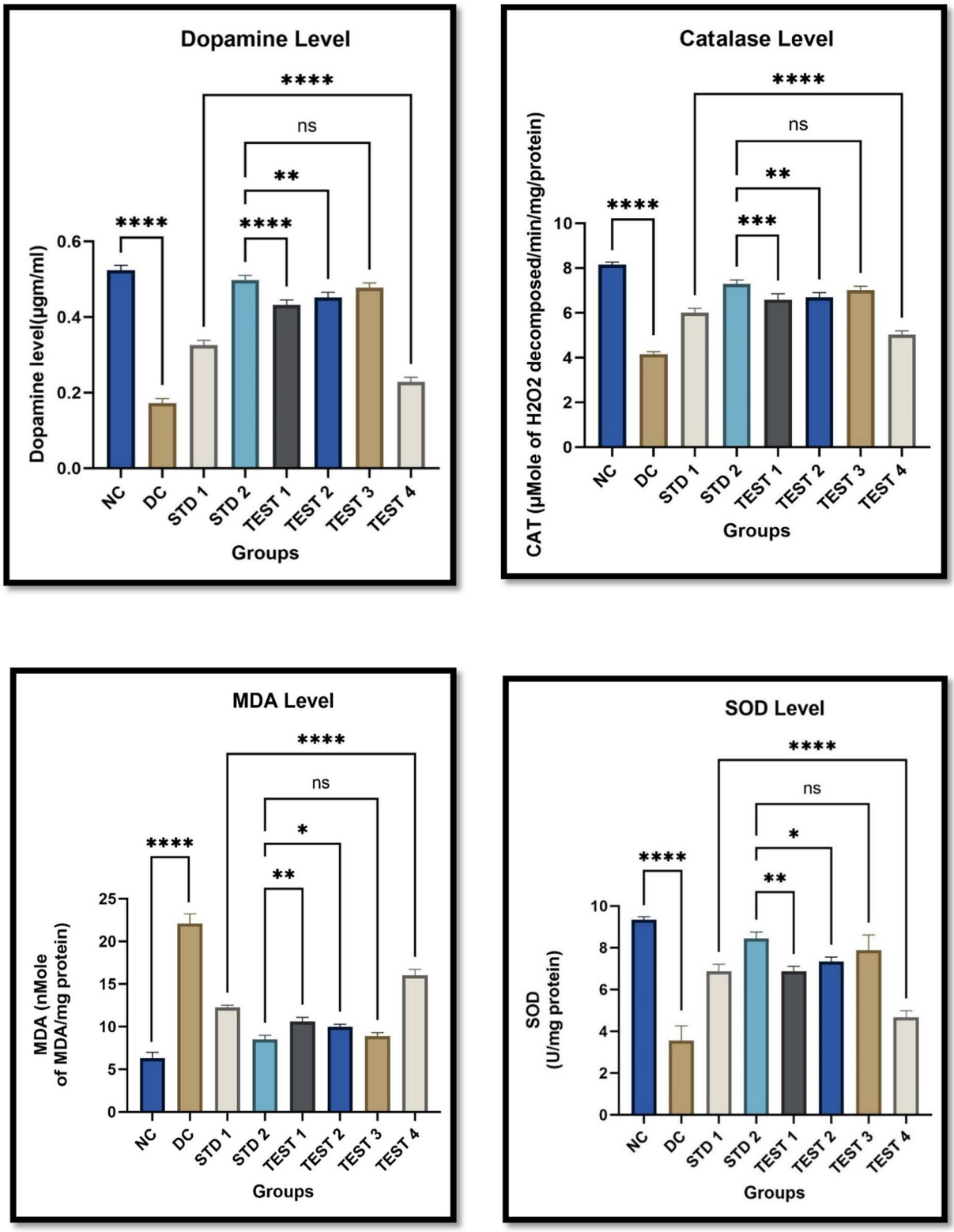


Fig. 11 Result of various biomarkers

Interestingly, the group treated with reserpine and METG (200 mg/kg) alone showed a lower SOD activity of 5.88 ± 0.325 U/mg protein, highlighting the importance of combined treatment for better antioxidant defense (Fig. 11).

These results suggest that combined treatments, particularly those including levodopa, significantly improve the antioxidant defense in the brain by enhancing SOD activity, thereby potentially mitigating oxidative stress.

Brain malondialdehyde level

Lipid peroxidation involves the degradation of lipid membranes by free radicals, initiating a chain reaction that produces malondialdehyde (MDA), a harmful end product that damages biomolecules and induces oxidative stress. In the lipid peroxidation assay, MDA reacts with thiobarbituric acid (TBA) to form TBARS, which are measured to determine the level of lipid peroxidation or MDA levels.

In our study, we evaluated several groups and their MDA levels in brain tissue homogenate. The normal control group had an MDA level of 6.321 ± 0.67 nMole/mg protein. The disease control group showed a significantly higher MDA level at 22.10 ± 1.12 nmol/mg protein. Treatment with reserpine and levodopa resulted in a reduction of MDA to 12.27 ± 0.25 nmol/mg protein.

Further combination treatments with selegiline, METG and NPMETG showed varying levels of effectiveness in reducing MDA levels. The reserpine + levodopa + selegiline group had an MDA level of 8.50 ± 0.48 nMole/mg protein. The reserpine + levodopa + METG (100 mg/kg) group had an MDA level of 10.60 ± 0.50 nMole/mg protein, while the reserpine + levodopa + METG (200 mg/kg) group had a slightly lower level of 9.98 ± 0.30 nMole/mg protein. The reserpine + levodopa + NPMETG (200 mg/

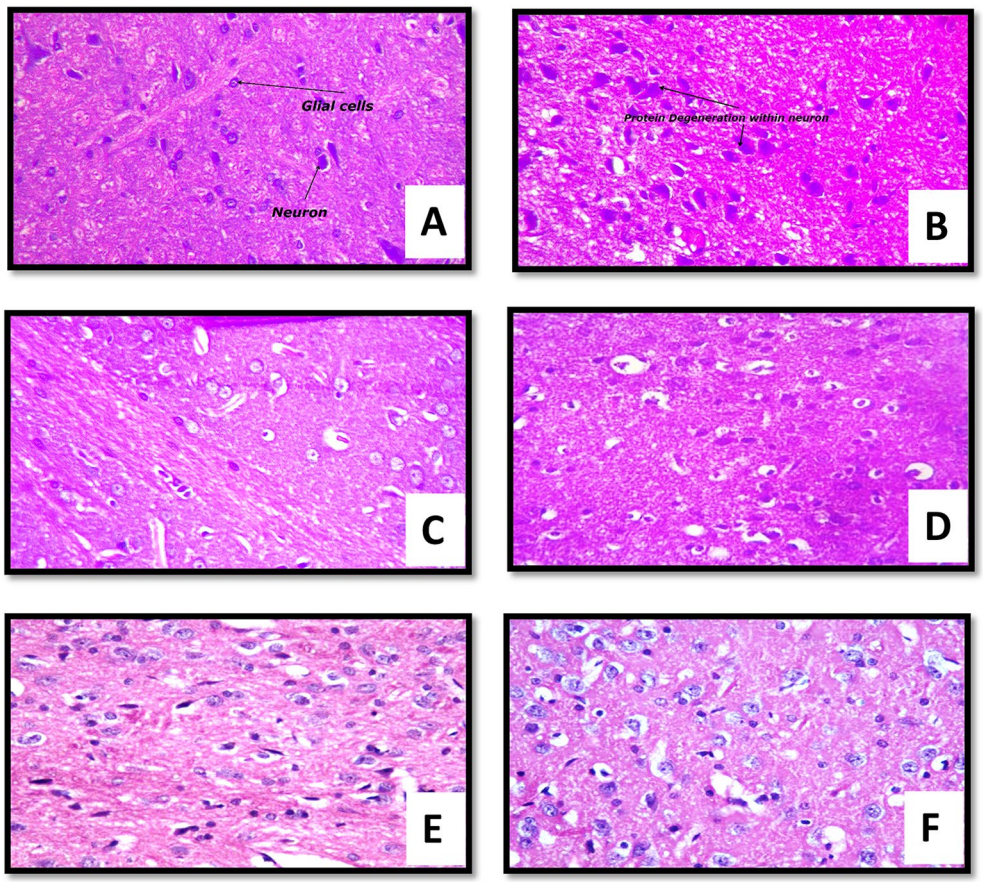


Fig. 12 Histopathological examination of mid brain sections stained with Hematoxylin and Eosin. *Figure A* shows healthy midbrain regions without any signs of oxidative stress or neuronal degeneration. *Figure B* shows the effects of reserpine treatment, which induces Parkinson-like symptoms. *Figure C* represents the group treated first with reserpine to induce Parkinson-like symptoms and then with levodopa and selegiline. *Figure D* shows the group treated with reserpine followed by a combination of levodopa and a 100 mg dose of *Tamarix gallica* extract. *Figure E* shows the group treated with reserpine and then with levodopa and a 200 mg dose of *Tamarix gallica* extract. The protective effects should be more significant than the 100 mg dose *Figure F* represents the group treated with reserpine and then with a nanosuspension of a 200 mg dose of *Tamarix gallica* extract, showing significant protection against the damage induced by reserpine

kg) group showed an MDA level of 8.91 ± 0.40 nmol/mg protein.

Interestingly, the group treated with reserpine and METG (200 mg/kg) without levodopa had a higher MDA level of 16.04 ± 0.68 nmol/mg protein. These results indicate that combining reserpine and levodopa with other compounds can significantly reduce lipid peroxidation, as evidenced by lower MDA levels in the brain tissue homogenate (Fig. 11).

Histopathology (Fig. 12)

Conclusion

The results of the current study suggest that *Tamarix gallica* is effective in improving Parkinson's symptoms and has neuroprotective and antioxidant effects.

Abbreviations

PD	Parkinson's disease
TG	*Tamarix gallica*
MDA	Malondialdehyde
TBA	Thiobarbituric acid
SOD	Superoxide dismutase
LPO	Lipid peroxidation
MAO-B	Monoamine oxidase B
DPPH	2,2-Diphenyl-1-picrylhydrazyl
CCSEA	Committee of Control And Supervision of Experiments on Animals
METG	Methanolic extract of *Tamarix gallica*
DA	Dopamine

Acknowledgements

Not applicable

Author contributions

All authors have studied the final manuscript for communication. The authors were responsible for study conceptualization, complete literature search, protocol development, data gathering, statistical analysis and development of the manuscript. The authors have read and approved the final version of the manuscript.

Funding

We would like to express our gratitude to the Student Startup Innovation Policy (SSIP) for funding and supporting our research work.

Availability of data and materials

The data used and analyzed during the present study are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

In this study, we followed the CCSEA (Committee of Control And Supervision of Experiments on Animals) guidelines for animal handling. Approval for all animal procedures was granted by the institutional Ethics Committee at the School of Pharmacy, RK University, with reference no. RKCP/COL/RE/23/135, on December 30, 2023. The experiments followed authorized protocols and the Guide for Care and Use of Laboratory Animals as per CCSEA guidelines.

Consent for publication

Not applicable.

Competing interests

The authors have no competing interests.

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Received: 26 June 2024 Accepted: 16 March 2025
Published online: 23 April 2025

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