International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 3, 2025
https://theaspd.com/index.php

Combining Network Pharmacology and Neuroprotection: The Role of Mucuna pruriens in Huntington's Disease Management

Pallavi V. Bhosle
School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India.
Sailesh J. Wadher
School of Pharmacy, Swami Ramanand Teerth Marathwada University, Nanded, Maharashtra, India.

ABSTRACT

Objective: The present study investigated the phytochemical composition and neuroprotective potential of Mucuna pruriens extract in treating Huntington's disorder (HD) by focusing on their antioxidant and anti-inflammatory properties through both in vitro and in vivo rat models.

Material and methods:Wistar rats were divided into five groups, with each group consisting of eight animals, and given Mucuna pruriens extract at 100, 200, and 400 mg/kg orally for 14 days. Huntington's disease was induced with 3-nitropropionic acid at 10 mg/kg/day intraperitoneally for two weeks. Network pharmacology identified target pathways, and antioxidant and anti-inflammatory effects were assessed with DPPH, ABTS, and mediator inhibition assays. Neuroprotection was evaluated through behavioural assessments and oxidative stress markers.

Results:Phytochemical and network pharmacology analyses of Mucuna pruriens extract identified high L-DOPA levels and multiple molecular targets relevant to Huntington's disease. The extract exhibited antioxidant and anti-inflammatory effects, reducing reactive oxygen species, lipid peroxidation, and inflammatory mediators. In a 3NP-induced HD model, Mucuna pruriens improved motor function, modulated oxidative stress, and preserved neuronal integrity, indicating its potential to mitigate HD progression.

Conclusion:Mucuna pruriens extract demonstrates significant neuroprotective effects against Huntington's disease through its antioxidant and anti-inflammatory properties. Future studies should explore its molecular mechanisms and clinical efficacy.

Keywords:Mucuna pruriens, Huntington's disease, neuroprotection, antioxidant, anti-inflammatory, L-DOPA, oxidative stress, Wistar rats, Network pharmacology, DPPH assay, ABTS assay

INTRODUCTION

Huntington's disease (HD) is a progressive neurodegenerative disorder affecting approximately 5 to 10 individuals per 100,000 people.[1] The disease typically manifests in mid-adulthood, with symptoms that worsen over time, leading to significant impairments in motor function, cognition, and mental health. HD is characterized by involuntary movements, cognitive decline, and psychiatric disturbances, all of which severely impact the quality of life and create a substantial caregiver burden.[2] The underlying genetic cause of HD is an expanded CAG repeat within the huntingtin (HTT) gene, leading to the synthesis of a mutated form of the huntingtin protein that contains an abnormal polyglutamine (polyQ) expansion. This mutation triggers a cascade of neurotoxic events that result in the progressive degeneration of specific neuronal populations, particularly in the striatum and cortex.[3]

To study HD, researchers often employ animal models, including those induced by 3-nitro propionic acid (3NP), a neurotoxin that mimics the neurodegenerative process seen in HD.[4] 3NP inhibits succinate dehydrogenase, causing mitochondrial dysfunction and energy depletion, which results in striatal neuron death and behavioral deficits akin to those experienced by HD patients.[5] These models enable the evaluation of potential therapeutic agents' neuroprotective effects, thus contributing to a deeper understanding of HD and the development of effective treatment strategies.

Given the serious nature of this disease and the lack of effective disease-modifying treatments, there is an urgent need for innovative therapeutic approaches. Recent research has increasingly focused on natural products as potential sources of neuroprotective agents, particularly those that possess

antioxidant and anti-inflammatory properties. Among these, *Mucuna pruriens*, a leguminous plant traditionally used in various medicinal systems, has garnered attention for its rich phytochemical composition, which includes L-DOPA, flavonoids, and alkaloids.[6] These bioactive compounds have been shown to exhibit neuroprotective effects, making Mucuna pruriens a promising candidate for addressing the challenges posed by neurodegenerative diseases, including Huntington's disease.[7]

The current study aims to investigate the phytochemical composition and neuroprotective effects of *Mucuna pruriens* extract in the context of HD, focusing on its antioxidant and anti-inflammatory mechanisms. By employing both in vitro and in vivo models, we seek to provide insight into the potential therapeutic benefits of this extract in alleviating the symptoms and progression of Huntington's disease.

MATERIALS AND METHODS

Network Pharmacology

Network pharmacology was conducted to explore potential mechanisms of L-DOPA in Huntington's disease (HD) treatment. The structures of L-DOPA were retrieved from the ChEMBL database, and HD-related targets were identified using the Swiss Target Prediction database. To screen drug-like phytoconstituents, the pharmacokinetic properties of compounds were evaluated using the SwissADME online tool. Compounds meeting the criteria of quantitative estimate of drug-likeness (QED) ≥ 0.18 and oral bioavailability ≥ 30% were selected for further analysis. These thresholds are consistent with prior studies in natural product-based drug discovery. Relevant biological pathways were analyzed using the KEGG database, with data organized in Excel and visualized in Cytoscape 3.7.2 to illustrate gene/protein and drug interactions.

Molecular Docking

The top five targets from network pharmacology were selected for molecular docking. Three-dimensional structures of these targets were retrieved from the Protein Data Bank (PDB), and L-DOPA was docked to evaluate binding interactions using Schrodinger's Maestro and Glide software, focusing on binding affinity as determined by docking scores and binding energy.

Plant Material Collection and Authentication

Fresh leaves of *Mucuna pruriens* were collected from verified sources and authenticated by the Botanical Survey of India. After air-drying at room temperature for 30 days, the plant material was powdered and stored for extraction.

Preparation of Extracts

The powdered Mucuna pruriens leaves were initially defatted with petroleum ether and then extracted with ethanol using a Soxhlet apparatus over a 60-hour period. Subsequently, chloroform extraction was performed, followed by aqueous extraction via cold maceration in water. All extracts were filtered, concentrated using a rotary evaporator, and stored under appropriate conditions.[8]

Organoleptic and Physicochemical Analysis

The organoleptic properties of *Mucuna Pruriens* leaves and bark were assessed for color, odor, taste, and texture. Physicochemical analyses included measurements of moisture content, pH, extractive values (using alcohol and water), loss on drying, and ash content (total, water-soluble, acid-insoluble, and sulfated) to evaluate purity and stability, were determined following WHO guidelines. [9]

Preliminary Phytochemical Screening

Phytochemical screening of all extracts was conducted to identify the presence of bioactive compounds, including alkaloids, carbohydrates, glycosides, phenolic compounds, flavonoids, proteins, saponins, sterols, acidic compounds, steroids, fixed oils, fats, and terpenoids.

Quantitative Estimation

The Folin–Ciocalteu reagent method was used to determine the total phenolic content, which was expressed in mg of gallic acid equivalents per gram of extract. Total flavonoid content was quantified via the aluminum chloride colorimetric method, with results expressed as mg of quercetin equivalents per gram of extract.

In-vitro antioxidant assay:

Determination of DPPH (2,2-diphenyl-1-picrylhydrazyl) radical-scavenging activity:

The DPPH free radical scavenging assay was used to evaluate antioxidant activity. A 0.1 mM DPPH solution in ethanol was mixed with extract solutions (1-5 µg/ml) and incubated in the dark for 30 minutes. Absorbance was measured at 517 nm, with ascorbic acid as the reference compound. [10]

Assay of hydrogen peroxide (H_2O_2) scavenging activity:

The H_2O_2 scavenging activity was evaluated using a modified method. A sample (10-320 µg/ml) was mixed with phosphate buffer (pH 7.4) and H_2O_2 (40 mM), then incubated at room temperature for 10 minutes. Absorbance was recorded at 230 nm, and ascorbic acid served as the positive control. [11]

Reducing power assay for antioxidant activity:

The reducing power was evaluated by mixing extract (10-320 µg/ml) with sodium phosphate buffer and potassium ferricyanide, followed by incubation at 50°C for 20 minutes. Trichloroacetic acid was added, and the mixture was centrifuged. The upper layer was then mixed with deionized water and ferric chloride, and absorbance was measured at 700 nm. The procedure was repeated three times, and EC_{50} was calculated from the concentration-absorbance graph. Ascorbic acid was used as the standard. [12]

Each assay was performed in triplicate, and results were statistically analyzed to confirm significance.

MTT Assay for Cytotoxicity Assessment:

The MTT assay was utilized to assess the cytotoxicity of *Mucuna pruriens* extracts by measuring cell viability after 24-hour exposure to various concentrations of the ethanolic and chloroform extracts from both leaves and bark. Results indicated cell viability levels exceeding 75%, suggesting the extracts' biocompatibility and potential for therapeutic use. Each assay was conducted in triplicate to ensure reliability.[13]

In Vivo Study

Experimental animals

Adult male Wistar rats (weighing 250–300 g) were housed under standard laboratory conditions (temperature: 23 ± 2°C, humidity: 55 ± 10%, light-dark cycle: 12:12 hours) with access to a standard pellet diet and water. The study protocol was approved by the Institutional Animal Ethics Committee (IAEC) and adhered to CPCSEA guidelines (animal house registration no. ????).

Induction of Huntington's disease-like symptoms

3-NP (10mg/kg) was repeatedly administered intraperitoneally for 14 days to induce the symptoms of HD.

Acute Toxicity Study

An acute toxicity study was conducted according to OECD guidelines using Wistar rats (150-200 g). The animals were randomly divided into three groups (n=3): Group I received 0.5% carboxymethyl cellulose (CMC) orally as the vehicle control, Group II was administered 300 mg/kg of the isolated phytoconstituent in 0.5% CMC orally, and Group III received 2000 mg/kg of the isolated phytoconstituent in 0.5% CMC orally. The rats were observed for 14 days for signs of toxicity and behavioral changes, with the maximum tolerated dose established to guide further experiments.

Experimental Design and Treatment Protocol

A total of five groups of Wistar rats (n=8 per group) were utilized for this study. Group I served as the vehicle control group and received no treatment. Group II was administered 3-NP at a dosage of 10 mg/kg via intraperitoneal injection for two weeks to induce neurological impairment. Groups III, IV, and V received the plant's ethanol extract (EEMP) in conjunction with 3-NP, at dosages of 100 mg/kg, 200 mg/kg, and 400 mg/kg, respectively, administered orally for the same duration.

Measurement of body weight

Body weight was noted on the first and last days of the experiment. Percentage change in body weight was calculated compared to the initial body weight on the first day of experimentation.

Behavioral assessment

The effect of Behavioral parameters was assessed on the 8^{th}, 11^{th}, and 14^{th} days.

Assessment of motor activity

Assessment of locomotor activity

Motor activity was measured using an automated electronic activity meter (Opto-Varimex 4, Columbus Instruments, USA). Infrared beams along the x and y axes detected horizontal and vertical movements, with each beam interruption recorded on a digital counter. Each animal was tested individually, and motor activity was recorded over 2 minutes.[14]

Assessment of movement analysis

3-NP treatment was associated with severe behavioral defects that prevent animals from walking. The severity of the 3-NP-induced motor abnormalities in these groups was evaluated using a quantitative neurological scale adapted from Ludolph et al. (1991): normal behavior; score 1, general slowness of displacement resulting from mild hind limb impairment; score 2, incoordination and marked gait abnormalities; score 3, hind limb paralysis; score 4, incapacity to move resulting from forelimb and hind limb impairment; score 5, recumbency.[15]

String test for grip strength

The rat was allowed to hold with the forepaws a steel wire (2 mm in diameter and 35 cm in length), placed at a height of 50 cm over a cushion support. The length/duration of time that the rat was able to hold the wire was recorded. This latency to the grip loss is considered as an indirect measure of grip strength (Shear et al., 1998)[16]

Limb withdrawal test

In this behavioral test, each rat was placed on a 20 cm high, 30×30 cm Perspex platform with four holes: two 5 cm holes for the hind limbs and two 4 cm holes for the forelimbs. The rat was positioned so that first the hind limbs and then the forelimbs were placed in the holes. Retraction times for the first and contralateral hind limbs were recorded, and the difference in retraction times was calculated as a measure of hind limb function and striatal degeneration. (Vis et al., 1999).[17]

Assessment of biochemical parameters

Biochemical tests were carried out immediately after behavioral observations on day 15 following 3-NP administrations.

Tissue preparation

All groups of rats were anesthetized with ether, and blood samples were collected for biochemical assays. Twenty-four hours after the final day of treatment (day 15), the animals were sacrificed by cervical dislocation. The striatum, midbrain, and cortex were then isolated and homogenized separately at 4°C in 0.1 M phosphate buffer solution (pH 7.4) for 15 minutes. The resulting supernatant was used for the estimation of biochemical markers, neuroinflammatory factors, and neurotransmitter levels.

Estimation of lipid peroxidation

Lipid peroxidation was assessed by measuring malonaldehyde (MDA) levels in tissue homogenates. Following Ohkawa et al., 0.1 ml of tissue sample was mixed with 2 ml of a thiobarbituric acid reaction mixture, boiled for 30 minutes, cooled, and centrifuged at 4830×g for 10 minutes. Absorbance was read at 532 nm (Shimadzu UV-1800). MDA levels were expressed as nmol/g tissue using an extinction coefficient of 1.56×10^5 M−1 cm−1.[18]

Estimation of reduced glutathione

Reduced glutathione (GSH) was measured using Ellman's method. Tissue homogenate was mixed with 10% trichloroacetic acid, centrifuged, and the supernatant was combined with phosphate buffer (pH 8) and DTNB reagent. Absorbance was measured at 412 nm, and GSH levels were expressed as nmol/g tissue using an extinction coefficient of 1.36×10^3 M−1 cm−1.[19]

Determination of catalase

Catalase was assayed as described by Sinha (1972). The reaction mixture (1.5 ml) contained 1.0 ml of 0.01 mol/l phosphate buffer (pH 7), 0.1ml of brain homogenate supernatant and 0.4ml of 2 mol/l hydrogen peroxide. The reaction was stopped by the addition of 2 ml of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in a 1:3 ratio). The absorbance was measured at 620 nm and expressed as μ mol of hydrogen peroxide consumed per min per mg protein. 2.7.5. [20]

Determination of superoxide dismutase

Superoxide dismutase (SOD) activity is measured according to a method described by Misra and Fridovich(1972). Auto oxidation of epinephrine at pH 10.4 was spectrophotometrically measured. In this method, the supernatant of the tissue was mixed with 0.8 ml of 50 mM glycine buffer, pH 10.4, and the reaction was started by the addition of 0.02 ml (−)-epinephrine. After 5 min the absorbance was measured at 480 nm (UV-1700 Spectrophotometer, Shimadzu, Japan). The activity of SOD was expressed as % activity of vehicle-treated control.[21]

Estimation of nitrite

The accumulation of nitrite in the supernatant, an indicator of the production of nitric oxide, was determined with a colorimetric assay with Greiss reagent (0.1% N-(1-naphthyl) ethylenediamine dihydrochloride, 1% sulfanilamide, and 2.5% phosphoric acid) as described by Green et al., 1982. Equal volumes of supernatant and Greiss reagent were mixed, the mixture was incubated for 10 min at room temperature in the dark and the absorbance was noted at 540 nm using a UV-1700 Spectrophotometer (Shimadzu, Japan). The concentration of nitrite in the supernatant was determined from a sodium nitrite standard curve and expressed as μmol per mg protein.[22]

Estimation of Protein

The protein content was measured by the biuret method using bovine serum albumin as standard.

Estimation of pro-inflammatory cytokines

The concentration of Interleukin-1β and tumor necrosis factor (IL-1β, and TNF-α) in tissue homogenate was analyzed by using ELISA antibody kits. The final concentration of inflammatory markers was analyzed from the standard curve (Barksby et al., 2007).[23]

Standard drugs and chemicals used

Standard drugs and chemicals for in vivo testing are primarily sourced from Sigma-Aldrich Ltd., and sodium nitroprusside is supplied by Molyhem Pvt. Ltd., India, while Nicotinamide comes from Finar India Ltd. 1,1,3,3-Tetramethoxypropane is provided by V.K. Chemicals, India, and carboxymethyl cellulose is available from various suppliers. lipid profile estimation kits were procured from Erba Diagnostics Pvt. Ltd. and other chemicals used in the present study were of analytical quality. All the drug solutions were freshly prepared before use.

Histopathological Analysis

Brain tissue was harvested from the sacrificed rats and fixed in a 10% neutral buffered formalin solution, dehydrated in ethanol, and embedded in paraffin. Sections of 5 μm thickness were prepared using a rotary microtome and stained with hematoxylin and eosin (H and E) dye for microscopic observations. Images were visualized and captured at 40X magnification.[24]

Statistical analysis

Data were presented as mean ± S.EM. for continuous variables, a t-test was performed to differentiate the mean difference. For comparison between more than two groups, the data were processed by one-way analysis of variance (ANOVA) followed by Tukey's multiple comparison test. $P<0.05$ was considered statistically significant. Statistical analysis was performed using SPSS version 21.

Results

Network Construction

Identification of Targets for Mucuna pruriens

The network pharmacology analysis for *Mucuna pruriens* (MP) identified a set of potential protein targets involved in the modulation of neurological pathways. The targets identified for L-DOPA, a principal compound of MP, include TH, EGFR, DRD2, ESR1, and GAPDH. These targets are known to play critical roles in pathways associated with neurodegenerative diseases such as Huntington's disease (HD).

Interaction Network of Targets

To understand the interactions among these targets, the STRING database was used to create an interaction network. This network, illustrated in (Figure 2,3) shows the connectivity between L-DOPA's targets, indicating their involvement in various biochemical pathways. The visualization helps identify high-confidence interactions that could be leveraged for targeted therapeutic strategies.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 3, 2025
https://theaspd.com/index.php

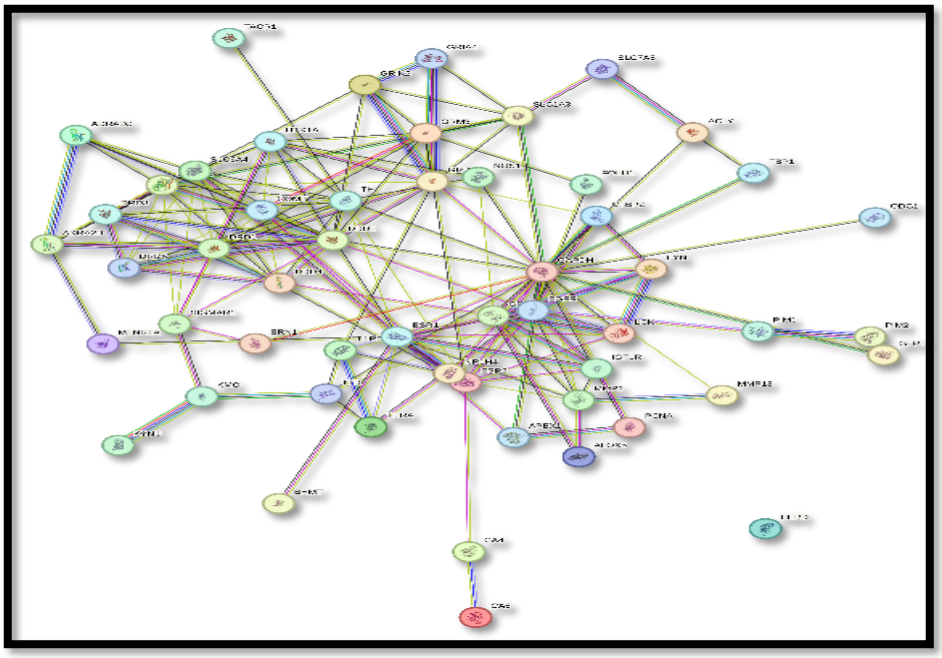


Figure 2. Interaction among common targets of L-DOPA and HD

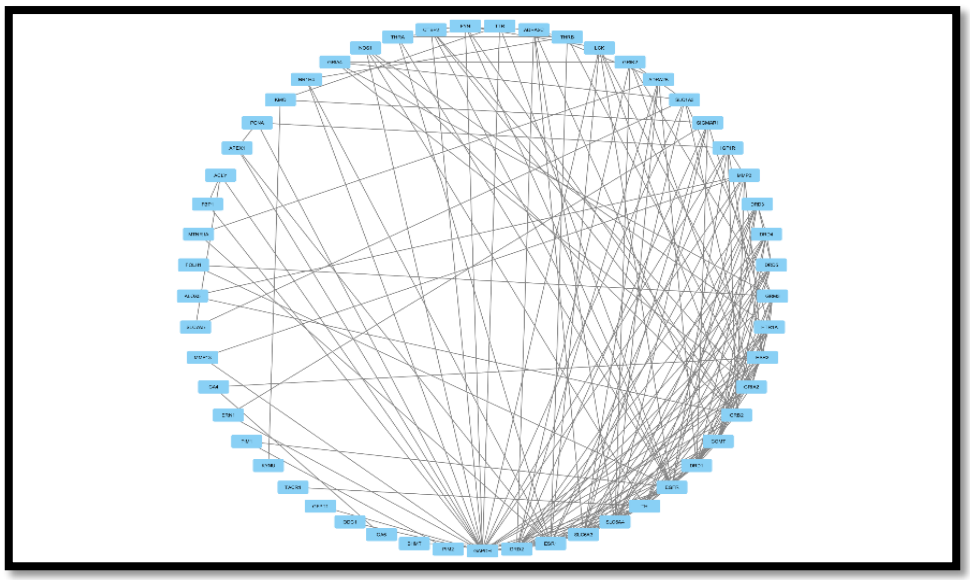


Figure 3: Network of L-DOPA with various targets of HD

Top Targets of L-DOPA

From the interaction analysis, L-DOPA demonstrated significant potential in modulating five key targets: TH, EGFR, DRD2, ESR1, and GAPDH. These targets were prioritized based on their central role in neurological processes, as shown in Figure 4. The ability of L-DOPA to influence these targets suggests its potential for modifying disease pathways relevant to HD.

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 3, 2025
https://theaspd.com/index.php

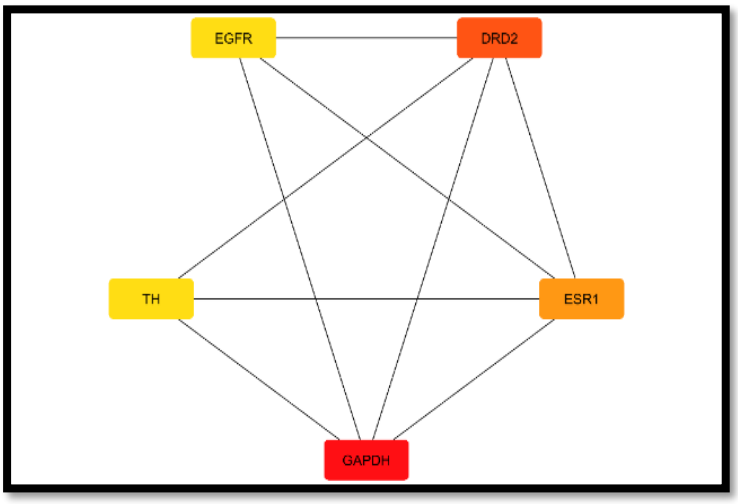
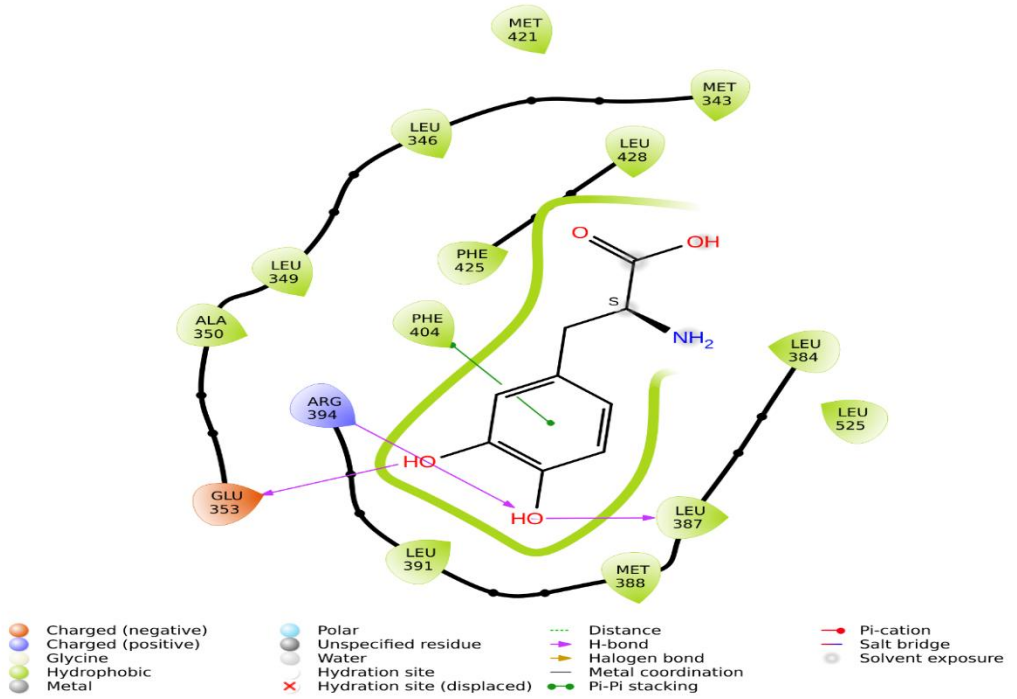


Figure 4.: Top 5 targets of L-DOPA in HD

Molecular Docking Results for L-DOPA



The molecular docking analysis provided insights into the binding interactions of L-DOPA with its identified targets (Table 2). The docking studies revealed notable binding affinities and interactions as follows:

ESR1 (PDB ID: 1SJO): A docking score of -7.307 was observed for the interaction between L-DOPA and ESR1. Hydrogen bonds were formed with ARG-394 and LEU-353, suggesting a strong binding potential that may be relevant in modulating estrogen-related pathways in neurological contexts. (Figure 5)

Interaction of L-DOPA with GAPDH (PDB ID: 2VYV)

L-DOPA exhibited strong binding with GAPDH, evidenced by a docking score of -6.592. Hydrogen bonds were formed with ARG-10 and ALA-94, alongside hydrophobic interactions involving ALA-180

and ILE-11. These results suggest L-DOPA's potential role in influencing GAPDH-related metabolic and neurodegenerative pathways. (Figure 6)

EGFR (PDB ID: 4I23): L-DOPA demonstrated a robust binding affinity, with a docking score of -6.704. The binding interactions included hydrogen bonds formed with the amino acid residues GLU-762 and LYS-745, supported by multiple hydrophobic interactions that contributed to the stability of the binding. (Figure 7)

DRD2 (PDB ID: 6CM4): The docking score for L-DOPA's interaction with DRD2 was -7.361. This interaction was characterized by hydrogen bonding involving key residues such as SER-193 and ASP-114, indicating a stable binding configuration that supports its neuroactive role. (Figure 8)

1SJO-L-DOPA
Figure 5.: Interaction of L-DOPA with ESR-1 (1SJO)

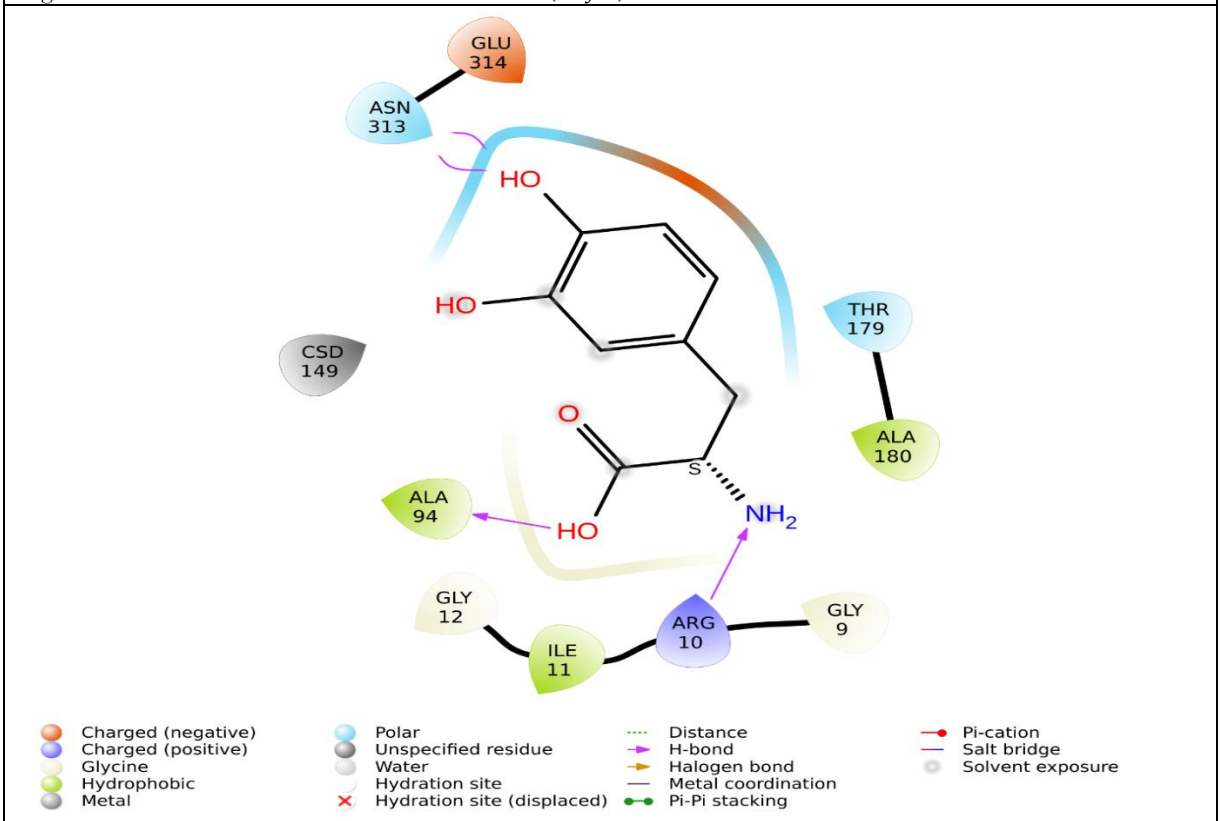


Figure 6: Interaction of L-DOPA with GAPDH (2VYV)

International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 3, 2025
https://theaspd.com/index.php

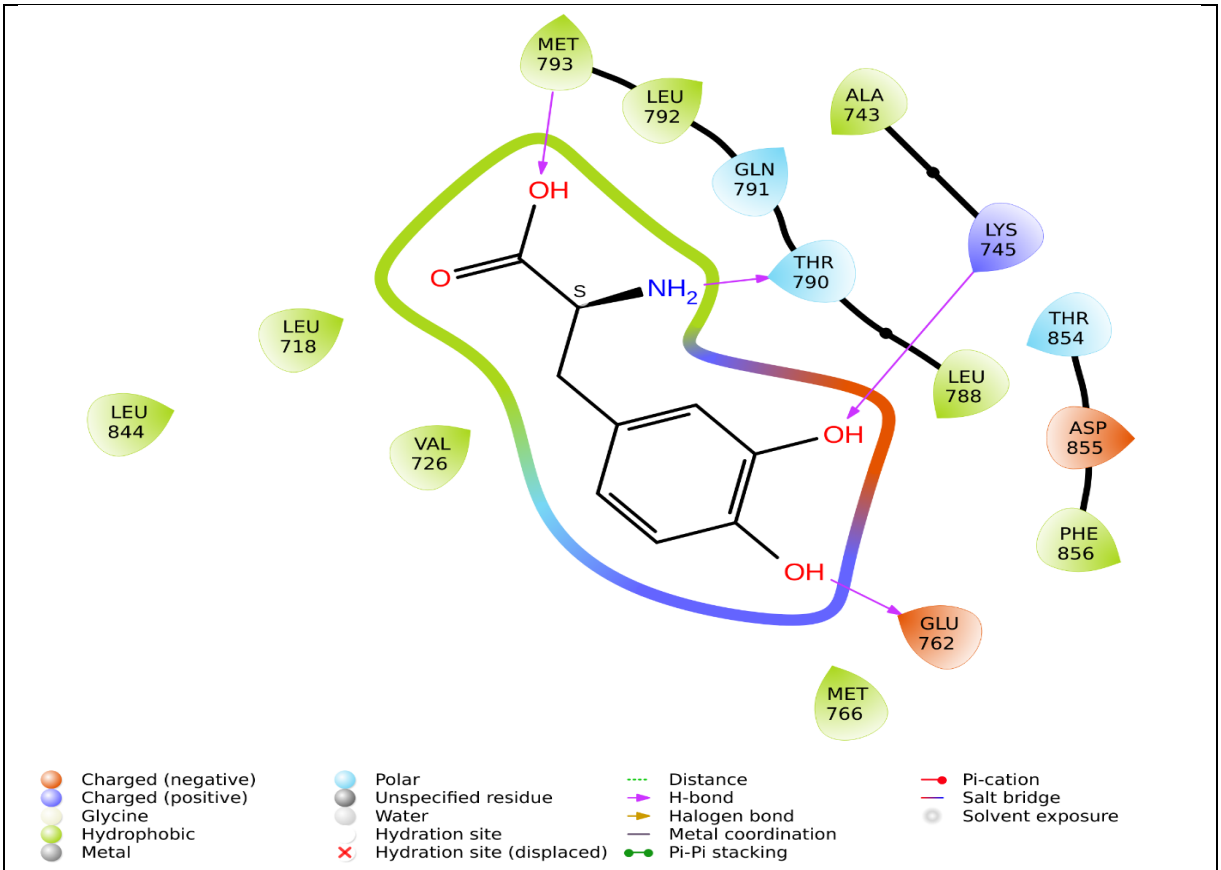


Figure 7: Interaction of L-DOPA with EGFR (4I23)

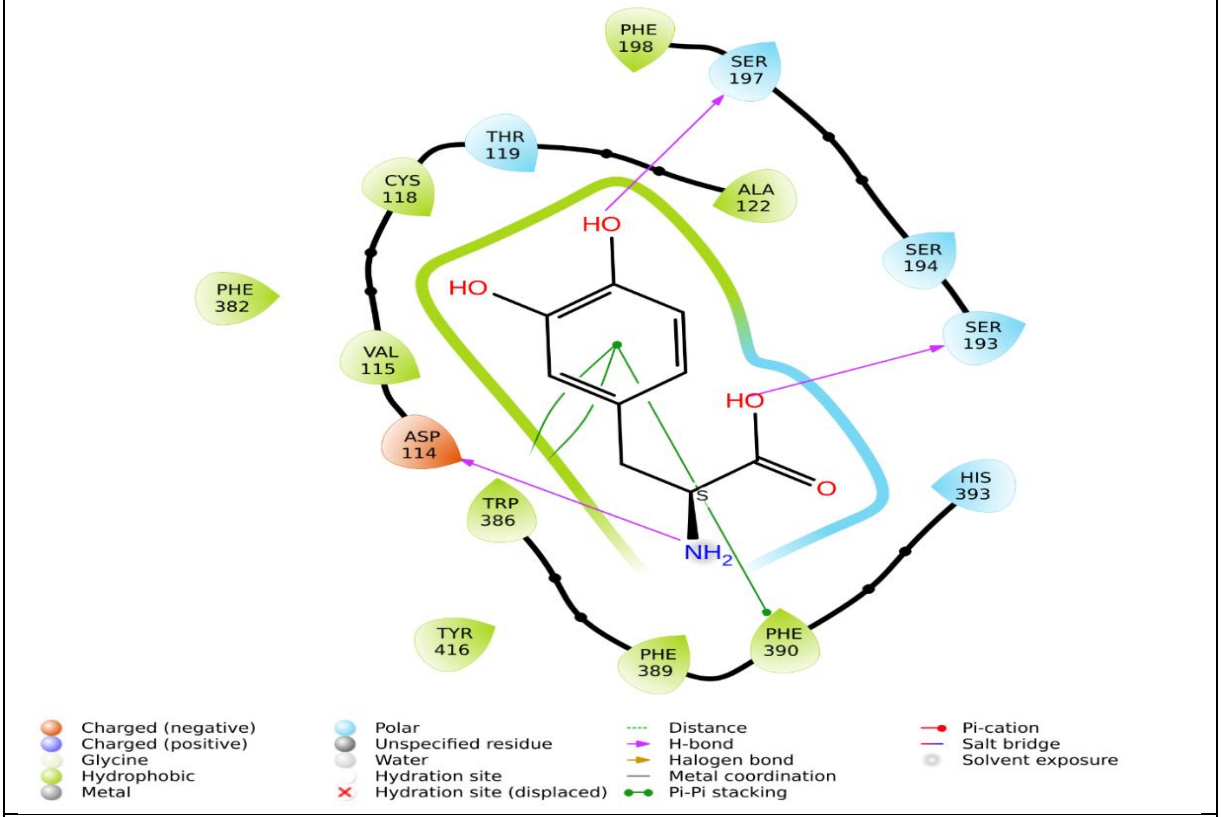


Figure 8.: Interaction of L-DOPA with DRD2 (6CM4)

Table 1.: Interactions of L-DOPA with various targets of HD

DRUGS NAME	Target Name &PDB IDs	Docking score (XP)	No. of Hydrogen bond	Hydrogen bond	Hydrophobic bond
L-DOPA	GAPDH 2VYV	-6.592	2	ARG-1O ALA-94	ALA-94 ALA-180 ILE-11
	DRD2 6CM4	-7.361	3	SER-193 SER-197 ASP-114	TYP-416 TRP-386 PHE-390 PHE-389 PHE-382 PHE-198 ALA-122 CYS-118 VAL-115
	ESR1 1SJO	-7.307	3	ARG-394 LEU-353 LEU-387	MET-343 MET-421 MET-388 LEU-346 LEU-349 LEU-428 LEU-384 LEU-525 LEU-391 LEU-387 ALA-350 PHE-425 PHE-404
	EGFR 4I23	-6.704	4	GLU-762 LYS-745 THR-790 MET-793	MET-793 LEU-792 LEU-788 LEU-718 LEU-844 VAL-726 MT-766 PHE-856- ALA-743

Organoleptic and Physicochemical Findings for *Mucuna pruriens*

The organoleptic evaluation of *Mucuna pruriens* leaves and bark revealed brown coloration, a pleasant aroma, a sweet-bitter taste, and a coarse texture.

Physicochemical analyses showed a moisture content of 8% for leaves and 7.5% for bark, with pH values ranging from 4.0 to 7.0. Alcohol extraction yielded the highest extractive values, at 70% for leaves and 78.2% for bark. Loss on drying was 0.599% for leaves and 0.456% for bark. Ash content analysis indicated water-soluble ash at 2.134% (leaves) and 1.658% (bark), acid-insoluble ash at 0.567% (leaves) and 0.982% (bark), and sulfated ash at 4.03% (leaves) and 1.03% (bark) respectively.

Phytochemical Analysis of Mucuna pruriens Leaf and Bark Extracts:

Ethanolic extracts of *Mucuna pruriens* leaf and bark exhibited the highest levels of total phenolics, flavonoids, and tannins compared to chloroform and water extracts. For the leaves, total phenolic

content ranged from 11.4-23.4 mg GAE/g, tannins 5.9-7.9 mg GAE/g, and flavonoids 14.7-30.18 mg RE/g, indicating a strong antioxidant profile.

For the bark, the total phenolic, tannin, and flavonoids content of ethanol, chloroform, and aqueous extracts ranged from 8.2-14.4, 0-7.2, and 11.6-18.11 g GAE/100 g extract, respectively.

In-vitro Antioxidant Assay

DPPH Radical-scavenging activity

The ethanolic leaf extract of *Mucuna pruriens* (ELEMP) exhibited the strongest DPPH radical-scavenging activity with an IC50 of 90.21 µg/ml. These results highlight ELEMP's superior antioxidant efficacy. (Table 2.)

Table 2.: DPPH radical-scavenging activity of ELEMP, CLEMP, EBEMP and CBEMP

Samples	Treatment	Dose Concentration	IC_{50} Values
1	Ascorbic Acid	(1-10µg/ml)	42.50 ± 0.74 µg/ml
2	ELEMP	(1-200µg/ml	102.19±5.62µg/ml
3	ELEMP	(1-400µg/ml)	90.21 ± 7.50 µg/ml
4	ELEMP	(1-600µg/ml)	74.50 ± 5.12µg/ml
5	CLEMP	(1-200µg/ml	108.22± 6.09 µg/ml
6	CLEMP	(1-400µg/ml)	95.14 ± 3.18 µg/ml
7	CLEMP	(1-600µg/ml)	87.29 ± 7.16 µg/ml
8	EBEMP	(1-200µg/ml)	103.22 ± 6.99 µg/ml
9	EBEMP	(1-400µg/ml)	96.47 ± 9.13µg/ml
10	EBEMP	(1-600µg/ml)	90.62 ± 7.11 µg/ml
11	CBEMP	(1-200µg/ml	108.17 ± 5.12 µg/ml
12	CBEMP	(1-400µg/ml)	99.89 ± 6.39 µg/ml
13	CBEMP	(1-600µg/ml)	93.43 ± 7.97 µg/ml

Data are represented as mean ± SD of three replicates.

"ELEMP (Ethanolic leaf extract of *Mucuna pruriens*), CLEMP (Chloroform leaf extract of *Mucuna pruriens*), EBEMP (Ethanolic bark extract of *Mucuna pruriens*), CBEMP (Chloroform bark extract of *Mucuna pruriens*)"

H_2o_2 Scavenging activity

The hydrogen peroxide scavenging activity of the extracts was determined at concentrations ranging from 10–160 µg/mL, with ascorbic acid as a control. Results were expressed as a percentage of scavenging activity. (Table 3)

Table 3.: H_2O_2-scavenging activity of ELEMP, CLEMP, EBEMP, and CBEMP

Samples	Treatment	Dose Concentration	% Inhibition
1	Ascorbic Acid	(100 µg/ml)	93.15 ± 2.18%
2	ELEMP	(1-20µg/ml	42.67 ± 2.45%
3	ELEMP	(1-40µg/ml)	51.43 ± 2.79 %
4	ELEMP	(1-80µg/ml)	72.14 ± 1.97%
5	ELEMP	(1-160 µg/ml)	77.15 ± 2.29%
6	CLEMP	(1-20µg/ml	39.75± 1.73 %
7	CLEMP	(1-40µg/ml)	44.29 ± 1.32%
8	CLEMP	(1-80µg/ml)	58.14 ± 2.36%
9	CLEMP	(1-160 µg/ml)	63.74 ± 2.18%
10	EBEMP	(1-20µg/ml)	37.51 ± 1.49%
11	EBEMP	(1-40µg/ml)	41.22 ± 1.74%
12	EBEMP	(1-80µg/ml)	54.27±1.67 %
13	EBEMP	(1-160 µg/ml)	58.34±1.83 %
14	CBEMP	(1-20µg/ml)	32.15 ± 1.22%
15	CBEMP	(1-40µg/ml)	38.09 ± 1.45%
16	CBEMP	(1-80µg/ml)	51.32 ± 2.10%
17	CBEMP	(1-160 µg/ml)	57.51 ± 1.67%

Data are represented as mean ± SD of three replicates.

"ELEMP (Ethanolic leaf extract of *Mucuna pruriens*), CLEMP (Chloroform leaf extract of *Mucuna pruriens*), EBEMP (Ethanolic bark extract of *Mucuna pruriens*), CBEMP (Chloroform bark extract of *Mucuna pruriens*)"

Reducing Power Assay

The in vitro reducing power assay revealed significant antioxidant activity in the ethanolic leaf extracts of *Mucuna pruriens* (ELEMP), with a maximum inhibition of 66.78% at 160 µg/ml. The bark extract showed a maximum inhibition of 55.44% at the same concentration, indicating the presence of potent biologically active compounds in these extracts. (Table 4)

Table 4.: Reducing power activity of different concentration of ELEMP, CLEMP, EBEMP, and CBEMP and ascorbic acid.

S. No	Conc. (µg/mL)	ELEMP	CLEMP	EBEMP	CBEMP	Ascorbic Acid
01	20	22.12±0.18	20.04±0.75	18.16±0.91	16.45±0.80	30.14±2.04
02	40	38.31±0.52	32.15±0.91	34.19±0.99	30.23±1.03	56.11±1.88
03	80	51.43±0.77	46.13±1.03	46.31±0.87	42.54±1.12	72.76±2.97

04	160	66.78±2.88	62.33±2.30	55.44±2.82	52.72±2.12	87.28±3.48

Data are represented as mean ± SD of three replicates.

Cytotoxicity Study

The cytotoxicity of the ethanolic extract of Mucuna pruriens was evaluated using the MTT assay, as shown in (Table 5). Results indicated no significant toxicity at concentrations up to 200 µg/mL, with cell viability remaining above 75%. This suggests that the extract is within a safe dosage range for further biological studies.

Table 5.: % Cell viability of ELEMP, CLEMP, EBEMP and CBEMP

Samples	Treatment	Dose Concentration	% Cell Viability
1	Control		100%
2	ELEMP	(1-50µg/ml)	91.34±2.42 %
3	ELEMP	(1-100µg/ml)	90.54 ± 5.30 %
4	ELEMP	(1-200µg/ml)	85.32 ± 2.17%
5	CLEMP	(1-50µg/ml)	88.12 ± 5.19%
6	CLEMP	(1-100µg/ml)	80.91± 5.67 %
7	CLEMP	(1-200µg/ml)	87.21 ± 5.14%
8	EBEMP	(1-50µg/ml)	82.44 ± 6.25%
9	EBEMP	(1-100µg/ml)	87.79 ± 6.13%
10	EBEMP	(1-200µg/ml)	81.67±3.25 %
11	CBEMP	(1-50µg/ml	85.77 ± 4.29%
12	CBEMP	(1-100µg/ml)	88.12 ± 6.11%
13	CBEMP	(1-200µg/ml)	83.13 ± 5.87%

Data are represented as mean ± SD of three replicates.

In-vivo Study

Acute Toxicity Evaluation of Ethanolic Extract of *Mucuna pruriens*

The acute toxicity evaluation of the ethanolic extract indicated no signs of toxicity or mortality in rats at doses up to 2000 mg/kg body weight over the 14-day monitoring period.

Effect of *Mucuna Purines* Leaves Extract on body weight in 3-nitro propionic acid-treated rats

During the treatment schedule, body weight was measured as the % change in weight by comparing the 14^{th}-day body weight with the 0-day body weight of the animals. Vehicle-treated animals showed no change in body weight, while 3-NP treatment significantly decreased body weight on day 5. Treatment with ELEMP (100, 200, and 400 mg/kg, p.o.) in 3-NP-treated rats significantly mitigated weight loss, especially at the higher dose(400mg/kg), with no significant effect observed at the lower dose(100mg/kg).

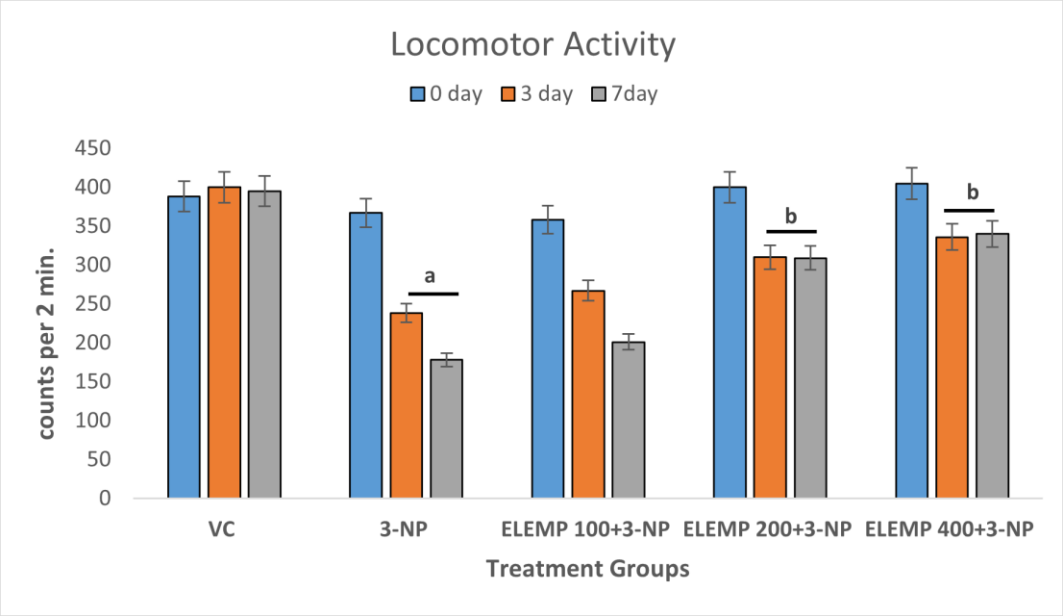
Table 6.: Effect of ELEMP on % change in body weight in 3-NP treated rats

Treatment (mg/kg)	% change in body weight
VC (Vehicle control)	4.88±0.68
3-NP	−26.76±4.35[a]
ELEMP 100+3-NP	−25.17±3.19
ELEMP 200+3-NP	−15.96±1.34[b]
ELEMP 400+3-NP	−10.47±0.90[b]

Data are presented as means ± S.E.M.
Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test; '[a]P<0.01 as compared to Vehicle control Group; [b]P<0.05 as compared to 3-NP control group.
Neurobehavioral study
Effect of *Mucuna Purines Leaves Extract* on Locomotor Activity in 3-nitro propionic acid-treated Rats
Table 7.: Effect of ELEMP on Locomotor activity in 3-NP treated rats

Treatment (mg/kg)	0 day	3 day	7day
VC (Vehicle control)	388 ± 5.45	400 ± 7.21	395 ± 6.68
3-NP	367 ± 8.32	238 ± 12.66[a]	178 ± 9.45[a]
ELEMP 100+3-NP	358 ± 11.98	267 ± 10.21	201 ± 14.67
ELEMP 200+3-NP	400 ± 19.29	310 ± 15.87[b]	309 ± 14.82[b]
ELEMP 400+3-NP	405 ± 14.87	336 ± 15.09[b]	340 ± 17.16[b]

Data are presented as mean ± S.E.M.
Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test; '[a]P<0.01 as compared to Vehicle control Group; [b]P<0.05 as compared to 3-NP control group.
'[a]P<0.01 as compared to Vehicle control Group; [b]P<0.05 as compared to 3-NP control group.



Graph 1. Effect of *Mucuna Purines Leaves Extract* on Locomotor Activity in 3-nitro propionic acid-treated Rats

Effect of *Mucuna purines* leaves extract on movement analysis in 3-nitro propionic acid-treated rats
After 2–3 days of 3-NP treatment, rats showed progressive motor impairments, including decreased movement and hind limb coordination, progressing to severe dystonic posturing and near-immobility.

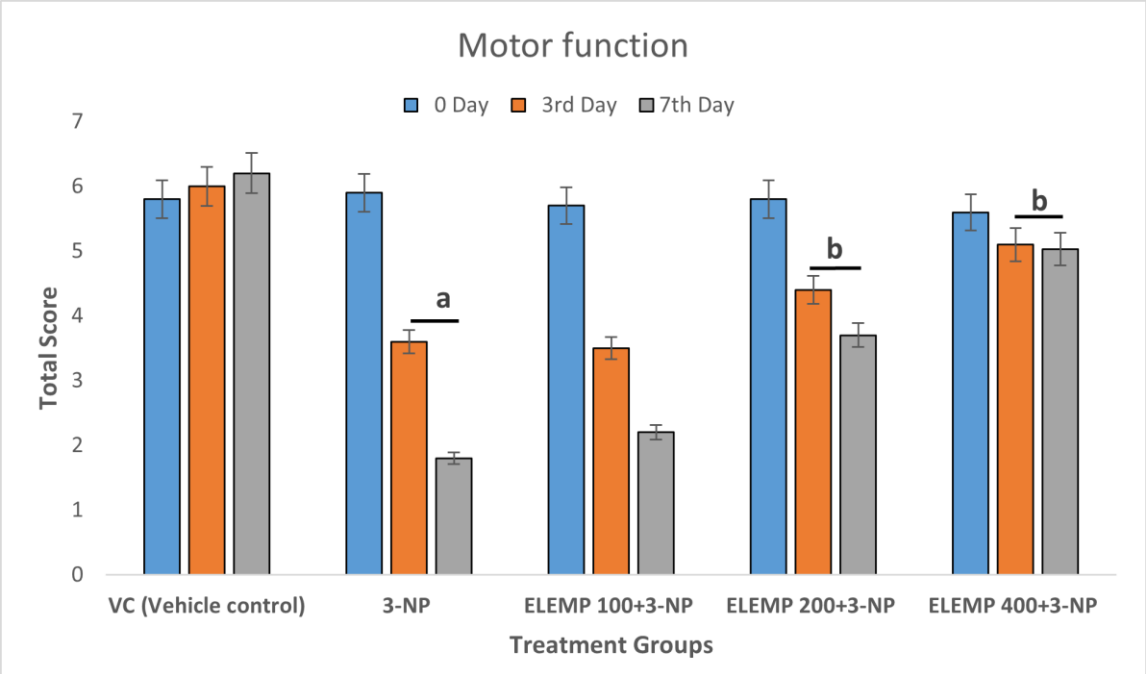
Mucuna pruriens ethanolic leaf extract (ELEMP) at 200 and 400 mg/kg (p.o.) significantly reduced these motor deficits, with the 400 mg/kg dose having a more pronounced effect. (Table 8)

Table 8.: Effect of ELEMP on Motor Function Scores in 3-NP treated rats

Treatment (mg/kg)	Total Score 0 Day	Total Score 3rd Day	Total score 7th Day
VC (Vehicle control)	5.8 ± 0.3	6.0 ± 0.7	6.2 ± 0.6
3-NP	5.9 ± 0.8	3.6±0.5[a]	1.8±0.4[a]
ELEMP 100+3-NP	5.7 ± 0.4	3.5±0.3	2.2±0.4
ELEMP 200+3-NP	5.8 ± 0.6	4.4±0.5[b]	3.7±0.4[b]
ELEMP 400+3-NP	5.6 ± 0.4	5.1±0.5[b]	5.03±0.6[b]

Data are presented as mean ± S.E.M.

Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test; '[a]P<0.01 as compared to Vehicle control Group; [b]P<0.05 as compared to 3-NP control group.



'[a]P<0.01 as compared to Vehicle control Group; [b]P<0.05 as compared to 3-NP control group

Graph 2. Effect of *Mucuna purines* leaves extract on movement analysis in 3-nitro propionic acid-treated rats

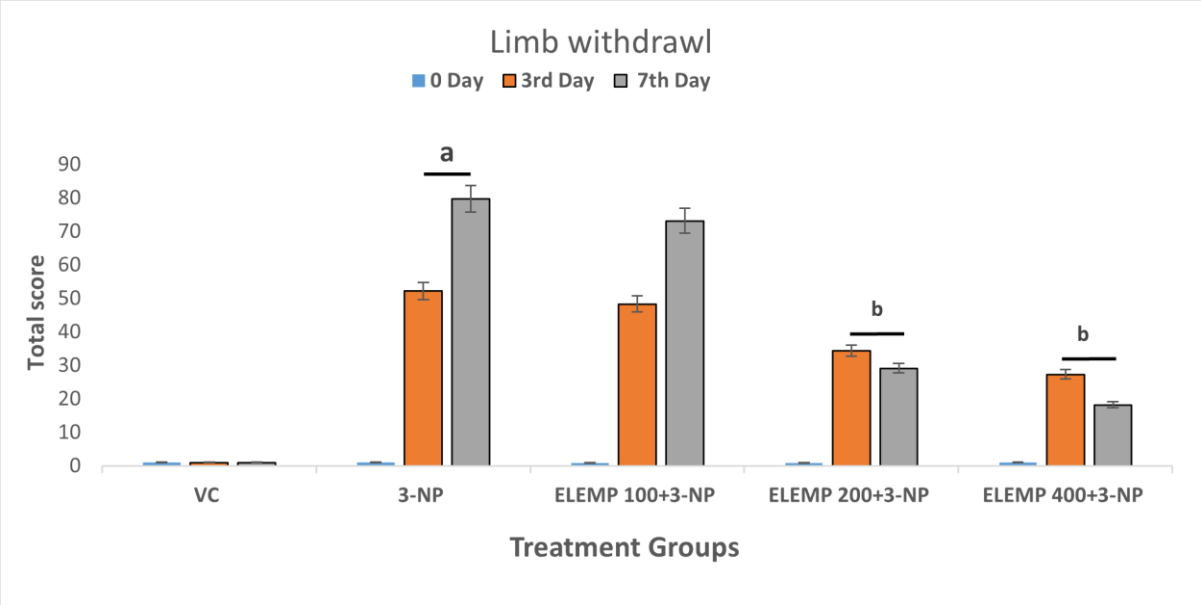
Effect of *Mucuna purines* Leaves Extract on the limb withdrawal test 3-nitro propionic acid-treated rats

The retraction time difference between the two hind limbs was significantly greater in 3-NP-treated rats than in vehicle controls, which quickly retracted both limbs. Treatment with *Mucuna pruriens* ethanolic leaf extract (ELEMP) at 200 and 400 mg/kg (p.o.) significantly improved limb retraction times in 3-NP-treated rats on days 3 and 7, with the higher dose showing a more pronounced effect. (Table 9)

Table 9.: Effect of ELEMP on Limb Retraction Time in 3-NP treated rats

Treatment (mg/kg)	Time (s) 0 Day	Time (s) 3rd Day	Time (s) 7th Day
VC (Vehicle control)	1.0 ± 0.1	1.0 ± 0.1	1.0 ± 0.1
3-NP	1.0 ± 0.1	52.16±4.16[a]	79.66±5.20[a]
ELEMP 100+3-NP	0.9± 0.1	48.29±5.39	73.17±4.28
ELEMP 200+3-NP	0.9 ± 0.1	34.36±2.33[b]	29.14±4.08[b]
ELEMP 400+3-NP	1.0 ± 0.1	27.32±2.90[b]	18.22±4.98[b]

Data are presented as mean ± S.E.M.
Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test; 'aP<0.01



as compared to Vehicle control Group; bP<0.05 as compared to 3-NP control group.
'aP<0.01 as compared to Vehicle control Group; bP<0.05 as compared to 3-NP control group.
Graph 3. Effect of *Mucuna purines* Leaves Extract on the limb withdrawal test 3-nitro propionic acid-treated rats

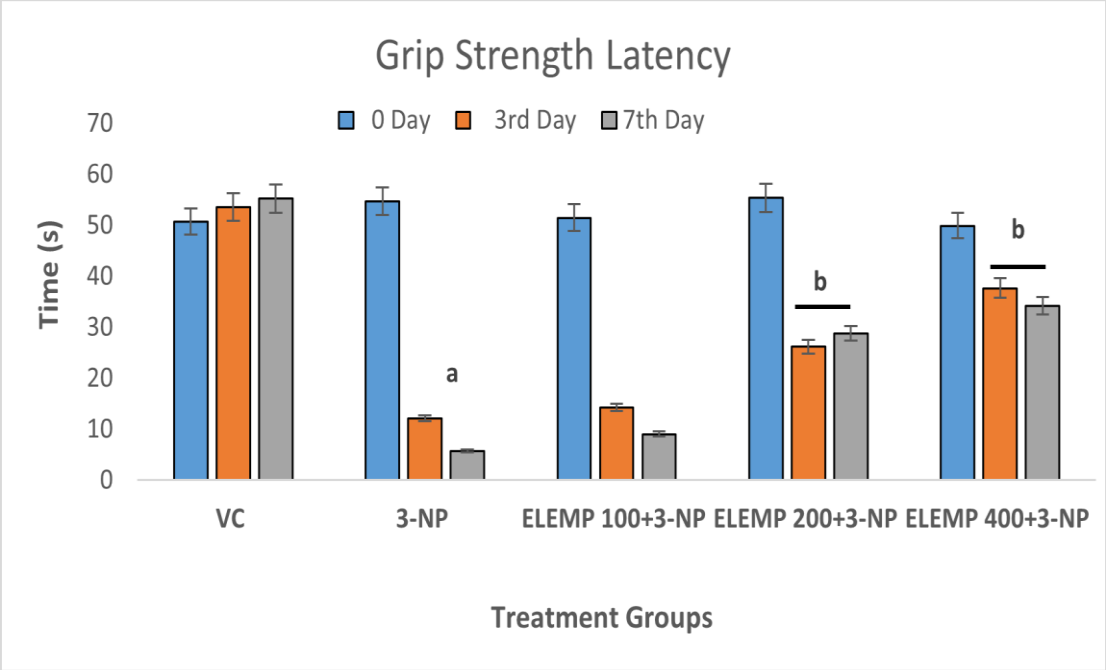
Effect of *Mucuna purines* leaves extract on the string test 3-nitro propionic acid-treated rats

The mean grip latency in rats treated with 3-NP was significantly lower compared to the vehicle group. A lower dose (100mg/kg) of ELEMP did not affect grip latency. However, administration of ELEMP (200 or 400 mg/kg, p.o.) significantly increased the latency to grip loss in 3-NP-treated rats compared to the 3-NP-only group. (Table 10)

Table 10.: Effect of ELEMP on Grip Strength Latency in 3-NP treated rats

Treatment Group (mg/kg)	Time (s) 0 Day	Time (s) 3rd Day	Time (s) 7th Day
VC (Vehicle control)	50.7 ± 4.15	53.59 ± 3.19	55.21 ± 5.7
3-NP	54.7 ± 3.89	12.12±0.07[a]	5.67 ±0.70[a]
ELEMP 100+3-NP	51.5 ± 3.67	14.28±5.14	9.05±0.66
ELEMP 200+3-NP	55.4 ± 5.87	26.16±3.18[b]	28.78±2.08[b]
ELEMP 400+3-NP	49.87 ± 3.39	37.67±4.13[b]	34.23±3.12[b]

Data are presented as mean ± S.E.M.
Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test; 'aP<0.01 as compared to Vehicle control Group; bP<0.05 as compared to 3-NP control group.



'[a]P<0.01 as compared to Vehicle control Group; [b]P<0.05 as compared to 3-NP control group.

Graph 4. Effect of *Mucuna purines* leaves extract on the string test 3-nitro propionic acid-treated rats

Biochemical studies

Effect of *Mucuna purines* leaves extract on brain oxidant and antioxidant levels in 3-nitropropionic acid-treated rats

The results of the behavioral studies were further supported by the results of biochemical parameters. Nitrite levels in the brains of rats were significantly increased following 3-NP administration in comparison to those of the Vehicle group, indicating 3-NP-induced oxidative damage. Treatment with ELEMP (200 or 400 mg/kg, p.o.) in 3-NP-treated rats significantly reduced lipid peroxidation, as evidenced by decreased TBARS levels, and restored antioxidant defenses by enhancing reduced glutathione (GSH) levels. Additionally, ELEMP treatment significantly restored catalase and superoxide dismutase (SOD) activity in the brain of 3-NP-treated rats. These findings suggest that ELEMP offers protective effects against oxidative stress induced by 3-NP in the brain. (Table 11)

Table 11.: Effect of ELEMP on Oxidative Stress Markers in 3-NP treated rats

Treatment Group	TBARS	GSH	SOD	CAT	Nitrite
VC (Vehicle control)	2.82 ± 0.07	5.92 ± 0.09	4.38 ± 0.06	3.51 ± 0.04	1.18 ± 0.08
3-NP	8.21 ± 0.80	1.67 ± 0.04[a]	1.32 ± 0.02[a]	1.09 ± 0.02[a]	5.19 ± 0.60[a]
ELEMP 100+3-NP	3.18 ± 0.09	1.92 ± 0.06	1.48 ± 0.03	1.27 ± 0.05	4.99 ± 0.55
ELEMP 200+3-NP	5.14 ± 0.47[b]	3.08 ± 0.04[b]	2.22 ± 0.03[b]	1.79 ± 0.03[b]	3.55 ± 0.43[b]
ELEMP 400+3-NP	6.72 ± 0.50	4.14 ± 0.07[b]	3.04 ± 0.04[b]	2.63 ± 0.05[b]	1.87 ± 0.20[b]

Data are presented as means ± S.E.M.

Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test; '[a]P<0.01 as compared to Vehicle control Group; [b]P<0.05 as compared to 3-NP control group.

Effect of *Mucuna purines* leaves extract on cytokine production in 3-nitropropionic acid-treated rats

Chronic administration of ELEMP (200 or 400 mg/kg, p.o.) in 3-NP-treated rats significantly reduced brain concentrations of pro-inflammatory cytokines IL-1β and TNF-α, compared to levels observed in the 3-NP-only group. These results indicate the anti-inflammatory potential of ELEMP in mitigating 3-NP-induced neuroinflammation. (Table 12)

Table 12.: Effect of ELEMP on Pro-inflammatory Cytokines in 3-NP treated rats

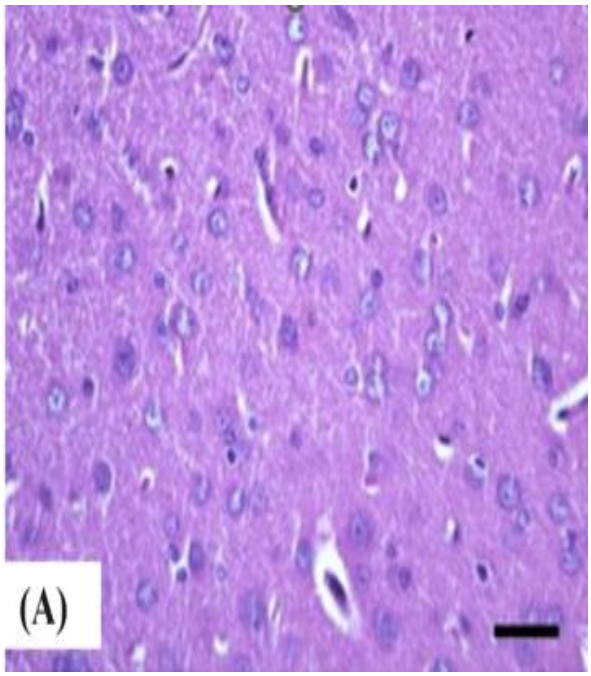
Group	IL-1 Beta (pg/mg tissue)	TNF-alpha (pg/mg tissue)
VC (Vehicle control)	170.08±11.68	120.67±14.27
3-NP	732.23±14.36[a]	621.57±42.49[a]
ELEMP 100+3-NP	721.67±20.18	604.34±26.29
ELEMP 200+3-NP	440.43±18.98[b]	403.49±16.55[b]
ELEMP 400+3-NP	320.87±23.67[b]	280.67±31.89[b]

Data are presented as means ± S.E.M.

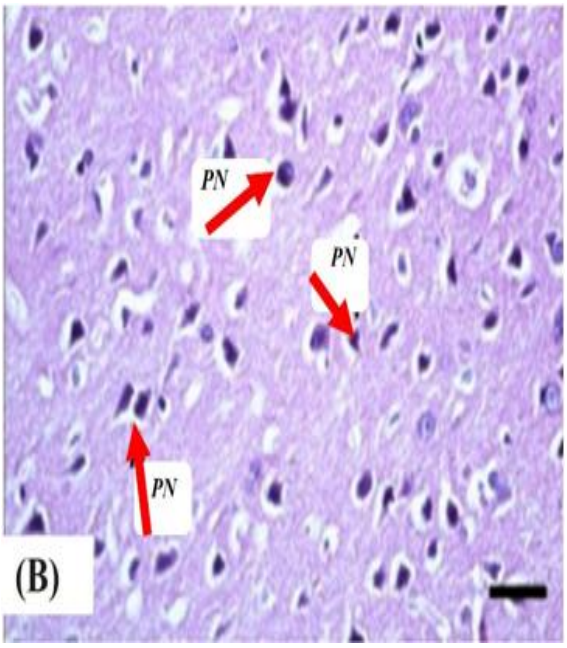
Data were analyzed by using one-way ANOVA followed by Tukey's multiple comparison test; "[a]P<0.01 as compared to Vehicle control Group; [b]P<0.05 as compared to 3-NP control group.

Effect of *Mucuna purines* leaves extract on 3-NP-induced histopathological changes in the striatum and cortex regions of rat brain

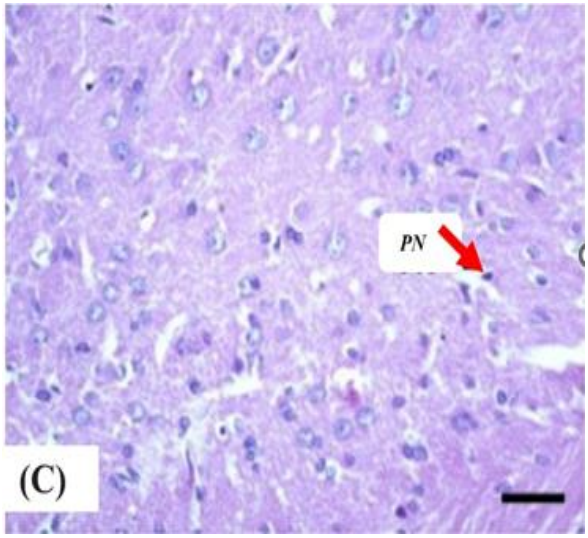
Histopathological changes in the striatum of control and 3-NP-treated animals are depicted in Fig. 9. The histological analysis revealed that control rats exhibited normal morphology in both the striatal and cortical regions. In contrast, rats treated with 3-NP displayed irregular, damaged cells with condensed and pyknotic nuclei in both regions (Fig. 9B). In the ELEMP + 3-NP (100 mg/kg, p.o.) group, a reduction in pyknotic nuclei was observed, suggesting partial protection (Fig. 9C). The ELEMP + 3-NP (200 mg/kg, p.o.) group showed a more pronounced decrease in pyknotic nuclei, indicating enhanced neuroprotective effects (Fig. 9D). Finally, the ELEMP + 3-NP (400 mg/kg, p.o.) group demonstrated the greatest reduction in pyknotic nuclei, with significantly better histological preservation in the striatum, highlighting the superior efficacy of the higher dose (Fig. 9E).



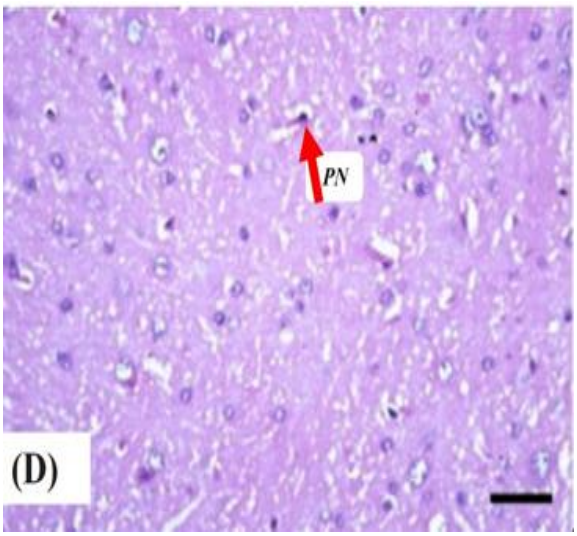
(A) Group I



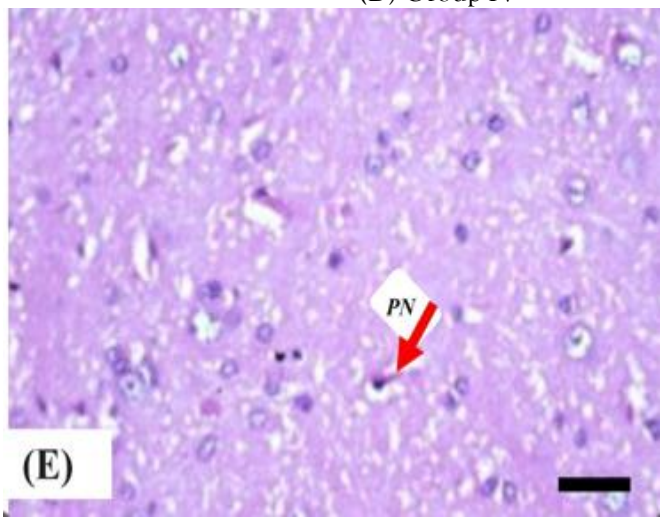
(B) Group II



(C) Group III



(D) Group-IV



(E) Group-V

Figure 9.: Effect of *Mucuna pruriens* Ethanolic Leaf Extract (ELEMP) on histopathological alterations in the striatum and cortex regions of control and 3-NP treated rats, focusing on changes induced by 3-NP administration to model Huntington's disease. Sections were visualized under a light microscope at a magnification of 40 x.

(A) Control rats displayed normal histology in the striatum and cortex regions. (B) Rats treated with 3-NP alone showed extensive damage, with condensed pyknotic nuclei observed in both striatal and cortical areas. (C) In the ELEMP + 3-NP (100 mg/kg, p.o.) group, the striatum exhibited a reduced number of pyknotic nuclei, indicating partial protection. (D) The ELEMP + 3-NP (200 mg/kg, p.o.) group demonstrated a more pronounced decrease in pyknotic nuclei, reflecting improved neuroprotective effects. (E) The ELEMP + 3-NP (400 mg/kg, p.o.) group showed the greatest reduction in pyknotic nuclei, with significantly better histological preservation in the striatum, underscoring enhanced efficacy at higher doses (scale bar—100 μm).

Discussion

The current study highlighted the neuroprotective effects of *Mucuna pruriens* (MP) on rats with 3-NP-induced neurotoxicity. The 3-NP neurotoxin model is widely used to induce symptoms similar to Huntington's disease (HD).[25] Research has shown that 3-NP-induced neurotoxicity mimics a wide range of biochemical, behavioral, and histopathological changes observed in HD.[26] As a mitochondrial toxin, 3-NP generates oxidative stress and irreversibly inhibits SD, disrupting the brain's metabolic processes.[27]

The present study highlights the potential of *Mucuna pruriens*, particularly its high L-DOPA content, in addressing Huntington's disease (HD) pathology through antioxidant effects, neuroprotection, and modulation of inflammatory pathways. Using network pharmacology, phytochemical analysis, and in vivo models, *Mucuna pruriens* showed significant efficacy in reducing HD symptoms, which suggests a multifaceted mechanism involving oxidative stress reduction, neurotransmitter regulation, and immune modulation.

Network pharmacology identified key molecular targets such as TH, EGFR, and DRD2, critical for neuronal health and dopaminergic regulation, which are significantly affected in HD. L-DOPA, a major component of *Mucuna pruriens*, plays a well-established role in enhancing dopamine synthesis, supporting neural signaling, and improving motor functions, which are compromised in HD.[28] Docking studies further validated the interaction of L-DOPA with these targets, suggesting that *Mucuna pruriens* can potentially stabilize dopaminergic signaling pathways disrupted in HD. This dopaminergic support is essential for maintaining motor coordination and cognitive stability, which are typically impaired in HD.

Phytochemical analysis confirmed the presence of potent antioxidants, including flavonoids and phenolics, which were further supported by strong radical scavenging activity in in-vitro assays. These antioxidants can effectively neutralize reactive oxygen species (ROS), a primary contributor to cellular damage in HD.[29] The high antioxidant capacity of *Mucuna pruriens* extract demonstrated through DPPH and hydrogen peroxide scavenging assays, indicates that the extract can mitigate oxidative stress in neural cells, thereby protecting them from HD-induced degeneration.

The In-vivo studies in the 3-nitro propionic acid (3-NP)-induced HD rat model revealed notable improvements in motor functions, oxidative stress markers, and body weight. In the present study, administering 3-NP led to a notable decrease in the animal's body weights. This weight loss could be attributed to metabolic disruptions and the degeneration of hypothalamic neurons. Additionally, 3-NP caused bilateral striatal lesions and bradykinesia, which likely contributed to reduced food intake, appetite, and subsequent weight loss.[30] However, treatment with MP significantly counteracted these weight changes in a dose-dependent manner. Behavioral assays, including locomotor activity and limb withdrawal tests, indicated that MP improved motor coordination and reduced neuromuscular impairments typically observed in HD models. Biochemical analyses supported these findings, showing reductions in lipid peroxidation levels and increases in antioxidant enzymes, specifically catalase and glutathione. The reduction in lipid peroxidation is a critical finding, as it points to decreased membrane damage from oxidative stress. Elevated levels of catalase and glutathione indicate a strengthening of the brain's endogenous antioxidant defences, which are often weakened in HD due to excessive oxidative stress.

In addition to antioxidant mechanisms, *Mucuna pruriens* exhibited significant effects on inflammatory cytokine levels, reflecting an immunomodulatory role. In HD, elevated levels of pro-inflammatory cytokines, such as TNF-α and IL-1β, exacerbate neuronal damage through inflammation. Treatment with *Mucuna pruriens* significantly reduced these cytokines, suggesting that its components help to suppress neuroinflammation. By lowering TNF-α and IL-1β levels, *Mucuna pruriens* may help reduce microglial activation and pro-inflammatory signaling in the brain, which are both known to contribute to the progressive neuronal damage seen in HD.

Histopathological analysis further supports the neuroprotective potential of *Mucuna pruriens*. In control rats, normal histology was observed in both the striatum and cortex regions. However, 3-NP treatment resulted in extensive damage, with irregular, damaged cells and condensed, pyknotic nuclei in both brain regions. In the ELEMP and 3-NP group treated with a lower dose, a reduction in pyknotic nuclei was observed, suggesting partial protection. With increasing doses of ELEMP and 3-NP, a more pronounced decrease in pyknotic nuclei was seen, indicating enhanced neuroprotective effects. The highest dose demonstrated the most significant reduction in pyknotic nuclei, with the best histological preservation in the striatum, underscoring the superior efficacy of the higher dose.

The combined effects of *Mucuna pruriens* on biochemical markers, cytokine levels, and histopathological changes highlight its potential to act as a broad-spectrum neuroprotective agent in HD. The reduction in oxidative stress, inflammation, and stabilization of dopamine pathways presents a compelling case for further investigation into *Mucuna pruriens* as a complementary therapeutic approach for HD. Future studies should aim to isolate individual bioactive compounds within *Mucuna pruriens* and examine their distinct roles in antioxidative, anti-inflammatory, and dopaminergic activities. Moreover, long-term studies on the safety and efficacy of these compounds in clinical settings would further elucidate the potential for *Mucuna pruriens* to be integrated into therapeutic regimens for HD.

Conclusion

The findings of this study indicate that *Mucuna pruriens* has significant neuroprotective capabilities against HD-related neurodegeneration. By reducing oxidative damage, modulating inflammatory responses, and supporting dopamine pathways, *Mucuna pruriens* shows promise as a plant-based therapy for managing HD symptoms.

---

[1] Gonzalez Rojas N, Cesarin M, Pekera G, Da Prata G, Etcheverry J, Gatto EM. Review of Huntington's disease: from basics to advances in diagnosis and treatment. J Neurorestor. 2022;12(3):93-113. doi: 10.14740/jnr721.

[2] Abdelfattah MS, Badr SEA, Lotfy SA, Attia GH, Aref AM, Abdel Moneim AE, et al. Rutin and Selenium Co-administration Reverse 3-Nitropropionic Acid-Induced Neurochemical and Molecular impairments in a mouse model of Huntington's Disease. Neurotox Res Neurotox Res. 2020;37(1):77–92.

[3] Huang, W., Chen, W., & Zhang, X. (2016). Huntington's disease: Molecular basis of pathology and status of current therapeutic approaches (Review). Experimental and Therapeutic Medicine, 12, 1951-1956. https://doi.org/10.3892/etm.2016.3566

[4] Túnez, I.; Tasset, I.; Pérez-De La Cruz, V.; Santamaría, A. 3-Nitropropionic Acid as a Tool to Study the Mechanisms Involved in Huntington's Disease: Past, Present and Future. *Molecules* **2010**, *15*, 878-916. https://doi.org/10.3390/molecules15020878

[5] Guo X, Disatnik M-H, Monbureau M, Shamloo M, Mochly-Rosen D, Qi X. Inhibition of mitochondrial fragmentation diminishes Huntington's disease–associated neurodegeneration. Published November 15, 2013.

[6] Pathania R, Chawla P, Khan H, Kaushik R, Khan MA. An assessment of potential nutritive and medicinal properties of *Mucuna pruriens*: a natural food legume. 3 Biotech. 2020 Jun;10(6):261. doi: 10.1007/s13205-020-02253-x. Epub 2020 May 20. PMID: 32477848; PMCID: PMC7239958.

[7] Zahra W, Birla H, Singh SS, Rathore AS, Dilnashin H, Singh R, Keshri PK, Gautam P, Singh SP. Neuroprotection by Mucuna pruriens in Neurodegenerative Diseases. Neurochem Res. 2022 Jul;47(7):1816-1829. doi: 10.1007/s11064-022-03591-3. Epub 2022 Apr 5. PMID: 35380400.

[8] Kaneria M, Kanani B, Chanda S. Assessment of effect of hydroalcoholic and decoction methods on extraction of antioxidants from selected Indian medicinal plants. Asian Pac J Trop Biomed. 2012 Mar;2(3):195-202. doi: 10.1016/S2221-1691(12)60041-0. PMID: 23569897; PMCID: PMC3609273.

[9]Quality Control Methods for Medicinal Plant Materials (2004). WHO Geneva, Indian edition p.28-37.

[10] Rubab, M., Chelliah, R., Oh, DH. (2022). Screening for Antioxidant Activity: Diphenylpicrylhydrazine (DPPH) Assay. In: Dharumadurai, D. (eds) Methods in Actinobacteriology. Springer Protocols Handbooks. Humana, New York, NY. https://doi.org/10.1007/978-1-0716-1728-1_61

[11] Jain A, Soni M, Deb L, Jain A, Rout SP, Gupta VB, Krishna KL. Antioxidant and hepatoprotective activity of ethanolic and aqueous extracts of Momordica dioica Roxb. leaves. J Ethnopharmacol. 2008 Jan 4;115(1):61-6. doi: 10.1016/j.jep.2007.09.009. Epub 2007 Sep 19. PMID: 17983713.)

[12] Mssillou I, Agour A, Hamamouch N, Lyoussi B, Derwich E. Chemical Composition and In Vitro Antioxidant and Antimicrobial Activities of *Marrubium vulgare* L. ScientificWorldJournal. 2021 Oct 31;2021:7011493. doi: 10.1155/2021/7011493. PMID: 34754277; PMCID: PMC8572620

[13] Gupta, Praveen & Patel, Shivani. (2020). In vitro antimitotic and cytotoxic potential of plant extracts: a comparative study of Mucuna pruriens, Asteracantha longifolia and Sphaeranthus indicus. Future Journal of Pharmaceutical Sciences. 6. 10.1186/s43094-020-00137-8.

[14] Kumar, P., Padi, S.S.V., Naidu, P.S., Kumar, A., 2006. Effect of resveratrol on 3 nitropropionic acid-induced biochemical and behavioural changes:possible neu roprotective mechanisms.Behav.Pharmacol.17,485–492.

[15] Ludolph,A.C.,He, F., Spencer, P.S.,Hammerstad, J., Sabri,M.,1991.3-Nitropropionic acid-exogenousanimalneurotoxinandpossiblehumanstriataltoxin.Can. J.Neu rol.Sci.18,492–498.

[16] Shear,D.A.,Dong, J.,Gundy,C.D.,Haik-Creguer,K.L.,Dunbar,G.L.,1998.Comparisonof intrastriatal injections of quinolinic acid and 3-nitropropionic acid for use in ani mal models of Huntington's disease. ProgNeuropsychopharmacolBiol.Psychiatry 22,1217–1240.

[17] Vis,J.C.,Verbeek,M.M.,Waal,R.M.,Donkelaar,H.J.,Kremer,H.P.H.,1999.3-Nitropropionic acidinducesaspectrumofHuntington'sdisease-likeneuropathologyinratstriatum. Neuropathol.Appl.Neurobiol.25,513–521.

[18] M. Akhtar, K.K. Pillai, D. Vohora, Effect of thioperamide on oxidative stress markers in middle cerebral artery occlusion model of focal cerebral ischemia in rats, Hum. Exp. Toxicol. 27 (2008) 761–767.

[19] A.V. Swamy, N.L. Patel, P.C. Gadad, B.C. Koti, U.M. Patel, A.H. Thippeswamy, D.V. Manjula, Neuroprotective activity of pongamia pinnata in monosodium glu tamate-induced neurotoxicity in rats, Indian. J. Pharm. Sci. 75 (2013) 657–663.
[20] Sinha,A.K.,1972.Colorimetricassayofcatalase.Anal.Biochem.47,389–394
[21] S. Marklund, G. Marklund, Involvement of the superoxide anion radical in the au toxidation of pyrogallol and a convenient assay for superoxide dismutase, Eur. J. Biochem. 47 (1974) 469–474.
[22] Green, L.C.,Wagner,D.A.,Glogowski, J., Skipper,P.L.,Wishnok, J.S.,Tannebaum, S.R., 1982.Analysisofnitrate,nitrite, and[15N]nitrate inbiologicalfluids.Ann.Bio chem.126,131–138.
[23] H E Barksby, S R Lea, P M Preshaw, J J Taylor, The expanding family of interleukin-1 cytokines and their role in destructive inflammatory disorders, *Clinical and Experimental Immunology*, Volume 149, Issue 2, August 2007, Pages 217–225, https://doi.org/10.1111/j.1365-2249.2007.03441.x
[24] Patel C, Thakur K, Shagond L, Acharya S, Ranch K, Boddu SH. Effect of Shorea robusta resin extract in 3-nitropropionic acid-induced Huntington's disease symptoms in Sprague-Dawley rats. Res Pharm Sci. 2023 Mar 10;18(3):303-316. doi: 10.4103/1735-5362.371586. PMID: 37593162; PMCID: PMC10427789.
[25] E. Brouillet, C. Jacquard, N. Bizat, D. Blum, 3-Nitropropionic acid: a mitochondrial toxin to uncover physiopathological mechanisms underlying striatal degeneration in Huntington's disease, J. Neurochem. 95 (2005) 1521–1540.
[26] M.F. Beal, E. Brouillet, B.G. Jenkins, R.J. Ferrante, N.W. Kowall, J.M. Miller, E. Storey, R.A. Srivastava, B.R. Rosen, B.T. Hyman, Neurochemical and histologic characterization of striatal excitotoxic lesions produced by the mitochondrial toxin 3-nitropropionic acid, J. Neurosci. 13 (1993) 4181–4192.
[27] P. Kumar, A. Kumar, Neuroprotective effect of cyclosporine and FK506 against 3 nitropropionic acid induced cognitive dysfunction and glutathione redox in rat: possible role of nitric oxide, Neurosci. Res. 63 (2009) 302–314.
[28] Pulikkalpura, H., Kurup, R., Mathew, P. *et al.* Levodopa in *Mucuna pruriens* and its degradation. *Sci Rep* **5**, 11078 (2015). https://doi.org/10.1038/srep11078
[29] Khan F, Garg VK, Singh AK, et al. Role of free radicals and certain antioxidants in the management of huntington's disease: a review. J Anal Pharm Res. 2018;7(4):386-392. DOI: 10.15406/japlr.2018.07.00256
[30] Danduga RCSR, Dondapati SR, Kola PK, Grace L, Tadigiri RVB, Kanakaraju VK. Neuroprotective activity of tetramethylpyrazine against 3-nitro propionic acid induced Huntington's disease-like symptoms in rats. Biomed Pharmacother. 2018 Sep; 105:1254-1268. doi: 10.1016/j.biopha.2018.06.079. Epub 2018 Jun 22. PMID: 30021362.