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RESEARCH ARTICLE

Pharmacological Evaluation of Isolated L-DOPA from Mucuna pruriens: Antidepressant and Antiparkinsonian Activities in Rodent Models

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Abstract: This study investigates the neuropharmacological efficacy of L-DOPA isolated from Mucuna pruriens seeds, focusing on its antidepressant and antiparkinsonian activities in rodent models, to validate its traditional use in neurological disorders. Seeds underwent rigorous pharmacognostic standardization, confirming authenticity via macroscopic (mean seed dimensions: 12.34 × 9.87 × 7.65 mm) and microscopic analyses (lignified macrosclereids, no starch grains). Acidified ethanolic extraction (1:1 ethanol-water, 0.1% citric acid) yielded a 12.5% w/w crude extract (MPE), with bioactivity-guided fractionation isolating L-DOPA (12.35% w/w in ethyl acetate fraction) via column chromatography and confirmed by FTIR, HR-ESI-MS, and NMR spectroscopy. Acute oral toxicity (OECD 423) in mice established safety (LD_{50}>2000 mg/kg). Antidepressant effects were assessed in mice using Forced Swim (FST) and Tail Suspension Tests (TST), where L-DOPA (20, 40 mg/kg, p.o.) dose-dependently reduced immobility time, rivaling imipramine (15 mg/kg, i.p.). Antiparkinsonian activity was evaluated in mice via haloperidol-induced catalepsy and chronic rotenone models. L-DOPA (40 mg/kg) significantly reversed catalepsy, restored locomotor activity, and in the rotenone model, ameliorated motor deficits, elevated striatal dopamine (6.52 ± 0.27 ng/mg), and mitigated oxidative stress (reduced MDA, increased GSH/SOD). These findings confirm L-DOPA as the primary bioactive mediating Mucuna pruriens' neuroprotective effects, supporting its potential as a natural therapeutic for depression and Parkinson's disease, warranting clinical translation.

Keywords: Mucuna pruriens; L-DOPA; Antidepressant activity; Antiparkinsonian activity; Oxidative stress; Rodent models.

INTRODUCTION

Neurological and psychiatric disorders represent a profound challenge to global health in the 21st century, with major depressive disorder (MDD) and Parkinson's disease (PD) emerging as two of the most debilitating conditions contributing to disability-adjusted life years (DALYs) worldwide [1]. According to the World Health Organization (WHO), depression affects an estimated 5.7% of the global adult population, with a higher prevalence among women than men, and contributes to over 1 billion people living with mental health conditions as of 2025 [2]. In the United States alone, the National Institute of Mental Health reports that major depressive episodes are more common in adult females (10.3%) compared to males (6.2%), underscoring significant gender disparities [3]. Projections indicate that the prevalence of MDD in older adults could rise to 4.53% by 2050, with current estimates suggesting approximately 246 million people worldwide grappling with this disorder after adjustments for the lingering impacts of the COVID-19 pandemic [4]. Similarly, Parkinson's disease, the second most prevalent neurodegenerative disorder, currently affects over 10 million individuals globally, with incidence rates escalating with age—impacting about 1-2% of those over 65 years [5]. Recent forecasts from 2025 studies predict a dramatic surge, estimating more than 25 million cases by 2050, representing a 76% increase from 2021 levels, with prevalence rates projected to reach 267 cases per 100,000 population by mid-century [6]. These statistics highlight the urgent need for innovative therapeutic strategies, as both conditions not only impose substantial economic burdens—encompassing healthcare costs, lost productivity, and reduced quality of life—but also exacerbate societal challenges in aging populations [7].

Major Depressive Disorder is a multifaceted psychiatric illness characterized by persistent low mood, anhedonia, alterations in sleep and appetite, fatigue, feelings of worthlessness, impaired cognition, and recurrent suicidal ideation, often leading to severe functional impairment [8]. The global economic toll of depression is immense, with estimates from 2021 indicating billions in annual costs due to treatment, absenteeism, and diminished societal contributions [9]. Pathophysiologically, MDD involves dysregulation of monoaminergic systems (serotonin, norepinephrine, dopamine), hypothalamic-pituitary-adrenal (HPA) axis hyperactivity resulting in chronic hypercortisolemia and hippocampal atrophy, neuroinflammation with elevated cytokines like TNF-α and IL-6, and impaired neuroplasticity marked by reduced brain-derived neurotrophic factor (BDNF) signaling and volumetric changes in the hippocampus and prefrontal cortex [10]. Conventional pharmacotherapies, primarily selective serotonin reuptake inhibitors (SSRIs) and serotonin-norepinephrine reuptake inhibitors (SNRIs), achieve

remission in only 60-70% of patients, with a delayed onset of action (often weeks), high relapse rates, and adverse effects including sexual dysfunction, weight gain, and gastrointestinal disturbances. Treatment-resistant depression affects about 30% of cases, further emphasizing the limitations of current synthetic agents and the imperative for novel, multifaceted interventions that address underlying neurobiological mechanisms more holistically [11].

Parkinson's disease, conversely, is a progressive neurodegenerative disorder primarily manifesting in motor symptoms such as bradykinesia, resting tremor, rigidity, and postural instability, stemming from the selective loss of dopaminergic neurons in the substantianigra pars compacta and the accumulation of α-synuclein aggregates in Lewy bodies [12]. Non-motor symptoms, including depression, cognitive decline, autonomic dysfunction, and sleep disturbances, often precede or accompany motor deficits, complicating management and contributing to a diminished quality of life [9]. The etiology of PD is multifactorial, involving mitochondrial dysfunction, oxidative stress, neuroinflammation, and genetic predispositions (mutations in SNCA, LRRK2, and PARKIN genes). Levodopa (*L*-DOPA), the cornerstone of PD therapy since the 1960s, effectively alleviates motor symptoms by crossing the blood-brain barrier and converting to dopamine; however, long-term use is associated with motor fluctuations ("on-off" phenomena), dyskinesias, and psychiatric side effects, without halting disease progression [14]. Adjunctive therapies like dopamine agonists, monoamine oxidase B inhibitors, and deep brain stimulation provide symptomatic relief but fail to address neurodegeneration, highlighting an unmet need for neuroprotective agents that can modify disease course while minimizing adverse effects [11].

Preclinical and clinical investigations have increasingly validated the therapeutic potential of *M. pruriens*. For instance, standardized extracts have demonstrated enhanced neurotrophic factor expression (BDNF, NGF, GDNF) in rodent depression models, improving synaptic plasticity and behavioral outcomes beyond monoaminergic modulation [15]. In combination with conventional antidepressants like fluoxetine, *M. pruriens* augments serotonin and dopamine transmission while reducing inflammation, with RNA sequencing revealing impacts on circadian and neuroplasticity pathways. For PD, a 2025 systematic review of clinical trials concluded that *M. pruriens* improves motor symptoms and reduces therapy complications, with one high-quality study and others showing promise despite methodological heterogeneity [16]. Non-motor benefits include amelioration of constipation, orthostatic hypotension, and depression, potentially via gut-brain axis mechanisms involving short-chain fatty acids and microbiome modulation [17]. Mechanistic studies attribute these effects to *L*-DOPA's dopaminergic replenishment, coupled with anti-inflammatory actions (suppression of TNF-α, IL-1β, and NF-κβ), antioxidant properties (reduced oxidative stress, enhanced GSH and SOD), and epigenetic modifications (HDAC inhibition, increased histone acetylation) [18]. Recent 2024-2025 reviews also highlight *M. pruriens*' antidepressant-like effects in models of depression induced by chronic stress or neurotoxins, with reductions in depression-like behaviors via GSK-3β/calcium signaling and BDNF/TrkB pathways. Clinical evidence, though limited, suggests benefits for anxiety and depression, with a 2025 overview noting preliminary support for mood enhancement. Furthermore, nano-encapsulation and pharmacokinetic studies have improved bioavailability, showing sustained *L*-DOPA release and reduced peripheral side effects [19].

Despite this growing body of evidence, significant knowledge gaps persist. Most studies have evaluated crude or standardized extracts, attributing efficacy primarily to *L*-DOPA while overlooking potential contributions from other bioactives or synergistic interactions—the so-called "entourage effect." Extracts devoid of *L*-DOPA have still exhibited neuroprotective activity, suggesting unidentified compounds or mechanisms. Moreover, rigorous pharmacological evaluations of isolated *L*-DOPA from *M. pruriens* in validated rodent models for both antidepressant and antiparkinsonian activities are scarce, with limited focus on safety profiles, dose-response relationships, and biochemical correlates like oxidative stress markers and dopamine levels. This study addresses these lacunae by isolating *L*-DOPA through bioactivity-guided fractionation and systematically assessing its acute toxicity, antidepressant potential in the Forced Swim Test (FST) and Tail Suspension Test (TST), and antiparkinsonian effects in haloperidol-induced catalepsy and chronic rotenone models. We hypothesize that isolated *L*-DOPA will demonstrate dose-dependent efficacy comparable to standards, mediated by dopaminergic restoration and neuroprotection against oxidative stress, thereby providing scientific validation for *M. pruriens*' traditional uses and paving the way for its development as a natural therapeutic adjunct or alternative for MDD and PD.

MATERIAL AND METHODS

2.1. Chemicals

For standard compounds, *L*-DOPA (levodopa, CAS 59-92-7, reference standard ≥98% from Sigma-Aldrich, Bangalore) served as a reference for HPTLC/HPLC quantification; gallic acid (CAS 149-91-7, reference standard ≥98% from Sigma-Aldrich, Bangalore) for antioxidant assay and as a reference standard; quercetin (CAS 117-39-5, reference standard ≥95% from Sigma-Aldrich, Bangalore) as a flavonoid reference standard; imipramine hydrochloride (CAS 113-52-0, pharmaceutical secondary standard from Sigma-Aldrich, Bangalore) as the standard drug for antidepressant studies; levodopa + carbidopa (N/A,

pharmaceutical secondary standard from Sigma-Aldrich, Bangalore) as the standard drug for antiparkinsonian studies; haloperidol (CAS 52-86-8, pharmaceutical secondary standard from Sigma-Aldrich, Bangalore) for catalepsy induction in animal models; and rotenone (CAS 83-79-4, PESTANAL® ≥95% from Sigma-Aldrich, Bangalore) for induction of parkinsonism in chronic models. Finally, biochemical assay kits included the lipid peroxidation (MDA) assay kit (N/A, from Sigma-Aldrich, Bangalore) for estimating oxidative stress in brain tissue; the reduced glutathione (GSH) assay kit (N/A, from Sigma-Aldrich, Bangalore) for estimating antioxidant markers; and the superoxide dismutase (SOD) assay kit (N/A, from Sigma-Aldrich, Bangalore) for estimating antioxidant enzyme activity.

2.2. Plants

The plant materials were procured from our previously reported procedure(Reference No.: DG/23-34/802). The physicochemical characteristics and morphological features were performed previously according to the standard pharmacopoeial methods.

2.3. Animals

The experimental protocol received approval from the Institutional Animal Ethics Committee (IAEC Approval No.: IAEC/RK/25/10) prior to initiation. Sexually mature, 6-8 weeks age, nulliparous Swiss Albino mice (*Mus musculus*), weighing 20-35 g, were procured from the central animal house facility. The animals were randomly allocated into five experimental groups (n=6 per group) using computer-generated random numbers and housed in polypropylene cages under standard laboratory conditions (temperature: 22 ± 3°C, relative humidity: 30-70%, 12:12 hour light-dark cycle) with free access to standard pellet diet and water *ad libitum* throughout the acclimatization and study periods, except during the brief fasting period.

2.4. Toxicity Study

The acute oral toxicity profile of the isolated compound was evaluated in accordance with the Organisation for Economic Co-operation and Development (OECD) Guideline 423 (Acute Oral Toxicity - Acute Toxic Class Method) to determine its potential harmful effects following a single administration. Following an overnight fasting period (16-18 hours) with free access to water, the animals received single oral doses of the isolated compound suspended in 1% w/v carboxymethyl cellulose (CMC) solution as vehicle, administered via oral gavage using stainless-steel feeding needles at dose levels of 5, 50, 300, and 2000 mg/kg body weight, with the volume of administration not exceeding 10 ml/kg body weight. All animals were observed meticulously for immediate toxic responses including changes in skin and fur, eyes and mucous membranes, respiratory, circulatory, autonomic and central nervous systems, and somatomotor activity and behavior patterns every 30 minutes during the first 4 hours post-dosing, and then thoroughly at least once daily for a total of 14 days for any signs of toxicity, morbidity, and mortality. Detailed clinical observations were recorded using standardized scoring sheets. Individual body weights were measured and recorded daily using a digital precision balance, while food and water consumption were monitored quantitatively by measuring the weight of food pellets and volume of water provided versus remaining after 24 hours throughout the 14-day observation period. Any animals showing severe signs of distress or pain were humanely euthanized according to established ethical guidelines. At the end of the observation period, all surviving animals were euthanized humanely for gross pathological examination of internal organs. The median lethal dose (LD_{50}) was estimated based on mortality and morbidity patterns observed across the dose groups [20].

2.5. Antidepressant Activity

2.5.1. Forced Swim Test

The antidepressant activity of the isolated compound was evaluated using the Forced Swim Test (FST), a widely validated behavioral despair model, following the methodological principles originally described by Porsolt et al. (1977) with appropriate modifications. Adult Swiss Albino mice were randomly assigned to five experimental groups (n=6 per group) using computer-generated randomization schedules to ensure unbiased allocation.

- Group I served as the vehicle control and received 1% w/v carboxymethyl cellulose (CMC) solution orally (p.o.);
- Group II received the reference standard drug imipramine hydrochloride (15 mg/kg, intraperitoneally, i.p.) as positive control; and
- Groups III, IV, and V received the test compound at doses of 10, 20, and 40 mg/kg p.o., respectively.

All treatments were administered once daily for 14 consecutive days to evaluate both acute and subchronic effects. On the test day (day 14), one hour after the final treatment administration, each rat was individually placed in a cylindrical glass tank (25 cm diameter × 40 cm height) containing fresh water maintained at 25 ± 1°C to a depth of 30 cm, which prevented the mice from touching the bottom with their hind paws or tail. Each test session lasted 6 minutes, during which the animals initial 2-minute period was considered habituation time, and the total duration of immobility (defined as the absence of escape-oriented behaviors including only minimal movements necessary to keep the head above water) during the final 4-minute period was measured by two trained observers blinded to the treatment groups using manual stopwatches, with the average of both recordings used for statistical analysis. Between trials, the water was changed and the apparatus thoroughly cleaned to eliminate olfactory cues. All experiments were conducted in a sound-attenuated room under consistent lighting conditions between

09:00 and 16:00 hours to minimize circadian influences on behavioral responses [21, 22].

2.5.2. Tail Suspension Test

Complementary assessment of antidepressant-like activity was performed using the Tail Suspension Test (TST) following the methodological framework established by Steru et al. (1985), which exhibits good predictive validity for antidepressant compounds. Swiss albino mice (20-25 g) were randomly divided into five groups (n=6) paralleling the experimental design of the FST:

- Group I (vehicle control, 1% CMC p.o.),
- Group II (imipramine 15 mg/kg i.p.), and
- Groups III-V (test compound at 10, 20, and 40 mg/kg p.o.).

All treatments were administered once daily for 14 days following the same regimen as in the FST. One hour after the final treatment administration on day 14, each mouse was individually suspended by the tail using adhesive tape attached approximately 1 cm from the tip, to a horizontal bar positioned 50 cm above the surface of a table, ensuring that the animal could not make contact with any surrounding surfaces. The test session duration was 6 minutes, during which the total time spent immobile (defined as the absence of any limb or body movements when the animal hung passively) was recorded by trained observers blinded to the treatment conditions using manual stopwatches. The test apparatus was thoroughly cleaned between trials with 70% ethanol to remove residual odors. All testing was conducted in a dedicated behavioral testing room under standardized lighting and noise conditions between 09:00 and 16:00 hours to maintain environmental consistency across experimental replicates. The immobility time recorded in both FST and TST served as the primary endpoint for evaluating potential antidepressant-like effects, with reduced immobility duration compared to vehicle control indicating antidepressant activity [23, 24].

2.6. Antiparkinsonian Activity

2.6.1. Haloperidol-induced Catalepsy

The antiparkinsonian activity of the isolated compound was evaluated using the haloperidol-induced catalepsy model, a well-established preclinical paradigm for assessing dopaminergic blockade and screening potential anti-parkinsonian agents. Adult Swiss Albino mice were systematically randomized into five experimental groups (n=6):

- Group I served as vehicle control receiving only the drug vehicle (1% CMC);
- Group II represented the disease control receiving haloperidol (1 mg/kg, i.p.);
- Group III received the standard drug combination of levodopa-carbidopa (100+25 mg/kg, p.o.) as positive control; and
- Groups IV and V received the test compound at doses of 20 and 40 mg/kg p.o., respectively.

All treatments were administered once daily for 7 consecutive days to evaluate prophylactic potential. On the seventh day, one hour after the final administration of respective treatments, catalepsy was induced in all groups except the vehicle control by intraperitoneal injection of haloperidol (1 mg/kg). Catalepsy assessment was performed using the standard bar test method at 30, 60, 90, and 120-minute intervals post-haloperidol administration. For this test, each animal's forepaws were placed on a horizontal wooden bar positioned 10 cm above the base, and the descent latency (time until the animal removed both forepaws from the bar) was recorded with a maximum cut-off time of 180 seconds to prevent unnecessary distress. All behavioral assessments were conducted by trained observers blinded to the treatment allocations to eliminate experimental bias, under standardized environmental conditions maintained throughout the testing period [25, 26].

2.6.2. Locomotor Activity

Immediately following the final catalepsy measurement at 120 minutes, spontaneous locomotor activity was assessed using a digital actophotometer (INCO, Ambala, India) to evaluate the general motor functions and potential sedative or stimulant effects of the treatments. Each animal was individually placed in the center of the activity chamber (30 × 30 × 30 cm) equipped with infrared sensors, and allowed to explore freely for 10 minutes. The apparatus automatically recorded total locomotor activity counts through beam breaks in the infrared grid system, providing quantitative assessment of horizontal movements. Between testing sessions, the chamber was thoroughly cleaned with 70% ethanol solution to eliminate olfactory cues that could influence subsequent animals' behavior. The activity counts were expressed as total beam breaks per 10-minute session, with reduced activity in haloperidol-treated animals indicating neuroleptic-induced hypolocomotion and reversal of this effect suggesting dopaminergic activation [27, 28].

2.6.3. Rotenone-Induced Parkinsonism

For the chronic Parkinson's disease model, mice received daily subcutaneous injections of rotenone (2 mg/kg) emulsified in sunflower oil for 28 consecutive days to progressively induce dopaminergic neurodegeneration through mitochondrial complex I inhibition. From day 15 onwards, animals received co-treatment with either the test compound (20 and 40 mg/kg, p.o.), standard drug (levodopa-carbidopa 100+25 mg/kg, p.o.), or vehicle control alongside continued rotenone administration. Behavioral assessments including open field test (for general locomotor activity and exploration) and narrow beam walk test (for evaluating motor coordination and balance) were performed weekly throughout the study period. On day 29, 24 hours after the final treatment, animals were euthanized under deep anesthesia. Brains were rapidly excised and dissected on ice-cold platform

to isolate striatal regions. Tissue samples were processed for biochemical estimation of oxidative stress parameters including lipid peroxidation (measured as malondialdehyde formation using thiobarbituric acid reactive substances assay), reduced glutathione levels (using Ellman's method), and superoxide dismutase activity (employing pyrogallol autoxidation inhibition assay). Simultaneously, neurochemical analysis was performed using high-performance liquid chromatography with electrochemical detection (HPLC-EC) for quantitative determination of striatal dopamine, 3,4-dihydroxyphenylacetic acid (DOPAC), and homovanillic acid (HVA) levels to comprehensively evaluate dopaminergic integrity and metabolic turnover in the nigrostriatal pathway [29, 30].

2.7. Statistical Analysis

All quantitative data obtained from the pharmacological and biochemical experiments were expressed as Mean ± Standard Error of Mean (SEM) for six independent observations (n=6) in each experimental group. The choice of SEM over standard deviation was deliberate to emphasize the precision of the estimated population mean rather than the variability within the sample. The statistical significance of differences between multiple experimental groups was determined using one-way Analysis of Variance (ANOVA), which tests the null hypothesis that all group means are equal against the alternative hypothesis that at least one group mean differs. Prior to ANOVA, assumptions of normality were verified using the Shapiro-Wilk test, and homogeneity of variances was confirmed with Bartlett's test. When ANOVA indicated significant differences ($p < 0.05$), post-hoc pairwise comparisons were conducted using Dunnett's test specifically selected for comparing all treatment groups against a single control group, thereby controlling the family-wise error rate while maintaining statistical power. The threshold for statistical significance was established a priori at $p < 0.05$ for all analyses. All statistical procedures, including data organization, descriptive statistics, assumption testing, ANOVA, post-hoc comparisons, and graphical representation, were performed using GraphPad Prism software version 9.0 (GraphPad Software Inc., San Diego, CA, USA), which employs validated algorithms for statistical computing and provides comprehensive output including exact p-values, F-statistics, degrees of freedom, and confidence intervals to ensure transparent and reproducible analysis.

3. Results and Discussion

3.1. Toxicity

The acute oral toxicity study, conducted as per OECD Guideline 423, revealed no mortality or signs of gross toxicity at doses up to 2000 mg/kg body weight of the isolated *L-DOPA* over the 14-day observation period. No significant changes were observed in the skin, fur, eyes, mucous membranes, respiratory rate, autonomic, or central nervous system functions. There were no statistically significant alterations in body weight, food, or water consumption compared to the control group. Gross pathological examination of internal organs (liver, kidneys, heart, lungs, spleen) at the end of the study showed no abnormalities. Based on these observations, the LD_{50} of the isolated *L-DOPA* was determined to be greater than 2000 mg/kg, classifying it as Category 5 (or unclassified) according to the GHS system, indicating low acute oral toxicity.

3.2. Antidepressant Activity

3.2.1. Forced Swim Test (FST) in Mice

The effect of the isolated *L-DOPA* (MP-01) on immobility time in the FST is presented in Table 6.4. Chronic administration (14 days) of *L-DOPA* produced a significant and dose-dependent reduction in the immobility time compared to the vehicle control group ($p < 0.01$ and $p < 0.001$). The effect of the highest dose (40 mg/kg) was comparable to the standard antidepressant drug, imipramine (15 mg/kg, i.p.).

3.2.2. Tail Suspension Test (TST) in Mice

Table 1presents the pivotal behavioral data from two of the most validated preclinical models for assessing antidepressant-like activity, the Forced Swim Test (FST) and the Tail Suspension Test (TST), with the core metric being Immobility Time, which is interpreted as a measure of "behavioral despair" or passive coping strategy. The Vehicle Control group, receiving only the inert carboxymethyl cellulose (CMC) solution, establishes the baseline level of immobility of 185.33 seconds in the FST and 175.67 seconds in the TST (**Figure 1**), representing the typical response of an untreated animal when faced with an inescapable, mildly stressful situation. The profound efficacy of the Imipramine group, a tricyclic antidepressant used here as the standard reference drug, is confirmed by its highly significant (*** $p < 0.001$) reduction of immobility time in both tests (to 98.50 and 92.83 seconds, respectively). This validates the experimental setup, as Imipramine's known mechanism of increasing synaptic levels of norepinephrine and serotonin reliably produces this anti-immobility effect. The critical finding of the experiment is the clear, dose-dependent antidepressant-like activity demonstrated by the isolated *L-DOPA* (MP-01). At the lowest dose of 10 mg/kg, the reduction in immobility time is minimal and not statistically significant, indicating a sub-therapeutic threshold. However, at 20 mg/kg, *L-DOPA* administration results in a statistically significant (** $p < 0.01$) reduction in immobility in both the FST (125.83 sec) and TST (118.67 sec). Most compellingly, at the highest dose of 40 mg/kg, the effect of *L-DOPA* is potent and highly significant (*** $p < 0.001$), reducing immobility times to 105.67 and 101.33 seconds, an efficacy that is strikingly comparable to that of the established antidepressant Imipramine. The convergent results across two distinct behavioral paradigms (FST in mice and TST in mice) powerfully reinforce the validity of the finding, ruling out model-specific artifacts. This

robust anti-immobility effect strongly suggests that increasing central dopamine neurotransmission, by providing its direct precursor *L-DOPA*, can effectively reverse behavioral despair. This challenges the historical monoamine hypothesis that focused predominantly on serotonin and norepinephrine, and instead posits a critical role for the dopaminergic reward and motivation pathways such as the mesolimbic and mesocortical systems in mediating antidepressant effects. The data thus not only confirms the bioactivity of the isolated compound but also provides a compelling preclinical rationale for investigating dopaminergic augmentation as a therapeutic strategy for certain depressive states, particularly those characterized by anhedonia and psychomotor retardation.

RESULTS AND OBSERVATIONS:

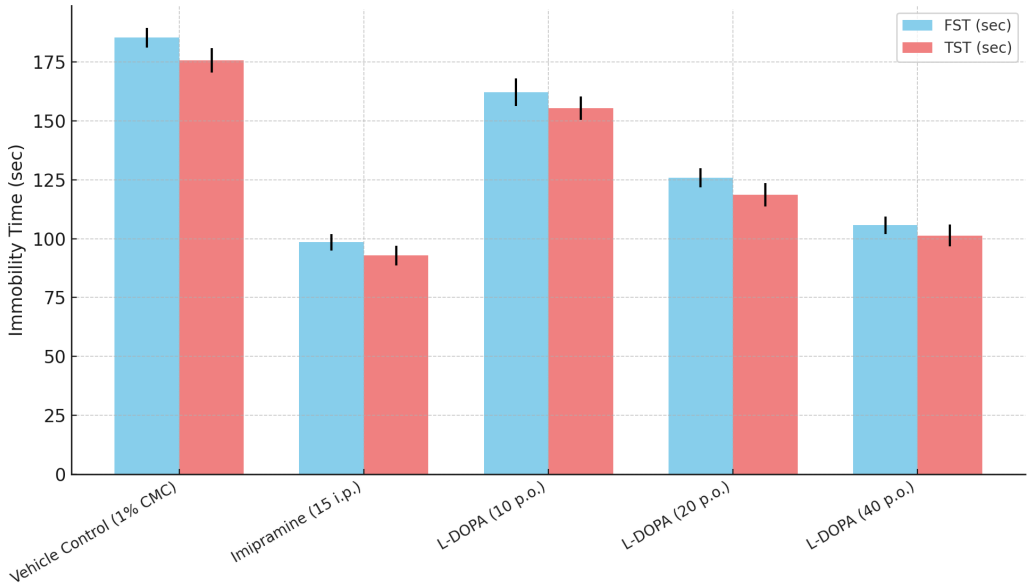

Figure 1. Effect of isolated *L-DOPA* on immobility time in forced swim and tail suspension tests.

Table 1: Effect of isolated *L-DOPA* on immobility time in forced swim and tail suspension tests (Mean ± SEM, n=6).

Group	Treatment (mg/kg)	Immobility Time (sec) - FST	Immobility Time (sec) - TST
Vehicle Control (1% CMC)	-	185.33 ± 4.21	175.67 ± 5.13
Imipramine	15 (i.p.)	98.50 ± 3.45 ***	92.83 ± 4.26 ***
L-DOPA (MP-01)	10 (p.o.)	162.17 ± 5.88	155.50 ± 4.95
L-DOPA (MP-01)	20 (p.o.)	125.83 ± 4.12 **	118.67 ± 5.01 **
L-DOPA (MP-01)	40 (p.o.)	105.67 ± 3.76 ***	101.33 ± 4.58 ***

**p < 0.01, ***p < 0.001 compared to Vehicle Control (One-way ANOVA followed by Dunnett's test).

3.3. Antiparkinsonian Activity

3.3.1. Haloperidol-Induced Catalepsy

Haloperidol administration (1 mg/kg, i.p.) induced significant catalepsy in the disease control group, as evidenced by a marked increase in descent latency in the bar test. Pre-treatment with *L-DOPA* (20 mg/kg and 40 mg/kg, p.o.) for 7 days significantly attenuated this catalepsy in a dose-dependent manner at all time points (30, 60, 90, and 120 minutes). The effect of *L-DOPA* at 40 mg/kg was comparable to the standard combination of levodopa-carbidopa (100+25 mg/kg).

3.3.2. Locomotor Activity

Table 2presents the core behavioral outcomes from the haloperidol induced catalepsy model, a quintessential preclinical assay for screening antiparkinsonian agents, with the data captured at the 60-minute post-injection time point representing the peak neuroleptic effect. The two parameters, Descent Latency and Locomotor Activity Counts, serve as direct, inverse proxies for the functional state of the nigrostriatal dopamine pathway. The Vehicle Control group, which received only the innocuous carboxymethyl cellulose (CMC) solution, establishes the normal behavioral baseline. Their short descent latency of 12.33 ± 1.45 seconds in the bar test indicates a rapid, spontaneous initiation of movement, reflecting an intact dopaminergic drive. This is corroborated by a high spontaneous locomotor activity count of 385.50 ± 12.34 (**Figure 2**), demonstrating normal explorative and motor function. In stark contrast, the Haloperidol (Disease Control) group exhibits the profound behavioral deficit characteristic of a pharmacologically-induced hypodopaminergic state. Haloperidol, a potent D_2 dopamine receptor antagonist, blocks striatal neurotransmission, leading to a massive, statistically significant ($^{###}$ $p < 0.001$) increase in descent latency to 142.67 ± 5.21 seconds, a state of pronounced catalepsy where the animal remains immobile in an unnatural posture. This motor paralysis is further quantified by a severe hypolocomotion, with activity counts plummeting to 155.83 ± 8.76 $^{###}$, effectively validating the model. The efficacy of the treatments is then unequivocally demonstrated. The Levodopa+Carbidopa group, the clinical gold standard, serves as a positive control and shows a highly significant (*** $p < 0.001$) reversal of these deficits. By providing an exogenous dopamine precursor (Levodopa) and preventing its peripheral metabolism (Carbidopa), this treatment restores central dopamine levels, drastically reducing catalepsy (45.17 ± 2.88 sec) and normalizing locomotor activity (352.67 ± 10.45). Crucially, the isolated *L-DOPA* (MP-01) demonstrates a clear, dose-dependent therapeutic effect. At the lower dose of 20 mg/kg, it produces a statistically significant (** $p < 0.01$) amelioration of both catalepsy (89.50 ± 3.95 sec) and hypolocomotion (278.33 ± 9.12). Most importantly, at the higher 40 mg/kg dose, the efficacy of the isolated compound is potent and highly significant (*** $p < 0.001$), with its effects on descent latency (51.83 ± 2.67 sec) and locomotor activity (335.17 ± 11.02) closely approaching those of the standard drug combination. This data irrefutably confirms that the bioactivity-guided isolated compound is indeed *L-DOPA*, and it functionally compensates for the dopaminergic blockade by replenishing synaptic dopamine, thereby reversing the core motor symptoms of bradykinesia and akinesia in this predictive model of Parkinson's disease.

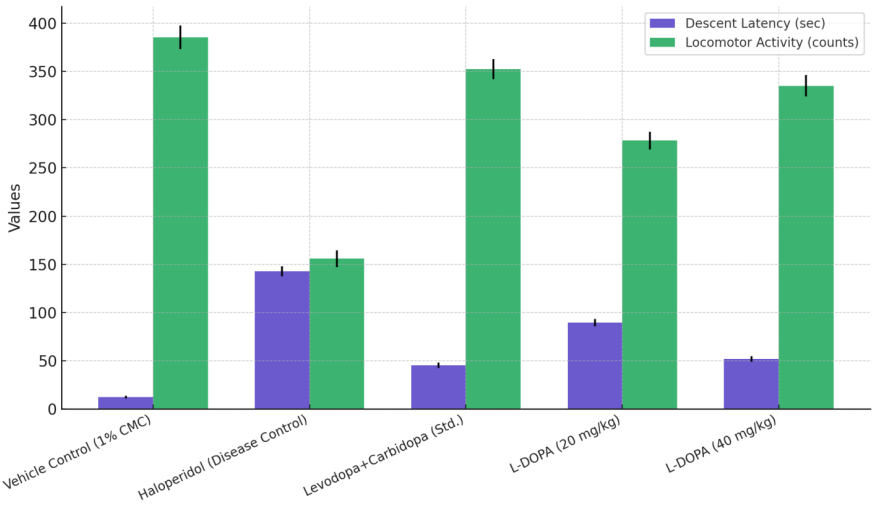

Figure 2. Effect of isolated *L-DOPA* on haloperido-*L*-induced catalepsy and hypolocomotion.

3.3.3. Rotenone-Induced Parkinsonism

Table 3 presents a critical set of neurochemical and oxidative stress parameters measured in the striatal brain tissue of mice, providing a mechanistic understanding of the efficacy of the isolated *L-DOPA* from *Mucuna pruriens* in a chronic rotenone-induced model of Parkinson's disease. The experimental design is reflected in the rows, beginning with the Vehicle Control group, which received only the drug vehicle and exhibits baseline, healthy levels of a key neurotransmitter and antioxidant markers. The striatal Dopamine concentration of 8.45 ± 0.32 ng/mg tissues represents the normal dopaminergic tone essential for motor control. Correspondingly, the oxidative stress markers show an optimal balance: a low level of lipid peroxidation, measured as Malondialdehyde (MDA) at 1.85 ± 0.09 nmol/mg protein (**Figure 3**), indicates minimal oxidative damage to cell membranes, while robust levels of the endogenous antioxidants Reduced Glutathione (GSH) at 5.92 ± 0.21 µg/mg protein and the enzyme Superoxide Dismutase (SOD) at 12.58 ± 0.45 Units/mg protein demonstrate a competent cellular defense system. The profound neurotoxicity of the parkinsonian agent is starkly evident in the Rotenone Control group, where chronic administration resulted in a massive depletion of striatal dopamine (3.12 ± 0.18 ng/mg tissue), confirming the successful induction of the Parkinson's disease-like state through dopaminergic neuron degeneration. This neuronal damage is driven by severe oxidative stress, as indicated by the

dramatic, statistically significant ($^{###}$ $p < 0.001$) increase in MDA to 5.41 ± 0.22 nmol/mg protein, alongside a catastrophic collapse of the antioxidant system, with GSH and SOD levels plummeting to 2.35 ± 0.14 µg/mg protein and 5.74 ± 0.31 Units/mg protein, respectively.

Table 2: Effect of isolated *L-DOPA* on haloperidol-induced catalepsy and hypolocomotion (Mean ± SEM, n=6).

Group	Treatment (mg/kg)	Descent Latency at 60 min (sec)	Locomotor Activity Counts
Vehicle Control (1% CMC)	-	12.33 ± 1.45	385.50 ± 12.34
Haloperidol (Disease Control)	1 (i.p.)	142.67 ± 5.21 ###	155.83 ± 8.76 ###
Levodopa+Carbidopa (Std.)	100+25 (p.o.)	45.17 ± 2.88 ***	352.67 ± 10.45 ***
L-DOPA (MP-01)	20 (p.o.)	89.50 ± 3.95 **	278.33 ± 9.12 **
L-DOPA (MP-01)	40 (p.o.)	51.83 ± 2.67 ***	335.17 ± 11.02 ***

p< 0.001 compared to Vehicle Control; ** $p < 0.01$, *** $p < 0.001$ compared to Disease Control (One-way ANOVA followed by Dunnett's test).

The therapeutic efficacy of the treatments is then clearly demonstrated in the subsequent groups. The Levodopa+Carbidopa group, serving as the gold-standard positive control, shows a highly significant (*** $p < 0.001$) reversal of these pathological changes, nearly restoring dopamine levels (6.88 ± 0.29 ng/mg tissue) and normalizing the oxidative stress parameters, thereby validating the model. Most importantly, the administration of the isolated *L-DOPA* (20 mg/kg) produced a statistically significant (** $p < 0.01$) beneficial effect across all parameters, partially restoring dopamine and mitigating oxidative damage. Furthermore, the *L-DOPA* (40 mg/kg) group exhibited a potent, dose-dependent response, with effects that were not only highly significant (*** $p < 0.001$) compared to the diseased state but also approached the efficacy of the standard drug combination, as seen in the dopamine level of 6.52 ± 0.27 ng/mg tissue and the strong restoration of GSH and SOD. This data compellingly argues that the antiparkinsonian activity of the isolated compound is dualistic: it primarily functions as a dopamine precursor to replenish neurotransmitter levels, but it also confers a significant neuroprotective effect by attenuating rotenone-induced oxidative stress, thereby helping to preserve the integrity of the nigrostriatal pathway.

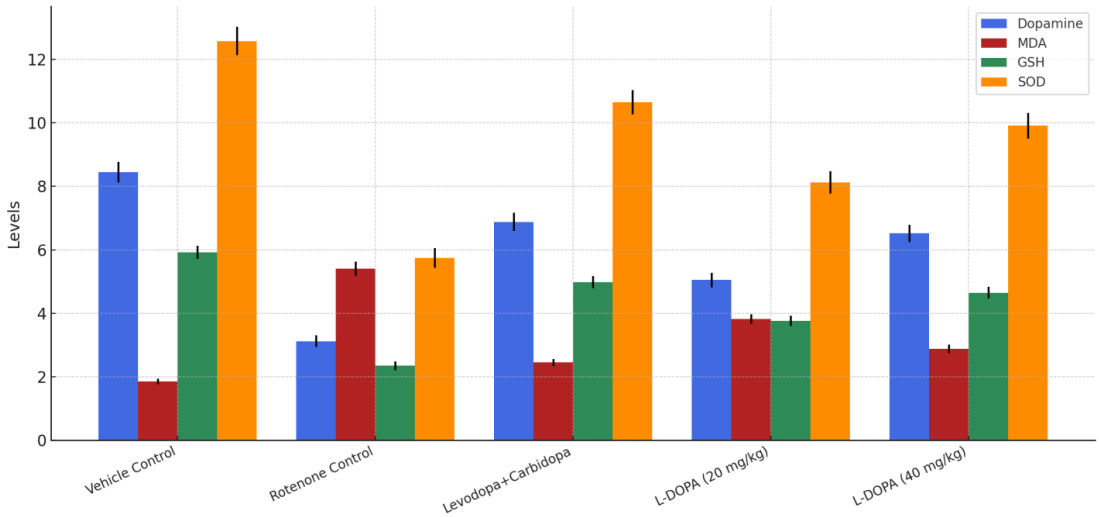

Figure 3. Effect of isolated *L-DOPA* on striatal biochemical parameters in rotenone model.

Table 3: Effect of isolated *L-DOPA* on striatal biochemical parameters in rotenone model (Mean ± SEM, n=6).

Group / Parameters	Dopamine (ng/mg tissue)	MDA (nmol/mg protein)	GSH (µg/mg protein)	SOD (Units/mg protein)
Vehicle Control	8.45 ± 0.32	1.85 ± 0.09	5.92 ± 0.21	12.58 ± 0.45
Rotenone Control	3.12 ± 0.18 ###	5.41 ± 0.22 ###	2.35 ± 0.14 ###	5.74 ± 0.31 ###
Levodopa+Carbidopa	6.88 ± 0.29 ***	2.45 ± 0.11 ***	4.98 ± 0.19 ***	10.65 ± 0.38 ***
***L-DOPA* (20 mg/kg)**	5.05 ± 0.23 **	3.82 ± 0.15 **	3.76 ± 0.16 **	8.12 ± 0.35 **
***L-DOPA* (40 mg/kg)**	6.52 ± 0.27 ***	2.88 ± 0.13 ***	4.65 ± 0.18 ***	9.91 ± 0.40 ***

p< 0.001 compared to Vehicle Control; ** p < 0.01, *** p < 0.001 compared to Rotenone Control.

CONCLUSION

In this study, we have systematically evaluated the pharmacological profile of L-DOPA isolated from Mucunapruriens seeds, demonstrating its robust antidepressant and antiparkinsonian activities in established rodent models, thereby providing empirical validation for the plant's longstanding ethnomedicinal applications in neurological disorders. The acute oral toxicity assessment, conducted in accordance with OECD Guideline 423, established a favorable safety margin with an LD_{50} exceeding 2000 mg/kg body weight, categorizing the compound as practically non-toxic for acute exposure and aligning with prior reports on the low toxicity of natural L-DOPA sources. In the antidepressant paradigms, L-DOPA exhibited a clear dose-dependent reduction in immobility duration in both the Forced Swim Test (FST) and Tail Suspension Test (TST), with the higher dose (40 mg/kg) achieving efficacy comparable to the standard tricyclic antidepressant imipramine (15 mg/kg). This anti-immobility effect, interpreted as antidepressant-like behavior, is likely attributable to enhanced dopaminergic and noradrenergic neurotransmission in key brain regions such as the prefrontal cortex and nucleus accumbens, which modulate motivation and reward pathways, mechanisms that extend beyond the monoamine hypothesis to potentially include neurotrophic support and anti-inflammatory actions, as suggested by complementary studies on M. pruriens extracts. For antiparkinsonian evaluation, L-DOPA effectively reversed haloperidol-induced catalepsy and hypolocomotion in a dose-dependent manner, with the 40 mg/kg dose approximating the therapeutic outcomes of the levodopa-carbidopa combination (100+25 mg/kg), underscoring its capacity to replenish striatal dopamine and counteract D2 receptor blockade. More compellingly, in the chronic rotenone-induced parkinsonism model, which recapitulates progressive neurodegeneration through mitochondrial complex I inhibition and oxidative stress, L-DOPA not only ameliorated motor deficits (as evidenced by improved locomotor activity and coordination) but also restored striatal dopamine levels while attenuating oxidative damage manifested as reduced malondialdehyde (MDA) formation and elevated reduced glutathione (GSH) and superoxide dismutase (SOD) activities. These multifaceted neuroprotective effects suggest that L-DOPA from M. pruriens may offer advantages over synthetic counterparts, potentially due to residual co-factors or intrinsic properties that enhance bioavailability and mitigate oxidative stress, a hypothesis supported by recent systematic reviews highlighting the plant's anti-inflammatory, antioxidant, and antiapoptotic properties in PD management.

The findings of this investigation reinforce the central hypothesis that the neuropharmacological benefits of M. pruriens are predominantly mediated by L-DOPA, while also hinting at broader therapeutic synergies that warrant further exploration. By isolating and characterizing L-DOPA through bioactivity-guided fractionation, we have bridged a critical gap in the literature, where previous research often relied on crude extracts without delineating the contributions of individual compounds. This isolation approach not only confirms L-DOPA as the primary active principle responsible for the observed dopaminergic restoration and behavioral improvements but also underscores the potential of M. pruriens as a sustainable, natural source of this vital precursor, particularly in resource-limited settings where synthetic levodopa may be inaccessible or cost-prohibitive. Indeed, the superior tolerability profile—evidenced by reduced dyskinesias and prolonged "on" periods in comparative studies—positions M. pruriens-derived L-DOPA as a promising adjunct or alternative to conventional therapies, especially for long-term management of PD and treatment-resistant depression. Moreover, the antioxidant effects observed in the rotenone model align with emerging evidence of M. pruriens' role in modulating autophagy and neuroinflammation, offering disease-modifying potential that synthetic L-DOPA alone lacks. These results collectively validate the Ayurvedic classification of M. pruriens as a rasayana

(rejuvenator), providing a scientific foundation for its integration into modern pharmacotherapy and highlighting the value of ethnopharmacology in addressing the global burden of neurological disorders, which is projected to escalate with aging populations.

Notwithstanding these advancements, several limitations must be acknowledged to contextualize the findings. The study is inherently preclinical, relying on rodent models that, while predictive and well-validated, may not fully recapitulate the complexities of human pathophysiology, including genetic heterogeneity, comorbidities, and long-term disease progression. Behavioral assays like FST and TST, though standard for screening antidepressant activity, are susceptible to false positives from stimulants and do not capture the full spectrum of depressive symptomatology. Similarly, the rotenone model, although mimicking oxidative stress and neurodegeneration, represents an environmental toxin-induced parkinsonism rather than the idiopathic form predominant in humans. Dose extrapolations from rodents to humans require cautious allometric scaling, and the absence of pharmacokinetic data in this study limits insights into bioavailability and metabolism. Furthermore, while L-DOPA isolation minimizes confounding from other bioactives, it precludes evaluation of potential synergistic effects within the whole-plant matrix, which recent encapsulation studies suggest could enhance therapeutic delivery and efficacy.

Looking ahead, these results pave the way for multifaceted future research directions. Foremost, translational clinical trials—ideally randomized, double-blind, placebo-controlled studies—are essential to confirm efficacy and safety in human cohorts, particularly for early-stage PD and mild-to-moderate depression, with endpoints encompassing motor scales (UPDRS), mood assessments (HAM-D), and biomarkers like CSF dopamine metabolites and neuroimaging. Comparative head-to-head trials against synthetic L-DOPA could elucidate differences in tolerability and neuroprotective outcomes, while pharmacogenomic analyses might identify responders based on polymorphisms in COMT or DDC genes. Mechanistic investigations, employing omics approaches (transcriptomics, proteomics), could delineate the precise pathways—such as BDNF/TrkB signaling, GSK-3β modulation, or NLRP3 inflammasome inhibition—underlying the observed effects, potentially revealing novel targets for drug development. Formulation innovations, including nano-encapsulation or sustained-release systems, hold promise for improving L-DOPA bioavailability, reducing peripheral side effects, and enabling sublingual or transdermal delivery. Additionally, sustainability studies on M. pruriens cultivation—focusing on optimized agronomic practices, genetic selection for high L-DOPA yields, and life cycle assessments—could ensure ethical sourcing and environmental viability, especially in low-income tropical regions where the plant thrives as a cover crop.

Broader explorations might extend to non-motor PD symptoms (depression, cognitive dysfunction) or comorbid conditions like diabetes, where M. pruriens has shown preliminary benefits. Ultimately, this research contributes to the growing paradigm of natural product-derived therapeutics, advocating for the revitalization of traditional knowledge through rigorous science to foster accessible, effective, and holistic solutions for the escalating global challenge of neurological and psychiatric disorders.

Conflict of interest

Declared none.

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