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Neuroprotective potential of *Vicia faba* seed extract in haloperidol-induced Parkinsonian rats

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Parkinsonian disorders are characterised by dopaminergic depletion and motor dysfunction, prompting growing interest in plant-derived dopamine precursors as supportive therapies. Although *Vicia faba* L. seeds are known to contain L-DOPA, evidence linking standardised extracts to functional neurobehavioral improvement, together with demonstrated central dopaminergic activity, remains limited. This study aimed to evaluate the neurobehavioral efficacy of a standardised hydro-alcoholic *V. faba* seed extract and assess its dopaminergic contribution in a haloperidol-induced Parkinsonian rat model. Extract-treated animals showed progressive improvements in paw withdrawal latency, locomotor activity, and exploratory behaviour across treatment levels compared with disease controls, with paw withdrawal time showing strong statistical significance ($p < 0.001$) and locomotor parameters also demonstrating statistically significant improvement across treatment groups. Chromatographic profiling indicated dopaminergic constituents, while brain tissue chromatographic profiles suggested the presence of centrally available levodopa-like constituents following treatment. By integrating behavioural findings with phytochemical and neurochemical evidence, this study supports the functional dopaminergic activity of *V. faba* extract beyond simple confirmation of its L-DOPA content and highlights its potential as a plant-derived adjunct for Parkinsonian symptom management. Further studies are needed to identify active constituents, clarify pharmacokinetics, and evaluate translational therapeutic relevance.

Keywords: Drug-induced parkinsonism, Levodopa, Neurobehavioral assessment, Parkinson's disease, RP-HPLC, *Vicia faba*

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Introduction

Parkinson's disease (PD) is the second most common neurodegenerative disorder worldwide, affecting more than 10 million individuals according to recent global estimates (2023–2024). It is clinically characterised by progressive motor impairment, including bradykinesia, resting tremor, rigidity, and postural instability, primarily resulting from degeneration of dopaminergic neurons in the substantia nigra pars compacta and subsequent depletion of striatal dopamine[1,2]. In addition to dopaminergic loss, PD involves complex pathological processes such as mitochondrial dysfunction, oxidative stress, neuroinflammation, α-synuclein aggregation, and impaired cellular clearance mechanisms, highlighting its multifactorial etiology[3,4].

Levodopa (L-DOPA) remains the gold standard for symptomatic management of PD because of its ability to cross the blood–brain barrier and restore central dopamine levels[5]. Although highly effective during early disease stages, long-term therapy frequently leads to complications including dyskinesias, motor fluctuations, and reduced therapeutic responsiveness, creating a need for adjunctive strategies that improve efficacy while minimising adverse outcomes[6,7].

Plant-derived dopaminergic sources have attracted increasing attention as potential complementary approaches in PD management[8]. Among these, *Vicia faba* L. (broad bean or fava bean) is recognised as a natural source of L-DOPA and has been traditionally consumed in various regions for neurological benefits[9,10]. In addition to levodopa, *V. faba* seeds contain flavonoids, polyphenols, and micronutrients with reported antioxidant and anti-inflammatory properties, suggesting possible neuroprotective effects beyond dopamine replacement[11,12]. However, variability in phytochemical composition due to cultivar differences, cultivation conditions, and processing methods underscores the need for standardised preparations when evaluating therapeutic potential[13].

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Safety considerations, such as the risk of hemolysis in individuals with glucose-6-phosphate dehydrogenase deficiency, also warrant controlled investigation[14].

Although the presence of L-DOPA in *V. faba* and its potential neuroprotective effects have been documented, most prior studies have focused on dietary intake, biochemical detection, or observational outcomes rather than examining standardised extracts in controlled experimental Parkinsonian models[9,15]. Importantly, few investigations have integrated behavioural assessment with phytochemical characterisation and evidence suggestive of central dopaminergic activity following administration of plant-derived preparations[16].

Therefore, the present study was designed to evaluate the neurobehavioral effects of a standardised hydro-alcoholic *V. faba* seed extract in a haloperidol-induced Parkinsonian rat model and to determine whether these effects are associated with indicators of dopaminergic activity. By linking behavioural outcomes with phytochemical and neurobiological evidence, this study aims to clarify the functional relevance of plant-derived dopaminergic support strategies and contribute to the development of standardised botanical adjuncts for Parkinsonian symptom management.

Materials and Methods

Materials and Chemicals

Dried *V. faba* seeds were obtained from online nurseries and authenticated by a qualified botanist prior to use. Ethanol was used for extract preparation, while methanol was employed for compound detection via UV–Visible spectrophotometry. Active pharmaceutical ingredients (APIs), including Levodopa and Carbidopa, were purchased from S.D. Fine Chemicals, Mumbai, along with all other necessary solvents and reagents.

Animals

Male Wistar rats (200 ± 25 g, n = 15) were housed under controlled environmental conditions with regulated temperature (21°C), humidity (55%), and a 12-hour light/dark cycle. The animals were randomly assigned to five experimental groups and provided ad libitum access to standard laboratory feed and water. Prior to the initiation of experimental procedures, they underwent a three-week acclimatisation period to ensure physiological and behavioural adaptation to the housing conditions. All experimental protocols were reviewed and approved by the Institutional Animal Ethics Committee (07/IAEC/CLPT/2024-25), ensuring compliance with ethical guidelines for animal research.

Experimental design

Haloperidol produces Parkinsonian-like motor impairment by blocking striatal dopamine D_2 receptors, thereby disrupting dopaminergic signalling and shifting the basal ganglia balance toward cholinergic overactivity, a neurochemical state known to generate rigidity, bradykinesia, and cataleptic responses in experimental models[17-19].

The animals were randomly assigned to five experimental groups. Group I (Disease Control) received Haloperidol (1 mg/kg, intraperitoneally) for one week. Group II (Standard) received Haloperidol (1 mg/kg, intraperitoneally) for one week followed by oral administration of Syndopa (Levodopa 20 mg/kg and Carbidopa 2 mg/kg). Group III (Test-1), Group IV (Test-2), and Group V (Test-3) received Haloperidol (1 mg/kg, intraperitoneally) for one week followed by oral administration of 2 mL, 3.5 mL, and 5 mL of *V. faba* seed extract, respectively. The extract volumes were selected based on preliminary exploratory observations and commonly reported phytopharmacological ranges, ensuring that the administered levels were safe, pharmacologically relevant, and appropriate for evaluating graded exposure across treatment groups.

Preparation of *V. faba* seed extract

Thirty-five grams of *V. faba* seeds were soaked in water for 24 hours to soften the outer layers and facilitate the removal of the seed coats. The peeled seeds were triturated into a uniform paste using a mortar and pestle. The paste was extracted using a hydro-alcoholic solvent system (ethanol: water = 8:2). The ethanol–water ratio was selected based on established phytochemical extraction principles, as moderately polar hydro-alcoholic mixtures are known to enhance the recovery of catechol-type constituents such as L-DOPA while preserving extract stability[20]. The pH of the mixture was adjusted to 8 using potassium hydroxide (KOH), and the preparation was allowed to stand for 24 hours to facilitate the extraction of soluble constituents. The mixture was then centrifuged at 4000 rpm for 20 minutes to separate the supernatant from solid residues, and the resulting supernatant was used for subsequent experimental analyses.

UV spectrophotometric analysis

UV–visible spectrophotometry was employed as a preliminary screening technique to assess the presence of catechol-type chromophores in the extract by comparison with catechol-containing reference standards (Levodopa and Carbidopa). Standard solutions of Levodopa and Carbidopa (10 mg/10 mL methanol) were scanned between 200 and 400 nm to obtain comparative spectra, and the extract was diluted appropriately and scanned under identical conditions. Because catechol derivatives in plant matrices display overlapping absorption bands in the UV region, this method was considered non-selective for differentiating individual compounds[21]. Accordingly, spectral observations were interpreted qualitatively and used only to support subsequent chromatographic evaluation rather than to confirm compound identity.

Brain extraction procedure

Brain extraction was performed in accordance with institutional ethical guidelines. Following euthanasia, the skull was dissected using sterilised instruments to expose the cranial cavity. The brain was carefully removed, preserving critical regions such as the cerebellum and brainstem. Immediately post-extraction, the brain was immersed in cold saline or phosphate-buffered saline (PBS) and kept on ice to maintain tissue integrity and prevent enzymatic degradation. The tissue was then trimmed and processed for subsequent experimental analyses.

Buffer preparation for mobile phase

The mobile phase buffers were prepared using phosphate and ortho-phosphoric acid solutions along with methanol. For the phosphate buffer, 68 mg of potassium dihydrogen orthophosphate (KH_2PO_4) was dissolved in 500 mL of HPLC-grade water, sonicated for 10 minutes, adjusted to pH 3 with orthophosphoric acid, filtered through a 0.45-micron membrane, and sonicated again to ensure uniformity. Methanol was used as received. The ortho phosphoric acid buffer was prepared by dissolving 0.2 mL of ortho phosphoric acid in 200 mL of HPLC-grade water, followed by 10-minute sonication in a water bath, filtration through a 0.45-micron membrane, and further sonication to maintain consistency. These buffers were utilised in the mobile phase for RP-HPLC analysis.

RP-HPLC analysis

Chromatographic analysis was performed using reverse-phase high-performance liquid chromatography (RP-HPLC) to compare retention characteristics of Levodopa and Carbidopa standards with those observed in plant extracts and biological samples. Separation was carried out on a C18 column maintained at 40°C using a mobile phase of methanol and potassium dihydrogen phosphate buffer (50:50, v/v) at a flow rate of 1.0 mL/min. Samples were filtered prior to injection and analysed under identical chromatographic conditions. The procedure was applied for comparative profiling and approximate estimation rather than for full quantitative bioanalytical validation[22].

Assessment of animal activity

To evaluate the therapeutic efficacy of treatments in alleviating Parkinsonian symptoms, a series of behavioural and motor activity tests was employed.

Catalepsy test

Paw Withdrawal Time (PWT) was recorded to assess motor rigidity. Catalepsy was assessed using the box test, in which each rat's forepaws were placed on a horizontal box positioned approximately 9 cm above the surface. The duration for which the imposed posture was maintained was recorded as paw withdrawal time, with shorter durations indicating reduced rigidity[23].

Actophotometer

Locomotor activity was assessed by recording the number of infrared beam interruptions over a 5-minute observation period[24].

Elevated plus maze

Anxiety-related behaviour was evaluated by measuring time spent in open and closed arms during a 5-minute trial[25].

IR Actimeter

Spontaneous locomotor and exploratory behaviour were quantified through beam-break counts across defined quadrants[26].

Data analysis

Statistical analyses were performed using GraphPad Prism software (version 10.4.2, La Jolla, CA, USA). All data are expressed as mean ± standard deviation (SD). One-way analysis of variance (ANOVA) was employed to evaluate differences between groups, followed by Tukey's Honest Significant Difference (HSD) post hoc test for multiple comparisons. A p-value of less than 0.05 was considered statistically significant. Graphs illustrating group-wise comparisons, error bars, and statistical annotations were also generated using the same software to facilitate interpretation of the results. Statistical significance was denoted in tables and figures using asterisk notation, while exact p-values are reported in the text where relevant.

Table 1 — Paw Withdrawal Time (Mean ± SD) across Treatment Groups

Condition	Disease Control	Standard	Test-1	Test-2	Test-3
03cm_30min	23.67 ± 4.73	8.00 ± 1.00***	14.67 ± 2.08**	12.33 ± 0.58**	8.33 ± 0.58***
03cm_60min	21.00 ± 4.36	7.67 ± 1.53***	15.33 ± 0.58**	12.00 ± 1.00**	7.67 ± 1.53***
03cm_90min	24.00 ± 4.00	6.33 ± 1.53***	15.67 ± 1.53**	11.00 ± 1.00**	7.00 ± 1.00***
09cm_30min	24.00 ± 3.61	14.67 ± 0.58***	27.33 ± 2.08	17.33 ± 2.31**	14.00 ± 1.00***
09cm_60min	25.67 ± 1.15	12.33 ± 0.58***	23.00 ± 2.65	18.33 ± 0.58**	13.67 ± 1.53***
09cm_90min	25.00 ± 1.73	12.33 ± 1.53***	21.67 ± 2.08	17.67 ± 1.15**	13.00 ± 1.00***

[Significance levels: *p < 0.05, **p < 0.01, ***p < 0.001 compared with Disease Control (ANOVA followed by Tukey's test).]

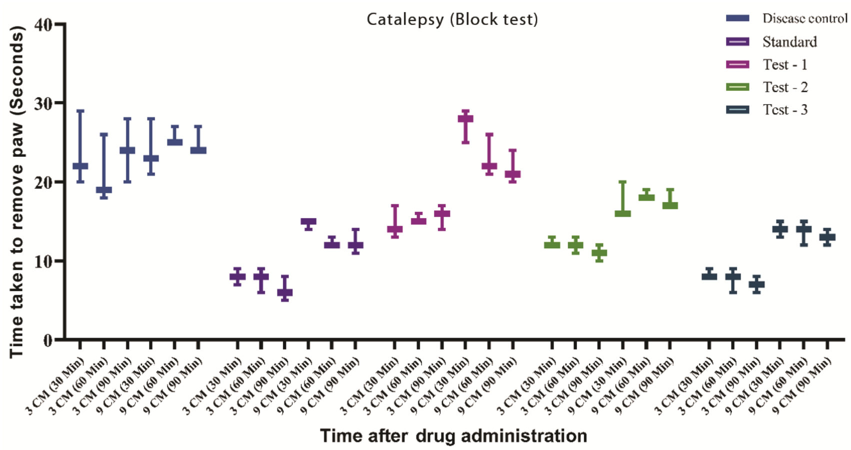


Fig. 1 — Graph for paw withdrawal time (Mean ± SD) across treatment groups.

Results

Assessment of anti-cataleptic activity using paw withdrawal time

The catalepsy test was conducted to evaluate motor function impairment through measurement of Paw Withdrawal Time (PWT). A decrease in PWT indicates improved motor coordination and reduced cataleptic symptoms.

The Disease Control group consistently exhibited the highest PWT values, confirming a sustained cataleptic state (Table 1 and Fig. 1). Treatment with the Standard reference drug resulted in a significant reduction in PWT across all time points, reflecting moderate anti-cataleptic activity. Among the test formulations, Test-3 demonstrated the most pronounced reduction in PWT, with values consistently lower than those observed in Test-1 and Test-2, and comparable to or better than the Standard group. These findings suggest Test-3 exhibits superior anti-cataleptic efficacy.

The differences among the groups were statistically significant in all experimental conditions, as evidenced by one-way ANOVA p-values < 0.0001. Post hoc Tukey's tests suggested that the reduction in PWT in Test-3 was statistically significant (p <0.05) when compared with the Disease Control and Test-1 groups in multiple conditions.

Locomotor activity using the actophotometer

The INCO Actophotometer quantifies locomotor activity in rodents using infrared beams or photoelectric sensors. Each movement interrupts the beams, triggering signals processed by a data acquisition system, providing precise and reproducible assessments for behavioural and neurological research.

Beam breaks reflect locomotor activity, where higher values indicate greater motor function. Across all time points, the Disease Control group showed the lowest activity, while the Standard and Test-3 groups showed significantly higher beam breaks, suggesting enhanced motor recovery or reduced catalepsy (Table 2 and Fig. 2).

One-way ANOVA showed statistically significant differences (p <0.001) among all treatment groups at each time point. Test-3 consistently demonstrated beam break counts close to or exceeding the Standard group, suggesting it is the most effective in restoring motor activity among the test formulations.

Table 2 — Number of Beam Breaks in 5 Minutes (Mean ± SD)

Time	Disease Control	Standard	Test-1	Test-2	Test-3
30 min	77.00 ± 5.00	130.00 ± 4.36***	82.33 ± 1.53	97.33 ± 4.16**	126.00 ± 6.24***
60 min	81.33 ± 14.22	148.00 ± 18.33***	95.67 ± 2.08*	109.33 ± 4.04**	142.33 ± 8.96***
90 min	79.67 ± 4.04	165.00 ± 18.08***	93.67 ± 4.16*	113.33 ± 4.73**	161.67 ± 12.22***

[Values are expressed as Mean ± SD. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. Significance levels: *p <0.05, **p <0.01, ***p <0.001 compared with the Disease Control group.]

Table 3 — Elevated Plus Maze – Duration in Arms (Mean ± SD, Minutes)

Condition	Disease Control	Standard	Test-1	Test-2	Test-3
Open – 30 min	1.00 ± 0.00	1.67 ± 0.58	1.33 ± 0.58	1.33 ± 0.58	1.00 ± 0.00
Open – 60 min	1.33 ± 0.58	1.67 ± 0.58	1.67 ± 0.58	1.33 ± 0.58	1.67 ± 0.58
Open – 90 min	1.00 ± 0.00	2.33 ± 0.58*	1.33 ± 0.58	1.33 ± 0.58	2.33 ± 0.58*
Closed – 30 min	4.00 ± 0.00	3.33 ± 0.58	3.67 ± 0.58	3.67 ± 0.58	4.00 ± 0.00
Closed – 60 min	3.67 ± 0.58	3.33 ± 0.58	3.33 ± 0.58	3.67 ± 0.58	3.33 ± 0.58
Closed – 90 min	4.00 ± 0.00	2.67 ± 0.58*	3.67 ± 0.58	3.67 ± 0.58	2.67 ± 0.58*

[Values are expressed as Mean ± SD. Statistical comparisons were performed using one-way ANOVA followed by Tukey's post hoc test. * *p*< 0.05 vs. Disease Control.]

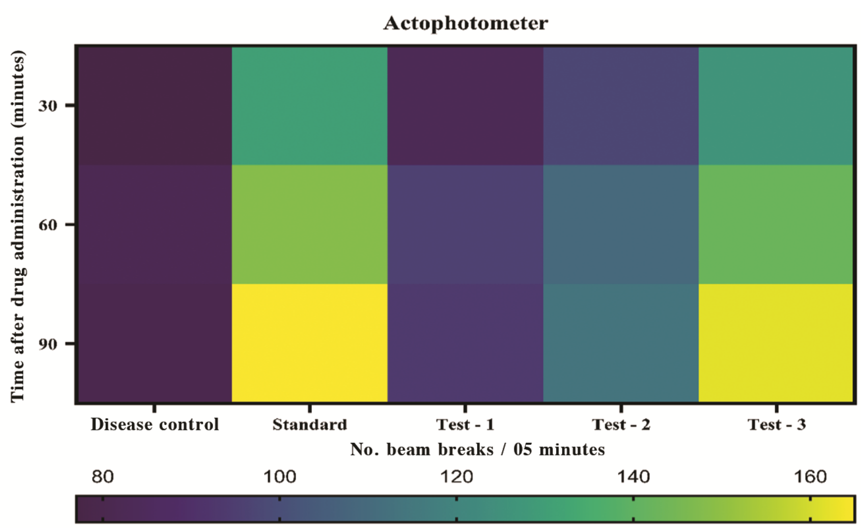


Fig. 2 — Graph for number of beam breaks in 5 minutes (Mean ± SD).

Anxiety activity using the elevated plus maze

In the elevated plus maze, increased time spent in open arms indicates reduced anxiety-like behaviour, while more time in closed arms suggests higher anxiety levels.

At the 90-minute mark (Table 3 and Fig. 3), significant differences were observed among groups (p = 0.0266) in both open and closed arm durations. Specifically, the Standard and Test-3 groups spent more time in open arms and less time in closed arms, suggesting an anxiolytic effect. No statistically significant differences were found at earlier time points (30 and 60 minutes), indicating that the anxiolytic-like effect emerged primarily at the later observation period.

Locomotor activity using the IR actimeter

The locomotor activity of animals was assessed at 30-, 60-, and 90-minute intervals using beam break counts across four quadrants, providing insight into motor coordination, exploratory behaviour, and drug efficacy.

Locomotor activity and post hoc statistical comparisons

The results, expressed as mean ± standard deviation, demonstrated clear group-wise differences over time. At 30 minutes, the Standard group exhibited the highest total activity (92.33 ± 7.24), followed closely by the Test-3 group (87.67 ± 17.53), both indicating robust and distributed locomotor engagement. Test-1 and Test-2 showed moderate activity levels (37.33 ± 45.38 and 50.33 ± 25.08,

Table 4 — Motor Activity Across All Quadrants (Beam Breaks per 5 Minutes)

Time Point	Group	Quadrant 1	Quadrant 2	Quadrant 3	Quadrant 4	Total Activity (Mean ± SD)
30 Min	Disease Control	8.00 ± 6.08	19.67 ± 17.10	0.00 ± 0.00	8.67 ± 15.01	36.34 ± 38.19
	Standard	15.00 ± 1.00	23.00 ± 1.00	27.00 ± 1.73	27.33 ± 3.51	92.33 ± 7.24
	Test-1	8.00 ± 6.08	13.33 ± 11.59	7.67 ± 13.28	8.33 ± 14.43	37.33 ± 45.38
	Test-2	12.00 ± 2.00	10.33 ± 8.96	21.00 ± 2.00	7.00 ± 12.12	50.33 ± 25.08
	Test-3	16.33 ± 2.08	21.33 ± 2.52	25.33 ± 2.52	24.67 ± 10.41	87.67 ± 17.53
60 Min	Disease Control	13.00 ± 4.58	11.00 ± 19.05	0.00 ± 0.00	16.33 ± 14.36	40.33 ± 37.99
	Standard	22.67 ± 2.08	17.33 ± 15.01	29.33 ± 1.53	21.67 ± 18.77	91.00 ± 37.39
	Test-1	17.67 ± 5.13	8.67 ± 15.01	0.00 ± 0.00	18.67 ± 16.20	45.00 ± 36.34
	Test-2	18.00 ± 3.61	6.00 ± 10.39	17.00 ± 15.13	17.67 ± 15.31	58.67 ± 44.44
	Test-3	20.33 ± 3.21	22.00 ± 8.89	29.67 ± 1.15	17.33 ± 15.82	89.33 ± 29.07
90 Min	Disease Control	27.00 ± 2.00	10.67 ±18.48	0.00 ± 0.00	20.00 ± 17.35	57.67 ± 37.83
	Standard	27.00 ± 2.00	30.67 ± 1.53	33.67 ± 3.51	24.67 ± 21.46	116.00 ± 28.50
	Test-1	20.67 ± 5.86	9.00 ± 15.59	0.00 ± 0.00	20.00 ± 17.35	49.67 ± 38.80
	Test-2	22.67 ± 4.16	7.00 ± 12.12	27.00 ± 3.46	19.33 ± 16.74	76.00 ± 36.49
	Test-3	24.33 ± 3.21	18.33 ± 15.95	32.67 ± 1.15	21.67 ± 19.09	97.00 ± 39.40

[Values represent mean ± standard deviation (SD) of locomotor activity measured as infrared beam breaks per 5-minute interval. Quadrant values indicate activity distribution within the arena, and total activity represents the sum of beam breaks across all quadrants at each time point.]

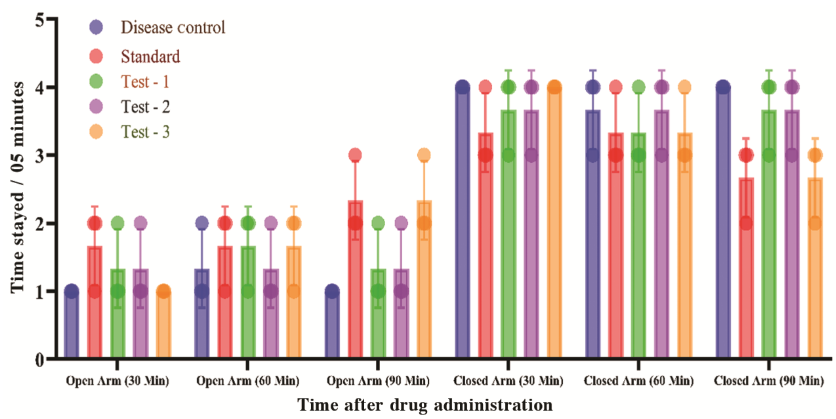


Fig. 3 — Elevated plus maze – duration in arms (Mean ± SD, Minutes).

respectively), while the Disease Control group demonstrated the lowest activity (36.34 ± 38.19), suggesting motor impairment (Table 4 and Fig. 4).

At 60 minutes, the Standard (91.00 ± 37.39) and Test-3 (89.33 ± 29.07) groups maintained high activity, implying sustained motor enhancement. Test-2 showed a notable improvement (58.67 ± 44.44), while Test-1 (45.00 ± 36.34) and Disease Control (40.33 ± 37.99) remained low.

By 90 minutes, the Standard group reached peak activity (116.00 ± 28.50), with Test-3 also achieving a high value (97.00 ± 39.40). Test-2 continued to improve (76.00 ± 36.49), whereas Test-1 (49.67 ± 38.80) and Disease Control (57.67 ± 37.83) remained lower, indicating persistent deficits or weaker motor stimulation.

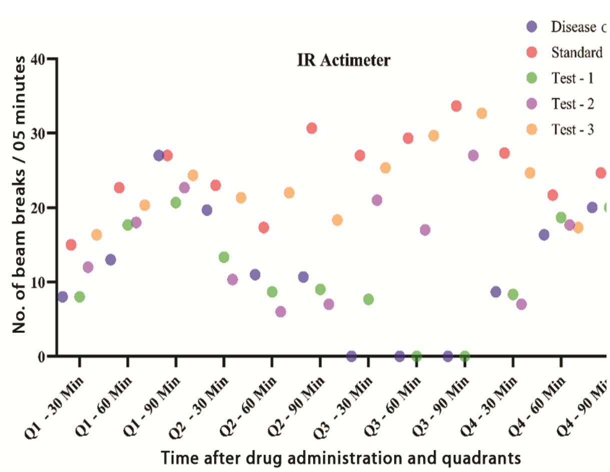


Fig. 4 — Motor activity across all quadrants (Beam breaks per 5 minutes).

Post Hoc analysis: One-way ANOVA with multiple comparisons

To statistically validate these observations, one-way ANOVA followed by post hoc comparisons was performed. Pairwise comparisons indicated differences in locomotor activity among treatment groups (Table 5), with the Standard and Test-3 groups showing higher activity compared with the Disease Control and Test-1/Test-2 groups. Disease Control animals demonstrated comparatively lower locomotor activity than treated groups, consistent with the expected pharmacological effects of the interventions. Comparisons between Test-3 and Test-1/Test-2 showed relatively larger mean differences (25.00 and 24.00), indicating greater improvement in locomotor performance with Test-3. In contrast, although the Standard vs. Test-3 comparison was statistically significant (mean difference = 4.00), the smaller magnitude of difference suggests comparable performance between these groups. Overall, these findings indicate that among the test formulations, Test-3 consistently enhanced locomotor activity and showed effects comparable to the Standard treatment across time points, supporting its potential utility for improving motor function in this experimental model.

Table 5 — Pairwise Comparisons Between Treatment Groups for Total Motor Activity

Group Comparison	Mean Difference	Significance
Disease Control vs Standard	39.66	***
Disease Control vs Test-1	18.66	***
Disease Control vs Test-2	19.66	***
Disease Control vs Test-3	43.66	***
Standard vs Test-1	-21.00	***
Standard vs Test-2	-20.00	***
Standard vs Test-3	4.00	***
Test-1 vs Test-2	1.00	***
Test-1 vs Test-3	25.00	***
Test-2 vs Test-3	24.00	***

[***p ≤0.001 pairwise comparisons between treatment groups (one-way ANOVA followed by post hoc testing)]

UV Spectrophotometric analysis of Levodopa and Carbidopa

UV spectrophotometric analysis was conducted to characterise the absorbance profiles of reference standards of Levodopa and Carbidopa and to compare them with those observed in *V. faba* seed extracts. Standard solutions of each compound (10 mg/10 mL in methanol) were scanned across the range of 200–400 nm using a LABINDIA UV3092 spectrophotometer.

Levodopa showed a characteristic absorption peak near 272 nm, while Carbidopa exhibited a peak near 271 nm. The seed extract displayed absorption within the same spectral region, indicating the presence of UV-absorbing constituents with catechol-type chromophores. Representative spectra are shown in Figs. 5 and 6.

A wavelength near 282 nm was observed within the overlapping spectral region of Levodopa and Carbidopa (Fig. 7). This region reflects spectral overlap between the two standards and was considered useful for qualitative comparison of their absorbance behaviour rather than for quantitative simultaneous estimation.

RP-HPLC detection of Levodopa and Carbidopa

The detection of Levodopa and Carbidopa was performed using reverse-phase high-performance liquid chromatography (RP-HPLC) in both plant extracts and rat brain homogenates. Chromatography was conducted on a Kinetex EVO C18 analytical column (50 mm × 4.6 mm, 5 µm) at 40°C, utilising a hydrophobic stationary phase to optimise separation.

The mobile phase consisted of methanol and 10 mM potassium dihydrogen phosphate (50:50, v/v),

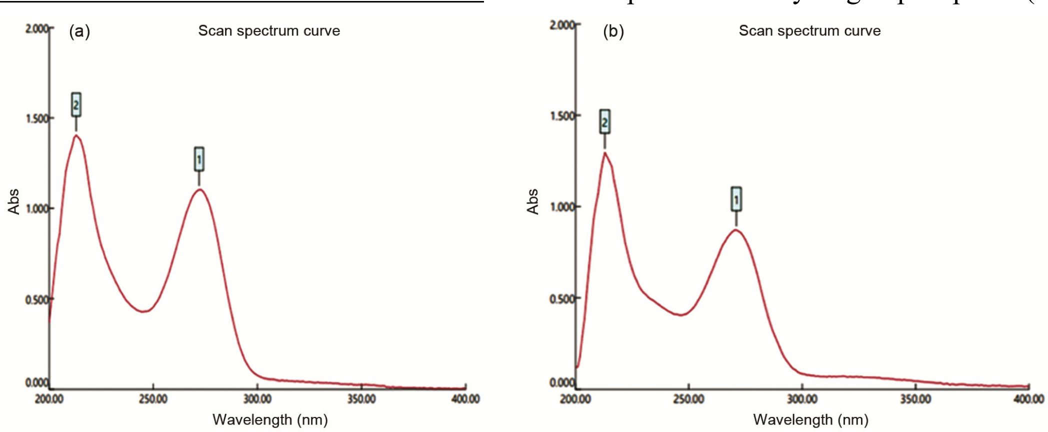


Fig. 5 — UV absorbance spectrum of (a) Carbidopa standard compared with the (b) *V. faba* seed extract, illustrating spectral overlap within the catechol absorption region.

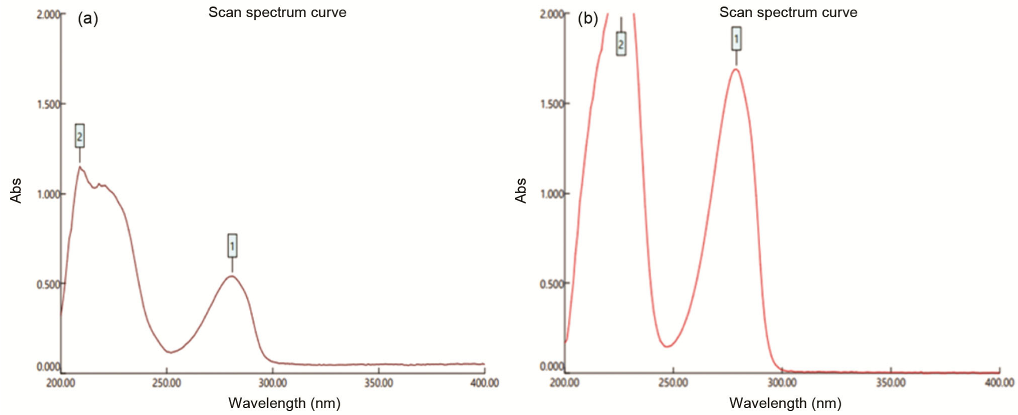


Fig. 6 — UV absorbance spectrum of (a) Levodopa standard compared with the (b) *V. faba* seed extract, showing characteristic catechol-type absorption behaviour.

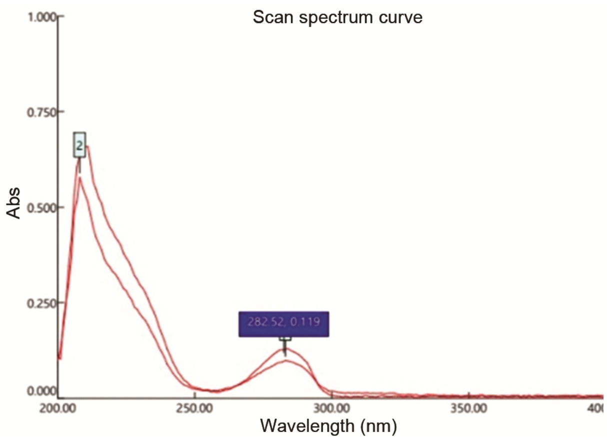


Fig. 7 — Overlapping UV spectra of Levodopa and Carbidopa standards showing a region of spectral convergence near 282 nm.

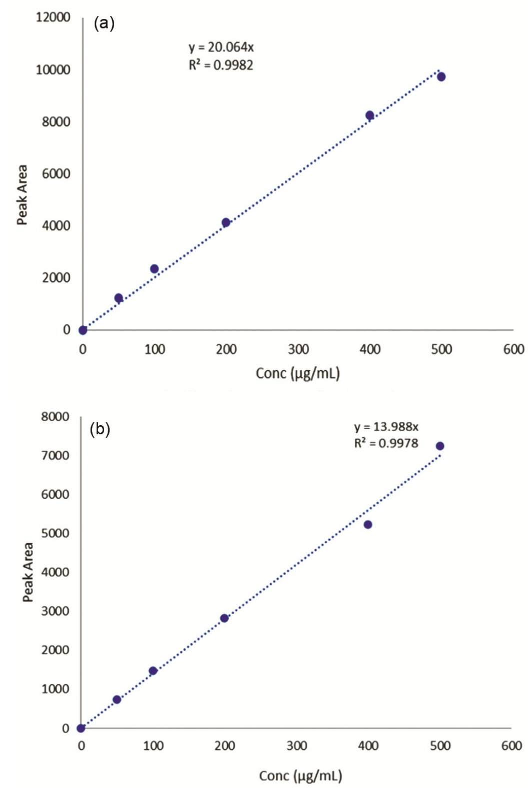


Fig. 8 — Calibration curve for (a) Levodopa, and (b) Carbidopa.

delivered at 1.0 mL/min to ensure efficient elution of the target analytes. Calibration curves were generated using spiked, drug-free rat brain homogenates within a concentration range of 50–500 µg/mL. The calibration experiments demonstrated excellent linear response ($r^2 > 0.999$), high accuracy (≈99–101%), and low variability (CV < 1%); however, the method was applied primarily for comparative chromatographic profiling rather than full quantitative bioanalytical validation (Fig. 8).

Chromatographic analysis of *V. faba* seed extract revealed peaks occurring within retention windows corresponding to levodopa standards, suggesting the presence of levodopa-like constituents. Rat brain homogenates collected after treatment similarly displayed peaks within the levodopa retention region, indicating central availability of compounds with comparable chromatographic behaviour. Peaks appearing within retention windows comparable to the carbidopa standard were also observed; however, without spectral confirmation or co-elution studies, these signals are interpreted only as chromatographic similarities rather than definitive evidence of carbidopa presence. The absence of clear carbidopa detection in brain homogenates is consistent with its known limited penetration across the blood–brain barrier, whereas levodopa undergoes carrier-mediated transport into the central nervous system. Table 6 presents calibration data for Levodopa, demonstrating consistent measurements with CVs ranging from

Table 6 — Calibration data for Levodopa

Concentration (µg/mL)	Measured (Mean, n=5)	CV (%)	Accuracy (%)
50	50.12	0.08	99.48
100	100.02	0.02	98.81
200	200.05	0.01	99.55
400	400.46	0.22	97.74
500	500.02	0.001	99

Table 7 — Calibration data for Carbidopa

Concentration (µg/mL)	Measured (Mean, n=5)	CV (%)	Accuracy (%)
50	50.12	0.08	99.50
100	100.09	0.01	99.14
200	200.12	0.08	99.78
400	400.34	0.01	99.42
500	500.04	0.02	99.92

0.001 to 0.47% and accuracies ranged from 100.00% to 100.60%, which falls within acceptable analytical variability and reflects minor experimental variation inherent to chromatographic measurements. Table 7 shows comparable results for Carbidopa, with CVs between 0.01% and 0.88% and accuracies ranging from 99.42% to 100.50%, consistent with acceptable analytical performance.

The retention times of standard API compounds were recorded as 2.91 ± 0.2 min for Levodopa and 3.37 ± 0.2 min for Carbidopa (Fig. 9). In a combined standard mixture, both drugs were detected at 2.18 min and 3.09 min, respectively (Fig. 10). Extracts from *V. faba* seeds showed peaks within retention regions corresponding to both Levodopa and Carbidopa standards, while rat brain extracts exhibited peaks within the retention region corresponding to Levodopa (Fig. 11), indicating the

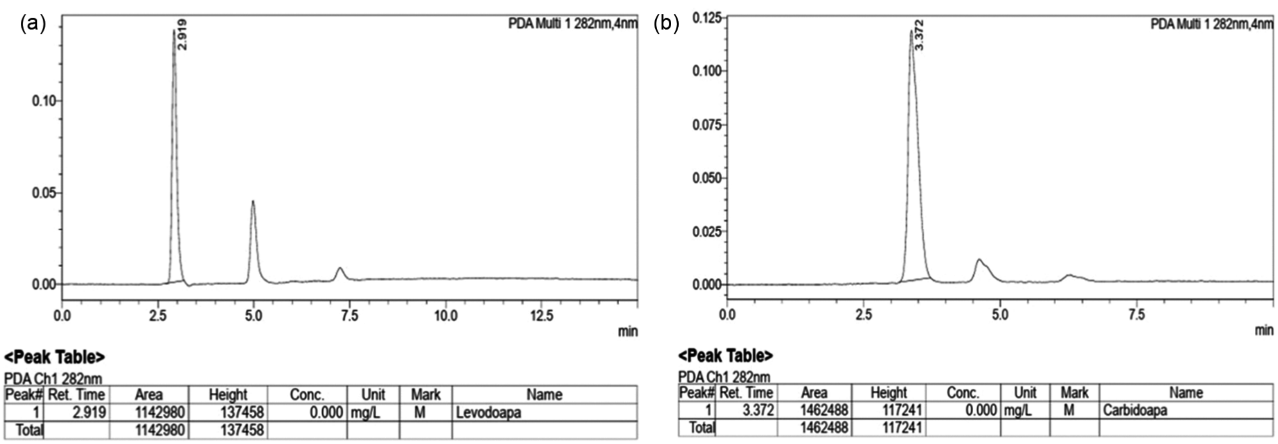


Fig. 9 — Chromatogram of (a) Levodopa, and (b) Carbidopa API.

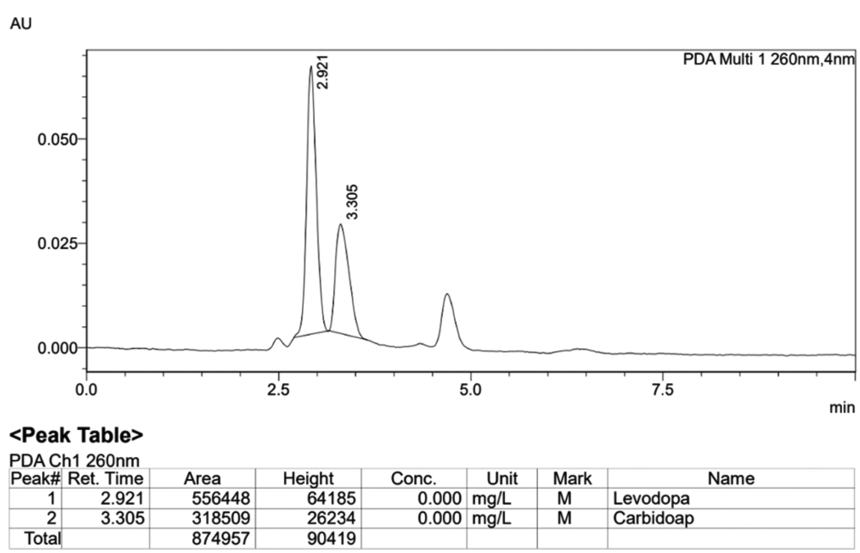
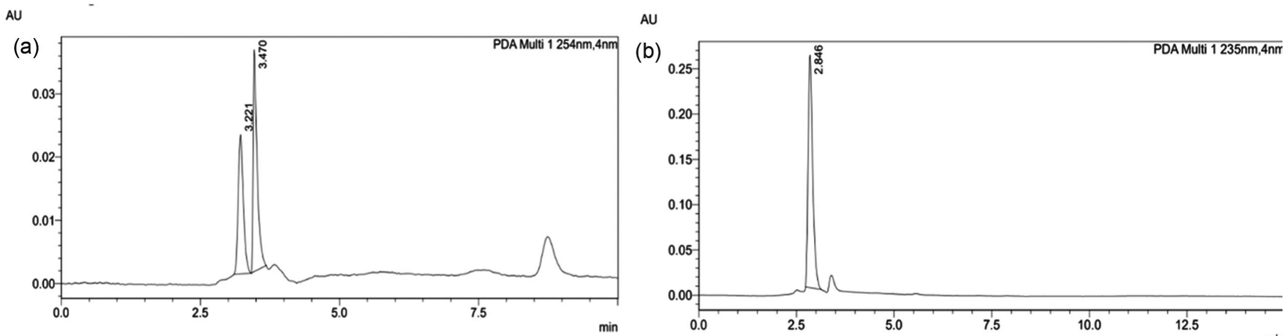


Fig. 10 — Chromatogram of Combined Mixture (Levodopa and Carbidopa).



(a) <Peak Table>
PDA Ch1 254nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	3.221	140820	22040	0.000	mg/L	M	Levodoapa
2	3.470	175039	35033	0.000	mg/L	M	Carbidopa
Total		315859	57073				

(b) <Peak Table>
PDA Ch1 235nm

Peak#	Ret. Time	Area	Height	Conc.	Unit	Mark	Name
1	2.846	2103621	256836	0.000	mg/L	M	Levodopa
Total		2103621	256836				

Fig. 11 — Chromatogram of (a) Levodopa and Carbidopa in seed extract, and (b) Levodopa in rat brain extract.

presence of chromatographically detectable constituents with similar retention behavior.

Discussion

The present study investigated the neurobehavioral effects of *V. faba* seed extract in a haloperidol-induced Parkinsonism model. Haloperidol produces motor impairment primarily through dopaminergic receptor blockade, thereby providing a pharmacological model to evaluate agents capable of modulating dopaminergic-mediated motor responses[17,18,27]. In this context, the extract produced progressively greater improvements in catalepsy, locomotor activity, and exploratory behaviour across increasing treatment levels.

A reduction in haloperidol-induced catalepsy is generally interpreted as evidence of improved dopaminergic signalling at the striatal level. The progressive decrease in paw withdrawal time observed in the treated groups, therefore, suggests that the extract enhanced dopaminergic-mediated motor responses rather than acting solely as a nonspecific stimulant. Similar motor improvements following dietary consumption of *V. faba* have been reported in Parkinson’s patients, supporting the relevance of the present experimental observations[9,28,29].

The restoration of locomotor activity observed in both actophotometric and infrared actimeter assessments further reinforces this interpretation. Rather than attributing the behavioural response to a single compound, it is likely that multiple phytoconstituents contributed to the observed effects. *V. faba* seeds are known to contain naturally occurring dopamine precursors, along with phenolic antioxidants and micronutrients, all of which have been implicated in supporting dopaminergic neurotransmission and reducing oxidative stress in neurodegenerative conditions[11,12,30]. The present results are therefore consistent with a multimodal mechanism involving both neurotransmitter support and neuroprotective activity.

Analytical evaluation of the extract provided complementary evidence supporting this interpretation. UV spectrophotometric analysis demonstrated absorption in the catechol region, while RP-HPLC chromatograms revealed peaks within retention regions corresponding to levodopa and related dopaminergic constituents[21,31].

Notably, RP-HPLC analysis of the seed extract also showed peaks within retention regions corresponding to both Levodopa and Carbidopa standards. Although this observation does not confirm the identity of individual compounds, it indicates the presence of constituents with similar chromatographic behaviour. Such findings are consistent with previous phytochemical reports describing *V. faba* as a natural source of dopamine precursors and structurally related catechol derivatives that may contribute to its biological activity.

In addition to motor outcomes, the extract influenced anxiety-related behaviour in the elevated plus maze. Since anxiety is a recognised non-motor symptom of Parkinson's disease, this finding suggests that the extract may modulate broader neurochemical pathways beyond dopaminergic transmission alone. Experimental work indicating possible interactions of *V. faba* with inhibitory neurotransmitter systems, including glycinergic mechanisms, provides a plausible explanation for these observations and supports the concept of multi-target activity.

Comparison with other plant-derived neuroprotective agents further supports the relevance of these findings.

Extracts from several botanical species rich in antioxidants or neurotransmitter precursors have demonstrated protective effects in haloperidol-induced models, while reviews of L-DOPA-containing legumes emphasise their therapeutic potential when appropriately standardised[32,33]. Within this context, the present study contributes by combining behavioural evaluation with chromatographic comparison in a controlled experimental model using a defined hydro-alcoholic extract.

Despite these encouraging findings, certain limitations should be considered. The analytical methods employed were suitable for comparative profiling but not for definitive compound identification or full quantitative validation. More selective techniques, such as LC-MS or targeted quantification, would be required to precisely characterise the phytochemical composition. Furthermore, the haloperidol model represents an acute pharmacological blockade rather than progressive neurodegeneration, and therefore cannot fully replicate the chronic pathology of Parkinson's disease. Future studies incorporating biochemical markers, long-term models, and controlled phytochemical standardisation will be necessary to clarify the therapeutic potential of *V. faba* preparations[8,34].

Overall, the results indicate that *V. faba* seed extract may improve Parkinsonian motor and behavioural deficits through combined dopaminergic support and neuroprotective actions. While additional validation is required, the findings support further investigation of standardised botanical preparations as adjunct approaches for Parkinsonian symptom management.

Conclusion

The present study demonstrates that a standardised hydro-alcoholic extract of *V. faba* seeds improved motor and behavioural deficits in a haloperidol-induced Parkinsonian model. Progressive reductions in catalepsy, together with the restoration of locomotor activity, were observed across increasing extract treatment levels, suggesting functional improvement in dopaminergic-mediated motor responses. No overt adverse behavioural or physiological effects were observed in treated animals during the study period, indicating that the administered extract volumes were well tolerated under the experimental conditions. Analytical evaluation indicated constituents with chromatographic characteristics consistent with dopaminergic precursors, supporting the biological relevance of the observed behavioural effects. While these findings reinforce the potential of *V. faba* as a botanical source of compounds relevant to Parkinsonian symptom modulation, further studies incorporating detailed phytochemical characterisation, mechanistic biomarkers, and long-term experimental models are required to clarify therapeutic applicability. Overall, the results support continued investigation of standardized *V. faba* preparations as potential adjunct approaches in Parkinson's disease management.

Limitations and future implications

This study has certain limitations. Phytochemical evaluation was primarily qualitative, and individual dopaminergic constituents were not isolated or quantified, limiting direct correlation between compound levels and behavioural effects. UV spectrophotometric analysis was non-selective, and although chromatographic profiling suggested the presence of constituents with retention behaviour similar to known dopaminergic agents, definitive structural confirmation was beyond the scope of this work. The study was also limited to short-term behavioural assessment without long-term toxicity evaluation or mechanistic biomarker analysis. Future research should therefore focus on compound characterisation, improved extract standardisation, chronic safety studies, and mechanistic investigations to better establish the therapeutic relevance of *V. faba* in Parkinsonian models.

Conflict of interest

The authors declare no conflict of interest.

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