Evaluation of antiparkinson activity of combination of aqueous extracts of *Mucuna pruriens (L.)* & *Camellia sinensis (L.)* in experimental animals.

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ABSTRACT

Introduction: Parkinson's disease (PD) is a progressive neurodegenerative disorder affecting the motor system, characterized by the loss of dopaminergic neurons in the substantia nigra pars compacta of the brain. Mucuna pruriens L. seeds are a natural source of L-DOPA, a precursor to dopamine, which helps compensate for the dopaminergic deficit in PD & the leaves of Camellia sinensis L. contain epigallocatechin gallate (EGCG), a catechol-O-methyltransferase (COMT) inhibitor that enhanced the bioavailability of L-DOPA by preventing its premature degradation and facilitating its passage across the blood-brain barrier (BBB).

Objective: The preset study was undertaken to investigate the antiparkinson activity of combination of aqueous extract of *Mucuna pruriens* (L.) (AEMP) and *Camellia sinensis* (L.) (AECS) in experimental animals.

Methods: Animals were divided into 8 groups (n=6). Group-1 received distilled water (5ml/kg, p.o.), Group-2 served as disease induced haloperidol (1mg/kg, i.p.), Group-3 as a standard control syndopa (10mg/kg, p.o.), Group 4 & 5 were administered AEMP (100mg/kg, p.o.) & AECS (50mg/kg, p.o.), respectively. Group 6, 7 & 8 were administered the combinations of AEMP: AECS, 30%:70%, 50%:50% and 70%:30%. Behavioural assessments included catalepsy and locomotor activity using catalepsy bar method and actophotometer respectively. Dopamine levels in brain tissue were measured, and histopathological analysis of brain was performed.

Results: Administration of AEMP and AECS in combination, significantly reduced haloperidol-induced catalepsy and improved locomotor activity. The most significant therapeutic effect was observed in the group receiving a 70:30 AEMP: AECS. *Mucuna pruriens (L.)*, a natural source of L-DOPA, crosses the blood-brain barrier and restores dopamine levels by modulating oxidative stress and inflammation-related targets such as Nrf2, SOD, cAMP signalling, and ERK/MAPK cascades. *Camellia sinensis (L.)*, is a multi-target agent, capable of modulating several key proteins involved in PD pathogenesis including alpha-synuclein (SNCA), caspases, NF-κB, BDNF, Nrf2, and Parkin. Biochemical analysis showed a marked restoration of dopamine levels in therapeutic groups. Histopathological evaluation confirmed the preservation of neuronal architecture and reduced neurodegenerative changes in extract-treated animals.

Conclusions: The combination of aqueous extract of Mucuna pruriens (L.) and Camellia sinensis (L.) significantly improved haloperidol-induced motor impairments and neurodegeneration in mice. Their synergistic effects, likely due to dopaminergic, antioxidant, and anti-inflammatory properties, highlight their potential as adjunct therapies for Parkinson's disease. Further studies are warranted to elucidate molecular mechanisms, assess long-term safety, and validate clinical relevance.

1. Introduction

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder characterized by motor symptoms and cognitive impairment. Over 80% of PD patients with cognitive impairment eventually develop Parkinson's disease dementia (PDD) (Putha et al., 2025). The disease is primarily characterized by the degeneration of dopamine-producing neurons in the substantia nigra pars compacta (SNpc) of the midbrain, which leads to hallmark motor symptoms including bradykinesia, resting tremor, rigidity, and postural instability (Morris et al., 2024, Radhakrishna et al., 2018, Vaillancourt et al., 2020). The substantia nigra, particularly the SNpc, plays a crucial role within the basal ganglia by modulating voluntary motor control through dopaminergic signalling via the nigrostriatal pathway. (Al-kuraishy et al., 2024). In PD, disruptions in this circuitry, including altered activity of D1 and D2 dopamine receptors, are central to both motor and behavioural symptoms (Sayyaed et al., 2023).

Drug-induced Parkinsonism is another concern, where medications like calcium channel blockers and certain neuroleptics block dopamine receptors or disrupt the blood-brain barrier, mimicking or exacerbating PD symptoms (Vanegas-Arroyave et al., 2024, Bohlega et al., 2013, Mena et al., 2006). The progression of PD is categorized into six stages, beginning in the lower brainstem and advancing to the cortex, with cognitive and motor symptoms worsening as more brain regions become involved (Braak et al., 2004). Haloperidol is a widely used antipsychotic medication with broad clinical applications, including the treatment of schizophrenia, acute psychosis, and agitation in various neuropsychiatric conditions. This adverse effect is primarily due to its potent antagonism of dopamine D2 receptors in the striatum, disrupting dopaminergic signalling critical for motor control (Katual et al., 2017).

While a definitive cure for Parkinson's disease (PD) remains elusive, preventive strategies are gaining attention through the identification of modifiable risk factors, early diagnostic tools, and neuroprotective interventions. (Armstrong et al., 2020).

Current treatments for Parkinson's disease (PD) are primarily symptomatic and do not reverse the underlying neurodegeneration. Moreover, these treatments are often accompanied by adverse effects such as dyskinesia, sedation, impulse control disorders, gastrointestinal issues, and cognitive disturbances (Demailly et al., 2024, Sveinbjornsdottir et al., 2016). Consequently, there is growing interest in exploring natural products, particularly medicinal herbs, as alternative therapeutic agents. Herbal medicines, known for their broad therapeutic properties and lower side effect profiles, present a promising avenue for developing cost-effective and safer treatments for PD (Jun et al., 2023). Mucuna pruriens (L.) and Camellia sinensis (L.). possess antiparkinson's activity. Mucuna pruriens L., commonly known as black velvet bean, is a tropical legume from the Fabaceae family and is widely used in traditional medicine, for its diverse therapeutic properties (Singh et al., 2023). It is rich in L-DOPA (3,4-dihydroxy-L-phenylalanine), a precursor of dopamine, making it especially relevant in the management of Parkinson's disease. The L-DOPA content in its seed's ranges from 3% to 6%, and the plant also contains various bioactive compounds such as alkaloids, saponins, tannins, and essential minerals (Singh et al., 2023). The wide range of pharmacological actions like, anti-parkinson's (Kamkaen et al., 2022), antioxidant (Diniyah et al., 2023), anti-inflammatory (Uchegbu et al., 2016), antibacterial (Shanmugavel et al., 2015), anti-tumor (Gamedze et al., 2024), anti-venom (Yee et al., 2009), antidiabetic (Verma et al., 2025), aphrodisiac (Sattayasai et al., 2022), antidepressant (Kaewmor et al., 2024).

Camellia sinensis (L.), an evergreen shrub from the *Theaceae* family, is the source of various types of tea—including green, black, and oolong—with green tea being the most recognized for its medicinal properties (Samanta et al., 2022, Namita et al., 2012). It is rich in polyphenols, particularly catechins, with epigallocatechin gallate (EGCG) being the most potent and abundant. EGCG is known for its strong antioxidant, anti-inflammatory, and neuroprotective effects, making it especially relevant for neurodegenerative disorders like Parkinson's disease (Azami et al., 2024). Notably, EGCG also functions as a catechol-O-methyltransferase (COMT) inhibitor, potentially enhancing L-DOPA bioavailability—a key aspect in Parkinson's therapy. With minimal side effects and broad

therapeutic potential, *Camellia sinensis* (*L.*) represents a promising natural agent in neuroprotective research (Putri et al., 2022). Pharmacological activities have been derived like, anti-parkinson's (Giri et al., 2020), antioxidant (Governa et al., 2022), antidepressant (Choudhary et al., 2025), hypertension(Chiang et al., 2021), anti-diabetic (Ansari et al., 2022), antimicrobial (Gopal et al., 2016), anti-inflammatory (Mota et al., 2015), antiproliferative (Esghaei et al., 2018), hepatoprotective (Shareef et al., 2022), antihyperlipidemic (Kongchain et al., 2020).

In the present study we evaluated the antiparkinson activity of combination of aqueous extracts of *Mucuna pruriens* (*L.*) and *Camellia sinensis* (*L.*) in haloperidol-induced parkinsonism using an experimental animal model. The study aimed to determine whether the combination of these plant extracts could provide neuroprotective benefits or symptom relief comparable to conventional treatment. To achieve this, we have conducted this study including phytochemical and pharmacological evaluations, measurement of dopamine levels in brain tissue, and histopathological analysis. We aimed to assess whether the addition of these botanical agents could mitigate dopaminergic depletion and improve pathological outcomes associated with drug-induced parkinsonism.

2. Materials & methods

2.1 Collection and authentication of plant material

The seeds of *Mucuna pruriens* (*L.*) and the leaves of *Camellia sinensis* (*L.*) were procured from Kirti Enterprises, Hadapsar, Pune. The botanical authentication of both plant materials was carried out by Dr. Harshad M. Pandit, Ph.D. (Botany), at Azad Nagar Gem CHS Ltd, Mumbai, MS, India. The authenticated specimen numbers were assigned as follows: *Mucuna pruriens* (*L.*) – Specimen #: SJP 24126286 and *Camellia sinensis* (*L.*) – Specimen #: SJP 24120806.

2.2 Preparation of extracts

The seeds of *Mucuna pruriens* (L.) were cleaned and ground into a fine powder to ensure uniformity for effective extraction. A total of 250 grams of the powder was soaked in 400 mL of distilled water for 72 hours via maceration to extract bioactive compounds. The mixture was then filtered, and the filtrate concentrated using a magnetic stirrer with a hot plate under controlled temperature and vacuum. The resulting aqueous extract was stored at 4 °C to maintain its stability and preserve active constituents (Yadav et al.,2013, Ahmad et al.,2012). Similarly, the leaves of *Camellia sinensis* (*L.*) were cleaned, dried, and ground into a fine powder. A total of 400 grams of the powdered leaves were extracted by maceration in 2.5 L of distilled water for 72 hours. The resulting extract was filtered and concentrated using the same method, then stored at 4 °C to maintain the integrity of heat- and oxidation-sensitive phytochemicals such as catechins and flavonoids for subsequent analysis or application (Sharma et al., 2012, Razavi et al., 2017).

2.3 Phytochemical analysis

The aqueous extract of *Mucuna pruriens (L.)* (AEMP) and *Camellia sinensis (L.)* (AECS) were subjected to preliminary phytochemical screening to identify the presence of key constituents such as alkaloids, glycosides, flavonoids, terpenoids, phenols, and tannins. (Tyagi et al., 2017, Shaikh et al., 2020).

2.4 Chemicals

Haloperidol was obtained from Divine laboratories, (Gujrat, India) and Syndopa tablet procured from Sun pharma, (Sikkim, India). All reagents were of analytical grade.

2.5 Animals

Healthy swiss albino mice weighing (25-30 grams) were used in the experiment, which were obtained from Crystal biological solutions, uralidevachi, Pune, Maharashtra. Total 96 albino mice were

employed, with 48 animals allocated to each behavioural model. They were maintained under standard laboratory conditions, including a temperature of 27 ± 2 °C, relative humidity of 45-55%, and a 12-hour light/dark cycle. All animals had free access to clean drinking water and a standard pellet diet provided ad libitum.

Animals were randomly divided into eight experimental groups (n=6) as follows;

- Group-1 received distilled water (5ml/kg, p.o.); this served as normal control.
- Group-2 received haloperidol (1mg/kg, i.p.); this served as disease control.
- Group-3 received standard drug syndopa (10mg/kg, p.o.).

Group 4-8: Mice were orally treated with various therapeutic treatments by single and combination of both crude drug extracts.

- Group-4 received aqueous extract of *Mucuna pruriens L.* (AEMP) (100mg/kg)
- Group-5 received aqueous extract of *Camellia sinensis L.* (AECS) (50mg/kg)
- Group 6: AEMP: AECS (30%:70%) (30mg/kg+ 35mg/kg= 65mg/kg)
- Group 7: AEMP: AECS (50%:50%) (50mg/kg+ 25mg/kg= 75mg/kg)
- Group 8: AEMP: AECS (70%:30%) (70mg/kg+ 15mg/kg= 85mg/kg)

2.6 Induction of parkinson's disease

Parkinson's disease was induced by intravenous injection of haloperidol 1mg/kg, for 1 week in alternate days prior the therapeutic treatment.

2.7 Catalepsy bar test

The catalepsy bar test was employed to measure the duration a mouse could maintain a fixed posture with both front limbs placed on a 4 cm high bar. A mouse was considered cataleptic if it remained immobile, and the time was recorded until it moved its paws or head, with a maximum cut-off time of 5 minutes. Assessments were conducted on the 1st, 7th, 14th, and 21st days following drug administration, noting the longest duration of immobility. All observations took place in a quiet room, and mice were returned to their cages between tests. (Ittiyavirah et al., 2014, Chaitra et al., 2016).

2.8 Actophotometer test

Each mouse was placed in the activity cage (Dolphin 2002-C V2) for 5 minutes to measure its locomotor activity. As the mouse moved and explored, it broke infrared light beams in the cage, which were automatically counted by a digital recorder. If the mouse stopped moving, the recorder paused, and resumed when the mouse began walking again. A "walk" was counted when the mouse moved using all four feet across the space between two opposite walls of the cage. The total time spent walking was also recorded (Gosavi et al., 2020).

2.9 Biochemical parameter

2.9.1 Dopamine level estimation

Whole brain was removed, weighed, and cut into fine pieces and homogenized in 5 mL of 5% HCl-butanol and centrifuged at 2000 rpm for 10 minutes. The supernatant was collected, mixed with 2.5 mL of heptane and 0.3 mL of 0.1 M HCl, shaken thoroughly, and centrifuged again. The lower aqueous phase was collected for dopamine analysis. For estimation, 0.02 mL of this phase was reacted with HCl, sodium acetate buffer (pH 6.9), and iodine solution. The oxidation reaction was halted with sodium thiosulphate in 5 M NaOH, followed by the addition of acetic acid and heating to 100°C for 6 minutes. After cooling, fluorescence was measured at 330–375 nm using a spectrofluorimeter, and dopamine levels were determined by comparing fluorescence with a blank and standard (Chaitra et al., 2016).

2.10 Histopathology of brain

The brain tissue was carefully isolated and immediately fixed in 10% neutral buffered formalin to preserve its structural integrity. Following fixation, the samples were processed and embedded in paraffin wax. Thin sections measuring 3–5 μ m in thickness were cut using a microtome, with serial sections of 3 μ m obtained for detailed histological analysis. The sections were stained using the haematoxylin-eosin (H&E) staining method to highlight cellular and tissue morphology. The stained

slides were then examined under a microscope(40x) to assess histopathological changes and structural alterations in the brain tissue (Chaitra et al., 2016, Chandrashekhar et al., 2010).

2.11 Statistical analysis

Statistical analysis of data was performed using one way ANOVA followed by Dunnett's multiple comparison test using Graph pad prism software 10.4.2. Data were expressed as Mean \pm SEM (n=6), and difference were considered as statistically significant *p<0.05, **p<0.01, ***p<0.001 when compared with disease treated group (Fowles et al., 2013).

3. Results

3.1 Physical properties

The aqueous extract of *Mucuna pruriens (L.)* (AEMP) appeared as a dark brown semisolid with a characteristic pungent aroma and taste. It was soluble in both water and alcohol, had a pH of 6.5, and yielded 5.6% w/w. In contrast, the aqueous extract of *Camellia sinensis (L.)* (AECS) was pale green, semisolid, and slightly turbid, with a distinct herbal or grassy aroma. It was also soluble in water and alcohol, had a pH of 5.0, and yielded 2.45% w/w.

3.2 Phytochemical analysis

The AEMP & AECS showed the presence of following phytochemicals like flavonoids, alkaloids, phenols, terpenoids, glycosides and tannis (Table 1 and Table 2).

Table 2

Table 1Phytochemical analysis of AEMP

Glycosides:

Tannis:

Borntrager's Test

Braymer's test

5

6

Sr	Test name	Phytochemicals
no		
1	Flavonoids:	Flavonoids
	Shinoda test	present
2	Alkaloids:	Alkaloids
	Wagner's test	present
3	Phenols: Ferric	Phenols present
	Chloride test	
4	Terpenoids:	Terpenoids
	Salkowski Test	Absent

Phytochemical analysis of AECS

	1	T
Sr	Test name	Phytochemicals
no		
1	Flavonoids:	Flavonoids present
	Shinoda test	
2	Alkaloids:	Alkaloids present
	Wagner's test	
3	Phenols: Ferric	Phenols present
	Chloride test	
4	Terpenoids:	Terpenoids Absent
	Salkowski Test	
5	Glycosides:	Glycosides present
	Borntrager's	
	Test	
6	Tannis:	Tannis present
	Braymer's test	

3.3 Behavioural models: Catalepsy bar and Actophotometer test

Glycosides

Tannis present

present

The catalepsy test showed that the disease control group (haloperidol, 1 mg/kg, i.p.) had a significant increase in immobility from day 7 to 21(###p<0.001), while the normal control (distilled water, 5 ml/kg, p.o.) showed baseline. The standard group (Syndopa, 10 mg/kg, p.o.) significantly reduced catalepsy (***p<0.001). AEMP (100 mg/kg, p.o.) (***p<0.001) showed reduction in immobility than AECS (50 mg/kg, p.o.) (**p<0.01), and the combination groups—Group 6 (65 mg/kg) (**p<0.01), Group 7 (75 mg/kg) (**p<0.01), and especially Group 8 (85 mg/kg) (***p<0.001) showed the most significant reduction. (Fig 1).

In the actophotometer test, the disease control group showed reduced locomotor activity (###p<0.001), while the normal and standard groups maintained high activity (***p<0.001). AEMP improved locomotion more than AECS (**p<0.01). Combination groups, particularly Group 8 (70% AEMP + 30% AECS, 85 mg/kg), showed the significant recovery in movement, indicating a strong synergistic effect (***p<0.001) (Fig 2).

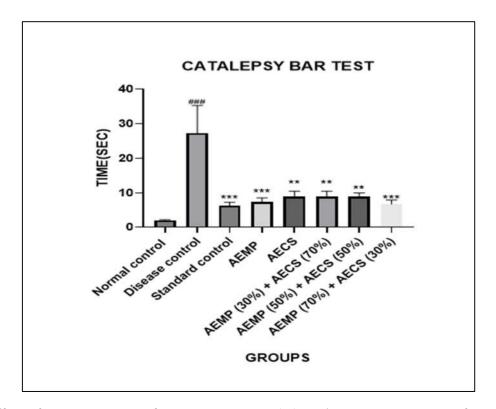


Fig. 1. Effect of aqueous extract of *Mucuna pruriens (L.)* seeds & aqueous extract of *Camellia sinensis (L.)* leaves and their combinations in haloperidol induced catalepsy in mice.

Values are expressed as Mean \pm SEM (n=6). Statistical analysis was carried out by One-way ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01, ***p<0.001 when compared with disease treated group *###p<0.001. AEMP: Aqueous extract of *Mucuna Pruriens L*. seeds, AECS: Aqueous extract of *Camellia Sinensis L*. leaves, i.p.: intraperitoneal route, p.o.: oral route.

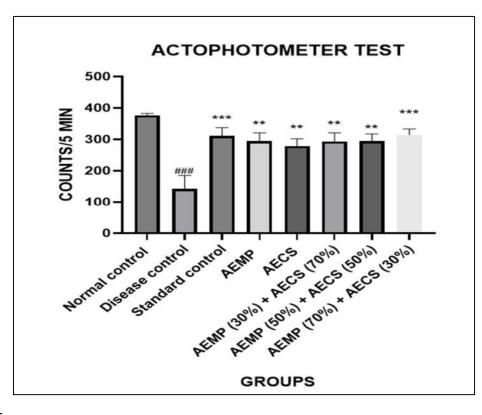


Fig. 2. Effect of aqueous extract of *Mucuna pruriens (L.)* seeds & aqueous extract of *Camellia sinensis (L.)* leaves and their combinations in haloperidol induced locomotor activity in mice.

Values are expressed as Mean \pm SEM (n=6). Statistical analysis was carried out by One-way ANOVA, followed by Dunnett's multiple comparison test *p<0.05, **p<0.01, ***p<0.001 when compared with disease treated group. AEMP: Aqueous extract of *Mucuna Pruriens L*. seeds, AECS: Aqueous extract of *Camellia Sinensis L*. leaves, i.p.: intraperitoneal route, p.o.: oral route.

3.4 Estimation of dopamine level in brain tissue

Table 5 shows the effect of AEMP and AECS on brain dopamine levels in Haloperidol-induced mice. The control group had normal dopamine levels (4.06 ng/g), while the Haloperidol group showed a significant decrease (1.95 ng/g) (###p<0.001). Syndopa (10 mg/kg) significantly restored dopamine (3.10 ng/g) (***p<0.001). AEMP (100 mg/kg) and AECS (50 mg/kg) both improved dopamine levels (2.98 ng/g and 3.00 ng/g, respectively) (**p<0.01). Combination treatments showed significant results, with Group 8 (85 mg/kg: 70% AEMP + 30% AECS) achieving the highest dopamine level (3.08 ng/g), demonstrating the best synergistic effect (***p<0.001) (Fig 3).

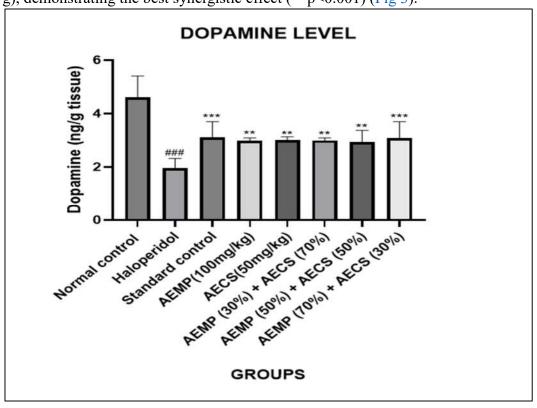


Fig. 3. Estimation of dopamine level in brain tissue by spectrofluorimetry in swiss albino mice

Values are expressed as Mean \pm SEM (n=6). Statistical analysis was carried out by One-way ANOVA followed by Dunnett's multiple comparison test *p<0.05, **p<0.01, ***p<0.001 when compared with disease treated group. AEMP: Aqueous extract of *Mucuna Pruriens L*. seeds, AECS: Aqueous extract of *Camellia Sinensis L*. leaves, i.p.: intraperitoneal route, p.o.: oral route.

3.5 Histopathology of brain tissue

Histological examination of brain tissues confirmed the biochemical and behavioural results. The normal control group showed no signs of damage. The disease control group (Haloperidol) exhibited significant brain damage, including neuronal shrinkage, vacuolation, and gliosis. The standard drug (Syndopa) showed mild neuronal damage, indicating some neuroprotective effect. In the test groups, Group 4 (AEMP) showed mild degeneration with neuronal aggregation and vacuolation. Group 5 (AECS) showed minimal vacuolation and gliosis, suggesting potential efficacy. Group 6 (AEMP 30%

+ AECS 70%) had mild to moderate degeneration. Group 7 (AEMP 50% + AECS 50%) showed mild degeneration but with some protective effects. Group 8 (AEMP 70% + AECS 30%) had minimal changes, indicating strong neuroprotective effects (Table 3 and Table 4).

Table 3 Histopathology of brain tissue (40x)

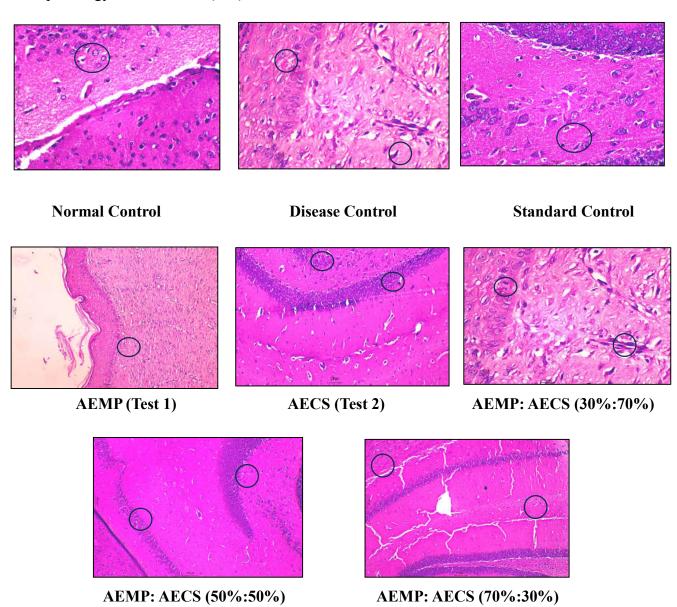


Table 4 Observations of histopathology of brain tissue

Sr	Groups	Histopathological Observations	Overall
no			Pathological Grade
1	Normal	Normal neuronal morphology, no	0
	Control	signs of degeneration	
2	Disease	Neuronal cells shrinkage,	Moderate (+++)
	Induced	vacuolation, gliosis	
3	Standard Drug	Mostly preserved neurons, slight	Minimal (+)
		vacuolation	
4	AEMP	Mild neuronal damage, improved	Mild (++)
	(Test 1)	architecture vs haloperidol	
		_	

5	AECS (Test 2)	Minimal degeneration, improved neuronal preservation	Minimal (+)
6	AEMP: AECS (30%: 70%) (Test 3)	Mild-to-moderate vacuolation, early gliosis, and some neuronal loss, especially due to reduced antioxidant defence	Mild (++) to Moderate (+++)
7	AEMP: AECS (50%: 50%) (Test 4)	Mild changes, including occasional vacuolated neurons and slight gliosis, better than AEMP alone	Mild (++)
8	AEMP: AECS (70%: 30%) (Test 5)	Well-preserved neurons, very few pathological changes	Minimal (+)

Note: Overall Grade score as- 0 = No Abnormality Detected, Minimal changes (+), Mild changes (+++), Moderate changes (++++), Severe changes (++++).

4. Discussion

The present study investigated the antiparkinsonian effects of aqueous extract of *Mucuna pruriens* (*L.*) seeds (AEMP) and *Camellia sinensis* (*L.*) leaves (AECS), individually and in combinations, in a haloperidol-induced models of Parkinson's disease in mice. These results demonstrated that both extracts exhibit significant neuroprotective and motor-restorative properties, as evidenced by improvements in behavioural parameters such as catalepsy duration and locomotor activity, supported by histopathological evaluation of brain tissue.

Cataleptic immobility was markedly reduced in all treatment groups compared to the disease control group (Haloperidol), with the most synergetic effect observed in the group receiving a 70:30 combination of AEMP and AECS. Similarly, locomotor activity, assessed via actophotometer, showed a substantial improvement in the group receiving a 70:30 combination of AEMP and AECS. Dopamine analysis supported the behavioral and histological findings, showing significant restoration of dopamine levels in extract-treated groups, especially those receiving combination therapy with higher AEMP proportions, indicating enhanced neuroprotective efficacy against haloperidol-induced dopaminergic depletion. Histological analysis reports that these behavioural outcomes, revealing preserved neuronal architecture and reduced neurodegenerative changes in extract-treated animals, particularly in groups receiving combination therapy.

These results suggest that the combination therapy of AEMP and AECS produces a profound neurorestorative effect. AEMP offers dopaminergic support through L-DOPA. L-DOPA interacts with enzymes like tyrosine hydroxylase and dopamine decarboxylase and also influences downstream dopaminergic pathways involving D1 and D2 receptors, cAMP signalling, and ERK/MAPK cascades. Chronic administration, however, is associated with long-term complications such as L-DOPA-induced dyskinesia (LID) and oxidative stress due to dopamine metabolism, which generates reactive oxygen species (ROS). While AECS provides strong antioxidant and anti-inflammatory actions via its polyphenolic content, notably EGCG. This catechin is known for their strong antioxidant & anti-inflammatory properties. EGCG is a multi-target agent, capable of modulating several key proteins involved in PD pathogenesis including alpha-synuclein (SNCA), caspases, NF-κB, BDNF, Nrf2, and Parkin. In the context of neurodegeneration, green tea extract showed to mitigate oxidative damage, modulate signalling pathways such as MAPK and NF-κB, and inhibit neuronal death. The dual-action mechanism—targeting both symptomatic relief and underlying neurodegeneration—makes this combination a promising candidate for further preclinical and clinical evaluation in Parkinson's disease.

Future research should aim to isolate the active principles and further delineate the underlying mechanisms. Based on these observations, we conclude that the combined aqueous extracts of AEMP and AECS, particularly at a 70:30 ratio (Test 5), possess potent anti-parkinsonian and neuroprotective potential without any observed toxicity, offering a promising phytotherapeutic alternative for Parkinson's disease management.

5. Conclusion

In the present study, aqueous extracts of *Mucuna pruriens (L.)* (AEMP) and *Camellia sinensis (L.)* (AECS), particularly in combination, significantly improved haloperidol-induced motor impairments and neurodegeneration in mice. The combination therapy, especially at 70% AEMP and 30% AECS, showed the most notable improvements in behavioural, biochemical (dopamine restoration), and histopathological outcomes. These effects are attributed to the dopaminergic action of AEMP (L-DOPA content) and the antioxidant properties of AECS (EGCG content). The findings support the therapeutic potential of this plant-based combination for Parkinson's disease, warranting further molecular and clinical investigations.

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Conflict of interest

The authors declare no conflict of interest.

References

- 1. Putha S, Gayam SR, Kasaraneni BP, Kondapaka KK, Nallamala SK, Thuniki P. Neuroscience-informed nomogram model for early prediction of cognitive impairment in Parkinson's disease. Neuroscience Informatics. 2025 Jun 1;5(2):100189.
- 2. Radhakrishnan DM, Goyal V. Parkinson's disease: A review. Neurology India. 2018 Mar 1;66(Suppl 1): S26-35.
- 3. Vaillancourt DE, Mitchell T. Parkinson's disease progression in the substantia nigra: location, location, location. Brain. 2020 Sep;143(9):2628-30.
- 4. Morris HR, Spillantini MG, Sue CM, Williams-Gray CH. The pathogenesis of Parkinson's disease. The Lancet. 2024 Jan 20;403(10423):293-304.
- 5. Al-kuraishy HM, Al-Gareeb AI, Albuhadily AK, Elewa YH, AL-Farga A, Aqlan F, Zahran MH, Batiha GE. Sleep disorders cause Parkinson's disease or the reverse is true: Good GABA good night. CNS Neuroscience & Therapeutics. 2024 Mar;30(3):e14521.
- 6. Sayyaed A, Saraswat N, Vyawahare N, Kulkarni A. A detailed review of pathophysiology, epidemiology, cellular and molecular pathways involved in the development and prognosis of Parkinson's disease with insights into screening models. Bulletin of the National Research Centre. 2023 May 23;47(1):70.
- 7. Sharma HS, Menon P, Lafuente JV, Muresanu DF, Tian ZR, Patnaik R, Sharma A. Development of in vivo drug-induced neurotoxicity models. Expert opinion on drug metabolism & toxicology. 2014 Dec 1;10(12):1637-61.
- 8. Braak H, Ghebremedhin E, Rüb U, Bratzke H, Del Tredici K. Stages in the development of Parkinson's disease-related pathology. Cell and tissue research. 2004 Oct; 318:121-34.
- 9. Blandini F. An update on the potential role of excitotoxicity in the pathogenesis of Parkinson's disease. Functional neurology. 2010 Apr 1;25(2):65.

10. Bohlega SA, Al-Foghom NB. Drug-induced Parkinson's disease. A clinical review. Neurosciences Journal. 2013 Jul 1;18(3):215-21.

- 11. Mena MA, De Yébenes JG. Drug-induced parkinsonism. Expert opinion on drug safety. 2006 Nov 1;5(6):759-71.
- 12. Katual MK, Jamwal S, Deshmukh R, Sharma A, Saini R, Kumar R, Harikumar sl. Study of haloperidol induced behavioural & bio-chemical abnormalities in rats: a novel tool in evaluation of anti-parkinsonian agents. 2017;7(3):15-30.
- 13. Armstrong MJ, Okun MS. Diagnosis and treatment of Parkinson disease: a review. Jama. 2020 Feb 11;323(6):548-60.
- 14. Vanegas-Arroyave N, Caroff SN, Citrome L, Crasta J, McIntyre RS, Meyer JM, Patel A, Smith JM, Farahmand K, Manahan R, Lundt L. An evidence-based update on anticholinergic use for drug-induced movement disorders. Cns Drugs. 2024 Apr;38(4):239-54.
- 15. Demailly A, Moreau C, Devos D. Effectiveness of continuous dopaminergic therapies in Parkinson's disease: A review of L-DOPA pharmacokinetics/pharmacodynamics. Journal of Parkinson's Disease. 2024 Jul 23;14(5):925-39.
- 16. Sveinbjornsdottir S. The clinical symptoms of Parkinson's disease. Journal of neurochemistry. 2016 Oct; 139:318-24.
- 17. Baby C, Kaur S, Singh J, Prasad R. Velvet bean (Mucuna pruriens): A sustainable protein source for tomorrow. Legume Science. 2023 Sep;5(3): e178.
- 18. Natarajan K, Narayanan N, Ravichandran N. Review on "*Mucuna*"-the wonder plant. Int J Pharm Sci Rev Res. 2012;17(1):86-93.
- 19. Lampariello LR, Cortelazzo A, Guerranti R, Sticozzi C, Valacchi G. The magic velvet bean of *Mucuna pruriens*. Journal of traditional and complementary medicine. 2012 Oct 1;2(4):331-9.
- 20. Yadav SK, Prakash J, Chouhan S, Singh SP. *Mucuna pruriens* seed extract reduces oxidative stress in nigrostriatal tissue and improves neurobehavioral activity in paraquat-induced Parkinsonian mouse model. Neurochemistry international. 2013 Jun 1;62(8):1039-47.
- 21. Uchegbu RI, Ahuchaogu AA, Mbadiugha CN, Amanze KO, Igara CE, Iwu IC, Ikechukwu-Nwankwo PC. Antioxidant, anti-inflammatory and antibacterial activities of the seeds of *Mucuna pruriens* (UTILIS). Am. Chem. Sci. J. 2016; 13:1-8.
- 22. Shanmugavel G, and Krishnamoorthy G. "In vitro evaluation of the antibacterial activity of alcoholic extract from *Mucuna pruriens* seed." (2015): 7-9.
- 23. Gamedze NP, Mthiyane DM, Mavengahama S, Singh M, Onwudiwe DC. Biosynthesis of ZnO nanoparticles using the aqueous extract of Mucuna pruriens (utilis): structural characterization, and the anticancer and antioxidant activities. Chemistry Africa. 2024 Jan;7(1):219-28.
- 24. Diniyah N, Bulgis UM, Marchianti AC. Antioxidant activity and phytochemical compositions of Mucuna pruriens L. in different conditions of time and temperature extraction. InIOP Conference Series: Earth and Environmental Science 2023 May 1 (Vol. 1177, No. 1, p. 012042). IOP

Publishing.

25. Yee FS. The protective effects of *Mucuna pruriens* seed extract against histopathological changes induced by Malayan cobra (Naja sputatrix) venom in rats. Tropical Biomedicine. 2009;26(1):80-4.

- 26. Verma R, Misra V, Bisen PS. Efficacy of Mucuna pruriens (L.) DC. in treating diabetes, Parkinson's disease, and erectile dysfunction—a review of clinical and preclinical trials. Exploration of Foods and Foodomics. 2025 Apr 23;3: 101083.
- 27. Choowong-In P, Sattayasai J, Boonchoong P, Poodendaen C, Wu AT, Tangsrisakda N, Sawatpanich T, Arun S, Uabundit N, Iamsaard S. Protective effects of Thai Mucuna pruriens (L.) DC. var. pruriens seeds on sexual behaviors and essential reproductive markers in chronic unpredictable mild stress mice. Journal of traditional and complementary medicine. 2022 Jul 1;12(4):402-13.
- 28. Kaewmor J, Rungruang S, Phunikhom K, Sattayasai J, Lahnwong C. Anti-anxiety and anti-depressive effects of Thai Mucuna pruriens seed aqueous extract against ethanol withdrawal syndrome in mice. Phytomedicine Plus. 2024 Nov 1;4(4):100630.
- 29. Ekanem AP, Obiekezie A, Kloas W, Knopf K. Effects of crude extracts of *Mucuna pruriens* (Fabaceae) and *Carica papaya* (Caricaceae) against the protozoan fish parasite Ichthyophthirius multifiliis. Parasitology Research. 2004 Mar; 92:361-6.
- 30. Namita P, Mukesh R, Vijay KJ. *Camellia sinensis* (green tea): a review. Global journal of pharmacology. 2012;6(2):52-9.
- 31. Samanta S. Potential bioactive components and health promotional benefits of tea (Camellia sinensis). Journal of the American Nutrition Association. 2022 Feb 3;41(1):65-93.
- 32. Azami S, Forouzanfar F. Therapeutic potentialities of green tea (Camellia sinensis) in ischemic stroke: Biochemical and molecular evidence. Metabolic Brain Disease. 2024 Feb;39(2):347-57.
- 33. Zhao T, Li C, Wang S, Song X. Green tea *(Camellia sinensis)*: A review of its phytochemistry, pharmacology, and toxicology. Molecules. 2022 Jun 18;27(12):3909.
- 34. Putri IS, Robbani TN, Divamillenia D, Pratiwi OG, I'tishom R. Potential neurogenesis and neuroprotective effects of epigallocatechin-3-gallate (EGCG) in green tea (Camellia sinensis) through microglia M2 induction process and NLRP3 inhibition as an innovation for ischemic stroke adjuvant therapy: A review. International Journal of Research Publications. 2022 Jan 2;92(1):8.
- 35. Governa P, Manetti F, Miraldi E, Biagi M. Effects of in vitro simulated digestion on the antioxidant activity of different Camellia sinensis (L.) Kuntze leaves extracts. European Food Research and Technology. 2022 Jan 1:1-0.
- 36. Choudhary D, Kaur R, Rani N, Kumar B, Singh TG, Chandrasekaran B, Rawat R, Eyupoglu V. Insights into in silico analysis to explore the multitarget antidepressant role of Camellia sinensis. Journal of Biomolecular Structure and Dynamics. 2025 Apr 28:1-3.

37. Chiang SS, Chen LS, Chu CY. Active food ingredients production from cold pressed processing residues of Camellia oleifera and Camellia sinensis seeds for regulation of blood pressure and vascular function. Chemosphere. 2021 Mar 1;267: 129267.

- 38. Ansari P, Hannan JM, Choudhury ST, Islam SS, Talukder A, Seidel V, Abdel-Wahab YH. Antidiabetic actions of ethanol extract of Camellia sinensis leaf ameliorates insulin secretion, inhibits the DPP-IV enzyme, improves glucose tolerance, and increases active GLP-1 (7–36) levels in high-fat-diet-fed rats. Medicines. 2022 Nov 11;9(11):56.
- 39. Gopal J, Muthu M, Paul D, Kim DH, Chun S. Bactericidal activity of green tea extracts: the importance of catechin containing nano particles. Scientific Reports. 2016 Jan 28;6(1):19710.
- 40. Mota MA, Landim JS, Targino TS, Silva SF, Silva SL, Pereira MR. Evaluation of the anti-inflammatory and analgesic effects of green tea (*Camellia sinensis*) in mice. Acta Cirurgica Brasileira. 2015 Apr;30(4):242-6.
- 41. Esghaei M, Ghaffari H, Esboei BR, Tapeh ZE, Salim FB, Motevalian M. Evaluation of anticancer activity of *Camellia sinensis* in the Caco-2 colorectal cancer cell line. Asian Pacific journal of cancer prevention: APJCP. 2018;19(6):1697.
- 42. Shareef SH, Ibrahim IA, Alzahrani AR, Al-Medhtiy MH, Abdulla MA. Hepatoprotective effects of methanolic extract of green tea against Thioacetamide-Induced liver injury in Sprague Dawley rats. Saudi journal of biological sciences. 2022 Jan 1;29(1):564-73.
- 43. Kongchian A, Keawboonlert N, Boonrak T, Lookyee S, Buasri K, Surongkul N, Tangpong J. Anti-hyperlipidemia and anti-obesity properties of Garcinia atroviridis and Camellia sinensis extracts in high-fat diet mice. Walailak Journal of Science and Technology (WJST). 2020 Oct 17;17(10):1126-38.
- 44. Tyagi T. Phytochemical screening of active metabolites presents in *Eichhornia crassipes* (Mart.) *Solms* and *Pistia stratiotes* (*L.*): Role in ethanomedicine. Asian Journal of Pharmaceutical Education and Research. 2017;6(4):40-56.
- 45. Shaikh JR, Patil M. Qualitative tests for preliminary phytochemical screening: An overview. International journal of chemical studies. 2020 Mar 1;8(2):603-8.
- 46. Ittiyavirah SP, Ruby R. Effect of hydro-alcoholic root extract of *Plumbago zeylanica l* alone and its combination with aqueous leaf extract of *Camellia sinensis* on haloperidol induced parkinsonism in wistar rats. Annals of neurosciences. 2014 Apr;21(2):47.
- 47. Fowles JR, Banton MI, Pottenger LH. A toxicological review of the propylene glycols. Critical reviews in toxicology. 2013 Apr 1;43(4):363-90.
- 48. Chaitra N, Joy A, Handral M. ANTIPARKINSON'S activity of *vigna vexillata* seed extract in haloperidol induced cataleptic rats. World Journal of Pharmaceutical Research. 2016 Apr 20;5(7):729-46.

49. Chandrashekhar VM, Ranpariya VL, Ganapaty S, Parashar A, Muchandi AA. Neuroprotective activity of *Matricaria recutita Linn* against global model of ischemia in rats. Journal of ethnopharmacology. 2010 Feb 17;127(3):645-51.

- 50. Gosavi DD, Kamdi AS, Kalambe SM, Bohra PN. "The spontaneous motor action of alcoholic excerpt of *Withania coagulans* fruits in Swiss albino mice by Actophotometer." (2020): 7:3:160-163.
- 51. Ahmad N, Rahman ZU, Akhtar N, Ali S. Testosterone like activity of ethanolic and aqueous extracts of Mucuna pruriens seeds and its effects on serum biochemical metabolites in immature male rats. Pak. Vet. J. 2012 Mar 1;32(1):60-4.
- 52. Kamkaen N, Chittasupho C, Vorarat S, Tadtong S, Phrompittayarat W, Okonogi S, Kwankhao P. Mucuna pruriens seed aqueous extract improved neuroprotective and acetylcholinesterase inhibitory effects compared with synthetic L-dopa. Molecules. 2022 Jan;27(10):3131.
- 53. Giri MA, Bhalke RD, Prakash KV, Kasture SB. Evaluation of Camellia sinensis, Withania somnifera and their Combination for Antioxidant and Antiparkinsonian Effect. Journal of Pharmaceutical Sciences and Research. 2020 Aug 1;12(8):1093-9.
- 54. Jun P, Zhao H, Jung IC, Kwon O, Han CH, Won J, Jang JH. Efficacy of herbal medicine treatment based on syndrome differentiation for Parkinson's disease: A systematic review and meta-analysis of randomized placebo-controlled clinical trials. Frontiers in Pharmacology. 2023 Feb 27; 14:1108407.