

Neuroprotective effects of *Moringa oleifera*, *Mucuna pruriens*, and *Silybum marianum* in a rotenone-induced Parkinson's disease model

Hoda Dakdouk, Iman Al Housseini, Samah Hachem, Jamilah Borjac*

Department of Biological Sciences, Faculty of Science, Beirut Arab University, Debbieh, Lebanon.

ARTICLE HISTORY

Received on: 21/08/2025
Accepted on: 11/12/2025
Available Online: 05/02/2026

Key words:

Parkinson's disease, *Moringa oleifera* Lam, *Mucuna pruriens* (L.) DC., *Silybum marianum* (L.) Gaertn., PI3K/AKT/mTOR.

ABSTRACT

Parkinson's disease (PD) is a progressive neurodegenerative disorder marked by the loss of dopaminergic neurons, impaired neurotransmission, oxidative stress, neuroinflammation, and the disruption of cellular signalling pathways. This research examined the neuroprotective efficacy of extracts from *Moringa oleifera* Lam. (MO), *Mucuna pruriens* (L.) DC. (MP), and *Silybum marianum* (L.) Gaertn. (SM) in a rotenone-induced PD mice model. Male Balb/c mice received daily intraperitoneal injections of rotenone (2.5 mg/kg) to induce PD, alongside oral administration of individual aqueous plant extracts (5%) for three weeks, noting that Levodopa (the usual positive control) was not included since our plant extracts were primarily tested during PD induction to explore their protective and preventive effects. Neurotransmitters (dopamine, serotonin, and norepinephrine) and inflammatory cytokines interleukin-6 (IL-6), tumor necrosis factor-alpha (TNF- α) were quantified using Enzyme-Linked Immunosorbent Assay. Reverse transcription polymerase chain reaction was used to measure *dopa-decarboxylase* (DDC) levels, nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), nuclear factor erythroid 2-related factor 2 (Nrf2), Phosphatidylinositol 3-Kinase (PI3K), activates protein kinase B (AKT), mammalian target of rapamycin (mTOR), and caspase-3. Protein levels of tyrosine hydroxylase (TH) were assessed using Western blot analysis. All three extracts significantly enhanced the expression of TH and DDC while reducing the concentrations of IL-6, TNF- α , NF- κ B, and caspase-3. MO and SM extracts stimulated Nrf2 activation. The PI3K/AKT/mTOR pathway was augmented, facilitating neuronal survival. MO, MP, and SM extracts exhibit neuroprotective advantages via modulating dopaminergic signalling, diminishing inflammation and apoptosis, and enhancing antioxidant pathways in PD. A primary limitation of the current study is the lack of phytochemical standardization and quantification of the administered extracts (e.g., L-dihydroxyphenylalanine in MP, silymarin in SM, and principal flavonoids in MO) by high-performance liquid chromatography or Liquid chromatography–mass spectrometry; this limitation constrains dose-response interpretation and reproducibility. Future work will include analytical standardization of extracts and quantitative correlation of active constituents with biological effects.

1. INTRODUCTION

Parkinson's disease (PD) is a progressive neurodegenerative disorder marked by motor symptoms like bradykinesia, stiffness, and tremors, which arise from the degeneration of dopaminergic neurons in the substantia nigra pars compacta [1]. In addition to defining motor impairments, PD encompasses various non-motor symptoms, including

cognitive deterioration, mood abnormalities, and autonomic dysfunction, underscoring its intricate etiology [2]. PD affects over 10 million individuals globally, with prevalence expected to double by 2040, largely driven by aging populations, genetic predispositions, and environmental exposures [3,4].

The underlying pathophysiology of PD involves oxidative stress, mitochondrial dysfunction, inflammation, and impaired cellular homeostasis, all contributing to dopaminergic neuronal death, impacting different brain functions [5]. In the brain, the substantia nigra plays a crucial role in the synthesis of dopamine, an essential neurotransmitter responsible for maintaining balance and controlling movements. Dopamine is produced from tyrosine, which is transformed into L-DOPA

*Corresponding Author

Jamilah Borjac, Department of Biological Sciences, Faculty of Science, Beirut Arab University, Debbieh, Lebanon. Email: j.borjac@bau.edu.lb

through tyrosine hydroxylase enzyme. This reaction is followed by the decarboxylation of L-Dihydroxyphenylalanine (L-DOPA) to dopamine, catalyzed by DOPA decarboxylase (DDC) [6]. The multifaceted nature of PD stems from the participation of other non-dopaminergic neurotransmitters, such as serotonin and norepinephrine, leading to a broader array of symptoms [7]. Recent studies have shown that deficits in the noradrenergic system led to motor and non-motor symptoms associated with behavioral and cognitive issues [8,9].

Environmental exposure to pesticides remains a significant public health concern, particularly in agricultural and rural regions where such chemicals are widely used. Among these, rotenone—a naturally derived pesticide from the roots of certain tropical plants—has been identified as a potent environmental toxicant. Although banned or restricted in some countries, rotenone is still used in various regions with a strong association to induce PD-like symptoms upon chronic exposure, where its mode of action relies on the inhibition of mitochondrial complex I [10], a selective mechanism that promotes the generation of reactive oxygen species (ROS), leading to increased oxidative stress, neuroinflammation, and degeneration of dopaminergic neurons [10,11]. The widespread use and environmental persistence of rotenone support its relevance as a real-world risk factor, making it a suitable agent for modelling environmentally induced PD in preclinical research.

Different studies show that oxidative stress and inflammation caused by microglia play a significant role in the ongoing damage of dopamine-producing neurons in PD [12–14]. Microglial cells transform in response to neural injury, known as microgliosis, characterized by cell proliferation, accumulation at damage sites, and significant phenotypic and functional alterations [15]. Alterations in microglial cells lead to increased production of ROS and the release of pro-inflammatory mediators such as interleukin-6 (IL-6) and tumor necrosis factor- α (TNF- α), resulting in exacerbated neuronal damage and neurodegeneration [16,17]. Different transcription factors control inflammation at the genetic level, including the nuclear factor kappa-light-chain-enhancer of activated B cells (NF- κ B), which plays a role in cell survival and inflammation [16]. Reversible oxidative damage affects cellular integrity, and this damage is eased by the master regulator, nuclear factor erythroid 2-related factor 2 (Nrf2). This transcription factor, responsive to redox alterations, resides in the cytoplasm under standard conditions and is activated by oxidative stress. When activated, Nrf2 translocates to the nucleus, where it attaches to antioxidant response elements and starts producing over 200 genes that help protect the cell from damage, enhancing the cell's ability to handle oxidative stress and inflammation [18].

The Phosphatidylinositol 3-Kinase (PI3K)/protein kinase B (AKT)/mammalian target of rapamycin (mTOR) pathway is a significant signaling cascade that regulates several biological processes. In PD, dysregulation increases vulnerability to neuronal death and disease progression. PI3K activates AKT, which subsequently activates mTOR, thus inducing various cellular responses, such as apoptosis inhibition, proliferation, and metabolic control [19].

Despite the availability of symptomatic treatment such as levodopa and dopamine agonists, there remains no cure or established therapy that halts disease progression. The search multitargeted, safe, and cost-effective neuroprotective agents has led to growing interest in plant-derived phytotherapeutics rich in antioxidant and anti-inflammatory compounds exhibiting pharmacological effects [20]. Among these plants, *Moringa oleifera* Lam. (MO) [21], *Mucuna pruriens* (L.) DC. (MP) [22], and *Silybum marianum* (L.) Gaertn. (SM) [23] stand out for their potent anti-inflammatory and antioxidant phytochemicals.

MO, referred to as the “miracle tree,” is indigenous to South Asia and thrives in tropical and subtropical regions. MO is renowned in medicine and nutrition [21]. Pharmacological studies show that MO extracts have essential health benefits and different biological actions such as antimicrobial [24,25], anti-inflammatory [26,27], antidiabetic [28], antioxidant, and anticancer actions [28,29]. Toxicological investigations demonstrate that extracts of MO derived from various plant parts—leaves, seeds, and stem bark—exhibit relative safety regarding toxicity at 2,000 mg/kg [30,31] and often show no deleterious effects even at doses of 4,000–5,000 mg/kg in rats [32].

MP is an herbaceous plant used in Ayurvedic medicine, recognized for its substantial concentration of L-DOPA, and employed in herbal therapy as a substitute for synthetic levodopa to mitigate the symptoms of PD [33]. Although raw seeds may exhibit toxicity due to L-DOPA and other anti-nutritional compounds such as tannins and tryptamines, extensive toxicological research and traditional usage confirm their safety following proper processing [22]. Additionally, these seeds possess pharmacological properties [34], including antioxidant [22], anti-inflammatory, and neuroprotective effects [35].

Silybum marianum (L.) Gaertn, or milk thistle, is a tall, spiny shrub with purple blooms that has been used for over 2,000 years in the treatment of liver, kidney, and gallbladder illnesses, as well as severe conditions such as jaundice and rheumatism [23]. It originated from regions in Europe, America, Africa, Australia, and Asia [36,37]. SM has been used in European salads and as a galactagogue, and it is acknowledged in Iranian traditional medicine as “Harshfbari” [38]. The main active ingredient is silymarin, a composite of flavonolignans [39]. Silymarin has potent antioxidant, anti-inflammatory, antidiabetic, hepatoprotective, nephroprotective, and anticancer effects [40,41]. Research indicates that silymarin induces apoptosis, elevates ROS levels, and inhibits NF- κ B and associated genes responsible for inflammation [42]. SM demonstrates no toxicity in humans, even at doses of 700 mg thrice daily for 24 weeks [43]. It is considered well tolerated, especially within a therapeutic dosage range; nevertheless, some recent studies suggest it may cause gastrointestinal symptoms, notably nausea and diarrhea [44].

These three medicinal plants share complementary mechanisms, enhancing antioxidant defense, reducing neuroinflammation, stabilizing mitochondrial function, and supporting cell survival, making them a comparative and combinatorial evaluation of particular interest in PD research.

This study examines the neuroprotective efficacy of aqueous extracts obtained from MO, MP, and SM in a rotenone-

induced mouse PD model. Although these plants exhibit antioxidant and neuroprotective properties, their comparative efficacy in environmentally induced PD models remains unclear. The research assesses the impact on neurotransmitter levels and related biochemical indicators and clarifies the fundamental molecular mechanisms, offering potential phytotherapeutic strategies to reduce PD progression linked to environmental exposure, providing an experimental basis for phytotherapy-based intervention in PD.

2. MATERIALS AND METHODS

2.1. Materials

Rotenone (Sigma-Aldrich®, cat # R8875-1G), Bradford 1x Dye Reagent (Bio-Rad®, cat # 500-0205), tyrosine hydroxylase antibody (MedchemExpress, HY-P80362), and Goat Polyclonal anti-Rabbit IgG (H+L) secondary antibody [HRP] (Novus Biologicals, NB7160). Primers were synthesized by Macrogen in Korea, following the design provided by NCBI/Primer Blast alignment. The phenyl methyl sulfonyl fluoride (PMSF) was sourced from Roche Diagnostics, lot # 70504525. RNeasy Plus Mini Kit from QIAGEN (cat # 74134), QuantiTect Reverse Transcription Kit from QIAGEN (cat # 205311), and QuantiNova SYBR Green PCR Kit from QIAGEN (cat # 208054). The Enzyme-Linked Immunosorbent Assay (ELISA) kits bought from Elabscience include E-EL-0033 for Serotonin, E-EL-0046 for Dopamine, E-EL-0047 for Noradrenaline, E-ELM0044 for Mouse Interleukin 6, and E-EL-M3063 for Mouse Tumor Necrosis Factor Alpha.

2.2. Ethics approval

The experimental protocol was approved by the Institutional Review Board (IRB) of Beirut Arab University on December 13th, 2023, with the code number (IRB number: 2023-A-0052-S-M-0553), and it followed the Animal Research: Reporting of *in vivo* Experiments (ARRIVE) guidelines as well as the Canadian Council on Animal Care (CCAC) guidelines.

2.3. Preparation of aqueous plant extracts

The plants selected for this experiment were chosen for their pharmaceutical significance in addressing various ailments in both *in-vitro* and *in-vivo* studies, attributed to their bioactive compounds with anti-inflammatory and antioxidant properties [45–47]. MO (the whole young aerial plant) and SM (seeds) were kindly provided by Dr. Mohammad Tarabulsi (PhD in Herbology and Homeopathy) following the cultivation and harvest of both plants under optimal conditions in El-Nabatieh, Lebanon. His authentication was based on morphological examination and phytochemical profiling to confirm key bioactive constituents consistent with each species. The specimens were assigned voucher codes NABTIEH-OCT.2023 and NABTIEH-MAY.2023, respectively, and voucher samples were deposited in the herbarium of Dr. Tarabulsi's research facility for future reference. The MP (krounchbeej) powder was acquired from Royal Herbal Land Pvt. Ltd. (Maharashtra, India, Pune-410401.2023), a certified herbal product supplier, and accompanied by batch and quality assurance documentation confirming botanical origin and purity. Plant powders were stored

in a cool, dry place, shielded from light, at appropriate humidity levels for under 6 months. The extraction method employed was based on traditional aqueous decoction techniques, which are commonly used in ethnopharmacology to preserve heat-stable bioactive compounds while mimicking common routes of human consumption. This method was selected to maintain the integrity of water-soluble phytoconstituents, such as polyphenols, flavonoids, and saponins, known for their neuroprotective potential. 5% aqueous solutions were prepared from each powdered specimen to standardize the preparation process. The 5% aqueous extract served as the stock solution, prepared by adding 5 g of the powdered seed or herb to 100 ml of boiling distilled water and subjected to a standardized heat extraction protocol: boiled for 10 minutes, then simmered for 30 minutes to ensure consistent extraction of active constituents. The mixtures were then filtered through Whatman No. 1 filter paper. Extracts were aliquoted and stored at –20 °C for 1 week before administration to maintain consistency and minimize degradation. The extraction procedure was performed consistently across all batches to ensure reproducibility and reduce variability. From this stock, the dose of 350 mg/kg body weight to be given was calculated based on the individual mouse's weight, which ranged between 20 g at the beginning of the experiment and 32 g at the end of the experiment. The corresponding volume ranging between 0.143 and 0.229 ml was drawn from the 5% extract and administered by oral gavage. We did not perform chromatographic quantification [High-performance liquid chromatography (HPLC)/Liquid chromatography–mass spectrometry (LC-MS)] of marker compounds for the extracts used in this experiment; therefore, the dose of specific active constituents (e.g., L-DOPA, silymarin components, or specific flavonoids) is not known. This limitation is acknowledged and described in the Discussion.

For *Moringa oleifera* Lam. (MO), 5 g of the pulverized dried young shoot and aerial part were added to 100 ml of distilled water, boiled for 10 minutes, then simmered for 30 minutes. Following filtration, the extract was aliquoted and stored at –20 °C for a maximum period of 1 week.

As for the *M. pruriens* (L.) DC. (MP), and *S. marianum* (L.) Gaertn. (SM), 5 g of each of the pulverized seeds were added to 100 ml of distilled water, boiled for 10 minutes, then simmered for 30 minutes. Again, after filtration, the extracts were aliquoted and stored at –20 °C for a maximum period of 1 week.

2.4. PD induction and plant treatment

The induction protocol was done by rotenone induction as described by Rocha *et al.*[48] with changes to the timing of disease onset based on observable Parkinsonian behaviors. PD mice displayed marked impairment in coordination, motor balance, and reflex control, as shown by significant increases in descent time in the pole climb test, shorter latency to fall in the rotarod test, and prolonged crossing time in the beam walk test compared to healthy controls in all experiments. MO, MP, and SM extracts significantly improved performance across all behavioral tests, indicating restored neuromuscular coordination. In rotarod and forced swim tests, all extracts reduced immobility

time and enhanced activity levels, emphasizing the enhanced motor and non-motor activities [48]. Rotenone, a chemical compound, is regarded as an environmental risk factor. It induces progressive neurodegeneration and replicates motor and molecular features of PD in mice by specifically inhibiting mitochondrial complex I. Rotenone (10 mg/ml in dimethyl sulfoxide) was diluted in sesame oil—a non-toxic [49], well-tolerated solvent suitable for its high lipophilicity—before intraperitoneal injection in Balb/c mice at 2.5 mg/kg daily for 21 consecutive days, these 3 weeks of rotenone injection is widely used to induce robust nigrostriatal dysfunction, behavioral deficits and biochemical markers of oxidative stress and inflammation in mice, permitting sensitive evaluation of neuroprotective interventions. The present study was designed to examine the preventive/neuroprotective potential of the extracts during toxin exposure; hence, a 3-week co-treatment paradigm was chosen to detect early mitigation of pathogenic cascades. Treatment duration was selected based on prior studies demonstrating that a 3-week period is sufficient to observe both behavioral recovery and molecular responses in rotenone-induced Parkinson’s models, while minimizing mortality and systemic toxicity [50]. The extract was administered orally by gavage once daily to control and PD model mice at a final dose of 350 mg/kg before rotenone administration. This dosage was selected based on previous studies demonstrating its efficacy in neuroprotection and safety in rodent models, and as evidenced by normal liver and kidney function parameters indicated in a preliminary study. Specifically, MO doses of 200–400 mg/kg have shown antioxidant and neuroprotective effects without adverse effects [51]. MP at 300–400 mg/kg has been used safely to restore dopaminergic function in PD models [52], and SM at 300–500 mg/kg is well-tolerated and effective in modulating oxidative stress and inflammation [53]. The 350 mg/kg dose was selected as a mid-range standard dose to ensure consistent exposure and comparability across all plant extracts. The plant extracts were administered concurrently with rotenone to assess their neuroprotective (preventive) effects during toxin exposure. Co-administration models are commonly used to test whether an intervention can block or attenuate the pathogenic cascade initiated by an environmental toxin (oxidative stress, mitochondrial dysfunction, inflammation), and thus to evaluate prophylactic potential in at-risk populations. A separate post-

treatment (therapeutic) paradigm was not included in this dataset due to resource constraints; such delayed-treatment studies are planned for future work to evaluate the extracts’ ability to reverse established neurodegeneration [54–56]. The weight of the mice was recorded every day during the entire trial duration, and the dose was normalized to each animal’s body weight and delivered by oral gavage. Preliminary toxicity evaluations were conducted to ensure safety, with no observable behavioral or physiological abnormalities at the chosen concentrations, as evidenced by normal liver and kidney function histology.

2.5. Experimental groups

Adult male mice (20–30 g), approximately 6–10 weeks of age, were obtained from the Beirut Arab University animal facility, following ethical approval from the Institutional Review Board (Approval Code: 2023-A-0052-S-M-0553). Mice were housed under controlled laboratory conditions, including a 12-hour light/dark cycle, regulated temperature and humidity, and access to a standard diet and water ad libitum. Animals were allowed to acclimate for 1 week before the start of the experiment. Following acclimatization, mice were randomly assigned to nine experimental groups, as detailed in Table 1, which outlines the treatment protocols for each group. Proper handling techniques were employed throughout the study to minimize stress and prevent injury. At the end of the 3-week experimental period, mice were anesthetized via intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg), followed by cervical dislocation. Brain tissues were immediately harvested, snap-frozen in liquid nitrogen, and stored at –80°C for subsequent analyses. Animals displaying signs of inflammation, deformities, or abnormal behavior were excluded from the study. To minimize variability and confounding factors, the same researcher conducted all experimental procedures, and mice were consistently housed in their original cages throughout the study.

2.6. Biochemical analysis

Mice were anesthetized and euthanized on the 22 days of the experiment. Following decapitation, the brains were promptly dissected, rinsed with 0.9% saline, weighed, and then snap-frozen at –80°C for later analysis. Whole brain tissues were homogenized in a lysis buffer (1 mM

Table 1. Experimental groups and treatment descriptions.

Group	Description
Control	Healthy mice receiving no treatment served as the negative control group
Vehicle	Healthy mice were administered sesame oil (vehicle used for rotenone to induce Parkinson’s disease) via injection
MO	Healthy mice were administered <i>Moringa oleifera</i> extract orally for 21 consecutive days.
MP	Healthy mice were administered <i>Mucuna pruriens</i> extract orally for 21 consecutive days.
SM	Healthy mice were administered <i>Silybum marianum</i> extract orally for 21 consecutive days
PD	Mice were intraperitoneally injected with rotenone for 21 consecutive days to induce a PD model
PD+MO	Rotenone-induced PD mice co-treated with <i>Moringa oleifera</i> extract for 21 days.
PD+MP	Rotenone-induced PD mice co-treated with <i>Mucuna pruriens</i> extract for 21 days.
PD+SM	Rotenone-induced PD mice were co-treated with <i>Silybum marianum</i> extract for 21 days.

Table 2. Primers sequences.

Primer	Forward (5'-3')	Reverse (5'-3')
<i>NF-κB</i>	GAAATTCCTGATCCAGACAAAAAC	ATCACTTCAATGGCCTGTGTGTAG
<i>Nrf2</i>	CAGCATAGAGCAGGACATGGAG	GAACAGCGGTAGTATCAGCCAG
<i>Caspase-3</i>	GGAGTCTGACTGGAAAGCCGAA	CTTCTGGCAAGCCATCTCCTCA
<i>DDC</i>	GGAGCCAGAAACATACGAGGAC	GCATGTCTGCAAGCATAGCTGG
<i>PI3K</i>	ATCATGCAAATCCAGTGCAA	CAGCTGTCCGTCATCTTTCA
<i>AKT</i>	ATCCCCTCAACAACCTTCTCAGT	CTTCCGTCCACTCTTCTCTTC
<i>mTOR</i>	AGAAGGGTCTCCAAGGACGACT	GCAGGACACAAAGGCAGCATTG
<i>GAPDH</i>	CATCACTGCCACCCAGAAGACTG	ATGCCAGTGAGCTTCCCGTTCAG

Gene Descriptions

§ NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells): A key transcription factor involved in the regulation of inflammation and immune responses.

§ Nrf2 (Nuclear factor erythroid 2 related factor 2): A transcription factor that regulates antioxidant response and protects against oxidative stress.

§ Caspase-3: A critical executioner in the apoptosis pathway, often used as a marker of programmed cell death.

§ DDC (Dopa Decarboxylase): An enzyme involved in dopamine biosynthesis, essential for converting L-DOPA to dopamine.

§ PI3K (Phosphoinositide 3-kinase): A signaling molecule involved in cell survival and growth, often dysregulated in neurodegeneration.

§ AKT (Protein Kinase B): A downstream effector of PI3K, playing a key role in promoting neuronal survival.

§ mTOR (Mammalian Target of Rapamycin): A central regulator of cell growth, metabolism, and survival; part of the PI3K/AKT/mTOR pathway.

§ GAPDH (Glyceraldehyde 3-phosphate dehydrogenase): Used as a housekeeping gene for normalization in gene expression analysis.

PMSF in 10 mM phosphate-buffered saline) at a 1:9 (w/v) ratio for biochemical and molecular analyses. Following centrifugation of the homogenates for 5 minutes at 6,000 × g, the supernatants were collected for further study and total protein quantification.

2.7. Protein quantification

The Western blot procedure was performed following the protocol outlined by Yang and Mahmood [57]. Protein samples combined with sodium dodecyl sulfate loading dye were separated on 12% acrylamide gels and transferred to polyvinylidene fluoride membranes utilizing a BIO-RAD electro-transfer system, following the manufacturer's guidelines. Membranes were subsequently blocked with a 5% solution of fat-free milk in bovine serum albumin for 1 hour at room temperature. After blocking, membranes were incubated overnight at 4°C with the primary antibody (HY-P80362, 1:1000) and subsequently allowed to equilibrate at room temperature for one additional hour. Following three washes with TBST buffer, membranes were incubated with the relevant secondary antibody (1:10,000) for 1 hour at room temperature, after which additional washing with TBST was performed. Protein detection utilized an enhanced chemiluminescence (ECL + H₂O₂) reagent and was visualized with the ChemiDoc MP Imaging System (Bio-Rad, USA). Band intensities were normalized to glyceraldehyde 3-phosphate dehydrogenase (GAPDH), serving as the loading control, and target protein expression was quantified accordingly.

2.8. Reverse transcription polymerase chain reaction

Total RNA was extracted from tissue homogenates using the RNeasy Mini Kit (QIAGEN®, USA; Cat. No. 74134), following the manufacturer's instructions. Following the provided protocol, the isolated RNA was reverse-transcribed into complementary DNA using the QuantiTect Reverse

Transcription Kit (QIAGEN®, USA; Cat. No. 205311). The gene expression levels were quantified using specific primers, as listed in Table 2. Where: $\Delta\Delta CT = \{\Delta CT \text{ (a target sample)} - \Delta CT \text{ (GAPDH gene)}\}_{\text{test}} - \{\{\Delta CT \text{ (a target sample)} - \Delta CT \text{ (GAPDH)}\}_{\text{control}}\}$.

2.9. Assessment of inflammatory markers and neurotransmitters

Commercial ELISA kits (Elabscience, USA) were employed to quantify the concentrations of proinflammatory cytokines, specifically IL-6 (Cat. No. E-EL-M0044) and TNF-α (Cat. No. E-EL-M3063), as well as key neurotransmitters including dopamine (E-EL-0046), norepinephrine (E-EL-0047), and serotonin (E-EL-0033), following the protocols provided by the manufacturer.

2.10. Statistical analysis

Statistical analyses were performed using GraphPad Prism software (version 10.4.1). Group differences were assessed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test for multiple comparisons. Normality was verified by the Shapiro–Wilk test. Data are presented as mean ± standard deviation (SD), with $n = 6$ mice per group, and statistical significance was considered at $p \leq 0.05$. Exact p -values and effect sizes were calculated where applicable to indicate the difference magnitude.

3. RESULTS**3.1. Effect of plant extracts on neurotransmitters**

The levels of dopamine, norepinephrine, and serotonin are illustrated in Figure 1 A–C, respectively.

In the PD group, dopamine levels were significantly reduced, showing a 1.7% decrease compared to the control group ($p < 0.0001$). Co-treatment with MO,

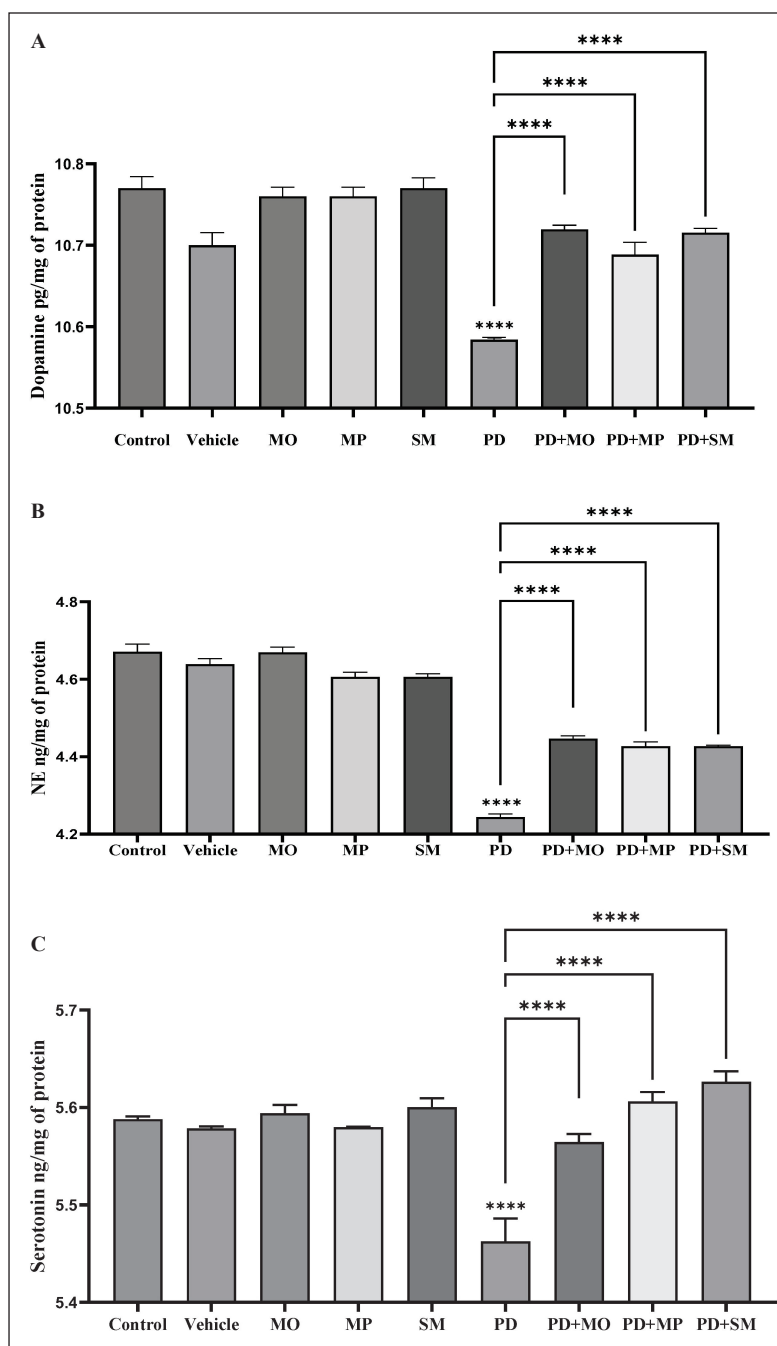


Figure 1. Effects of *Moringa oleifera* Lam. (MO), *Mucuna pruriens* (L.) DC. (MP), and *Silybum marianum* (L.) Gaertn. (SM) on dopamine (pg/mg protein) (A), norepinephrine (NE) (ng/mg protein) (B), and serotonin (ng/mg protein) (C) levels in mouse brain tissue. Data represent mean $\pm$ SD of 6 mice per group ($n = 6$). Statistical significance was evaluated using one-way ANOVA followed by Tukey's post hoc test. **** $p < 0.0001$ indicates significant differences compared to the rotenone-treated group.

MP, and SM extracts significantly reversed this reduction, with dopamine levels increasing by approximately 1.3%, 1.1%, and 1.2%, respectively, compared to the PD group ($p < 0.0001$).

Similarly, norepinephrine levels were markedly decreased in PD mice, showing a 7.7% reduction relative to the control group ($p < 0.0001$). Co-treatment with MO, MP,

and SM extracts significantly restored norepinephrine levels, resulting in an increase of 7.9%, 2.8%, and 3.0%, respectively, versus the PD group ($p < 0.0001$).

Serotonin levels were also declined significantly in the PD group compared to controls, with 3.2% decrease ($p < 0.0001$). Mice co-treated with MO, MP, and SM extracts led to significant improvements elevating serotonin levels by

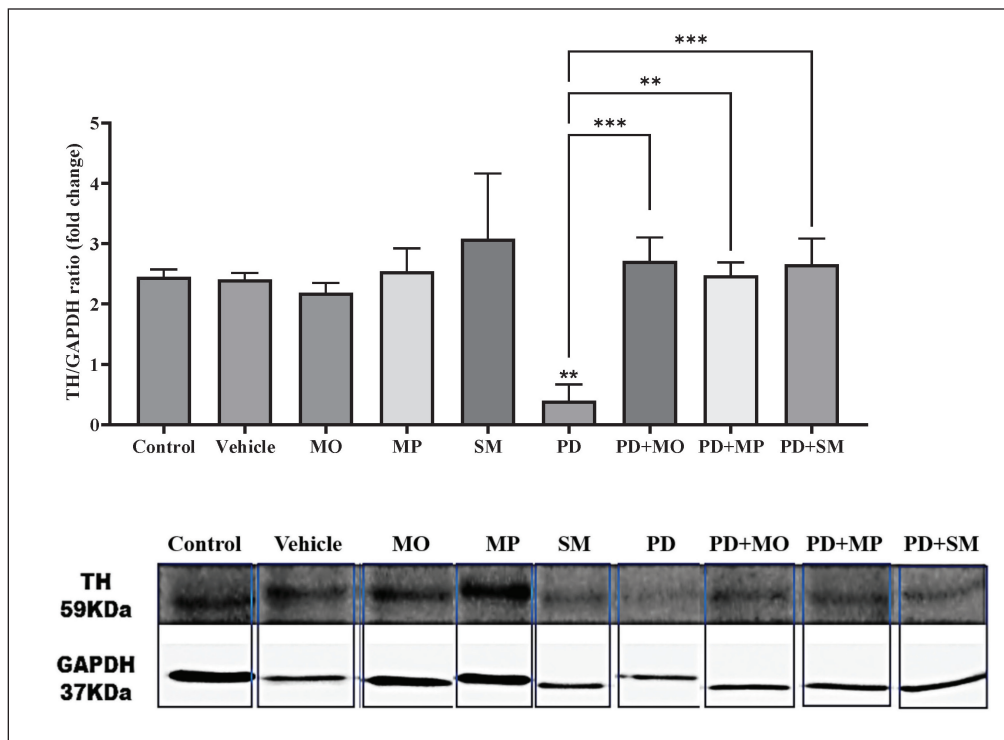


Figure 2. Effects of *Moringa oleifera* Lam. (MO), *Mucuna pruriens* (L.) DC. (MP), and *Silybum marianum* (L.) Gaertn. (SM) on the expression of Tyrosine hydroxylase (TH). TH protein levels were assessed using western blotting and quantified with ImageJ. Expression was normalized to GAPDH and reported as fold-change relative to control. Data are mean $\pm$ SD ($n = 6$). One-way ANOVA followed by Tukey's post hoc test was used for statistical analysis. ** $p < 0.01$ and *** $p < 0.001$ indicate significant differences compared to the rotenone-treated group.

approximately 3.1%, 1.8%, and 2.2%, respectively, compared to untreated PD mice ($p < 0.0001$).

3.2. Effect of plant extracts on TH expression level

TH levels across the experimental groups are illustrated in Figure 2. Induction of PD resulted in a significant reduction in TH expression, showing approximately a 4-fold decline in comparison to the control group ($p < 0.01$). Co-treating PD mice with the three extracts markedly restored TH levels. Specifically, MO, MP, and SM led to 4.33-fold ($p < 0.001$), 4.00-fold ($p < 0.01$), and 4.50-fold ($p < 0.001$), respectively, relative to the PD group. These levels were comparable to, or even exceeded, those observed in the control group.

3.3. Effect of plant treatments on pro-inflammatory cytokines (IL-6 and TNF- α)

Baseline levels of IL-6 and TNF- α remained relatively stable among the control, vehicle, MO, MP, and SM groups, with IL-6 levels ranging between 5.62 and 8.61 pg/mg of protein and TNF- α levels from 0.45 to 0.80 pg/mg of protein. In contrast, PD induction triggered a pronounced inflammatory response, marked by 53% elevation ($p < 0.0001$) in IL-6 and 45% increase ($p < 0.0001$) in TNF- α , compared to the control group. Co-treatment with MO, MP, and SM extracts substantially attenuated these cytokines upregulation ($p <$

0.0001). Specifically, IL-6 levels reduced to 39% MO, 58% MP, and 44% SM, while TNF- α levels significantly decreased by 32.7%, 51.0%, and 27.3%, respectively, in comparison to the PD group. Minor elevations in TNF- α and IL-6 observed in control or vehicle groups likely reflect normal biological variability or mild effects of the vehicle solvent, and are not statistically significant. These slight differences do not affect the overall conclusion of significant cytokine elevation in the PD group and the subsequent reduction by plant extracts. These modulatory effects are graphically represented in Figure 3A and B.

3.4. Effect of extracts on DDC gene expression

According to Figure 4, the healthy mice treated with MO, MP, or SM, DDC mRNA levels were significantly elevated compared to untreated controls, suggesting that these extracts can pharmacologically stimulate dopaminergic metabolism even in the absence of pathology. A significant downregulation of DDC gene expression was observed in PD mice, with a 4.12-fold decrease relative to the control ($p < 0.01$), confirming the impact of rotenone-induced neurodegeneration as illustrated in Figure 4. Treatment with plant extracts markedly reversed this decline. Specifically, MO induced a 32.85-fold increase, MP resulted in a 7.51-fold enhancement, and SM led to a 22.13-fold increase in DDC expression levels compared to the untreated PD group (all $p < 0.0001$).

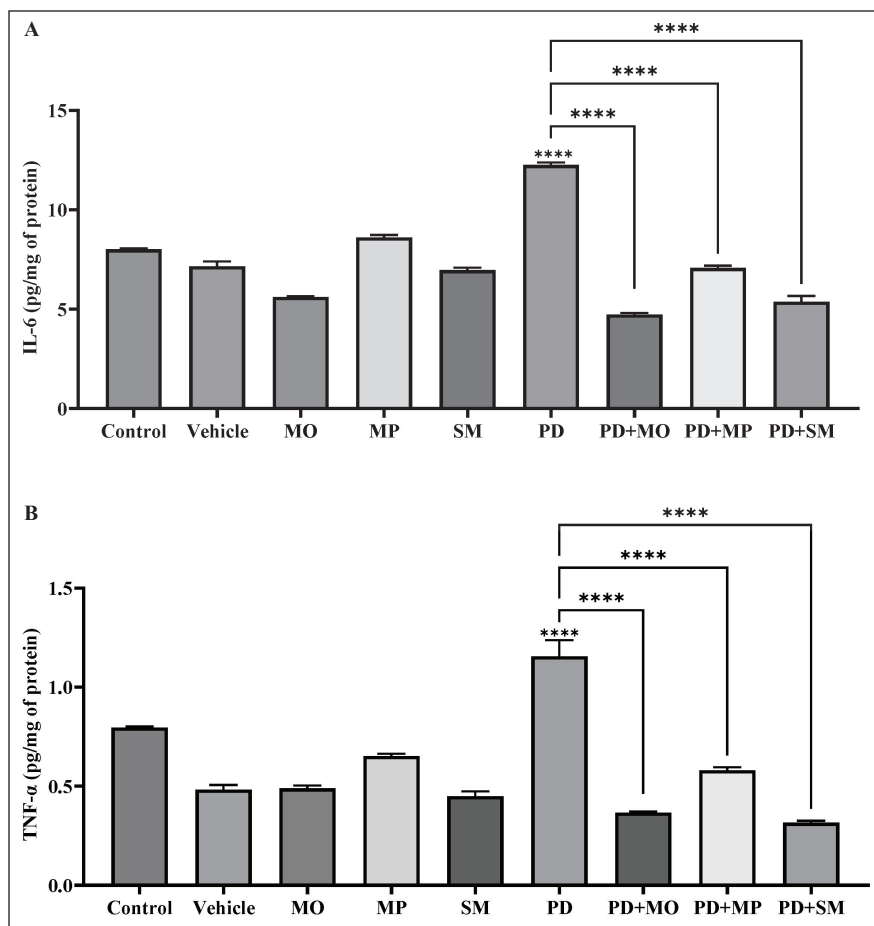


Figure 3. Effects of *Moringa oleifera* Lam. (MO), *Mucuna pruriens* (L.) DC. (MP), and *Silybum marianum* (L.) Gaertn. (SM) on the level of proinflammatory cytokines interleukin-6 in pg/mg of protein (IL-6) (A) and tumor necrosis factor-alpha in pg/mg of protein (TNF-α) (B) in mice brain tissues. Data are means of 6 mice per group $\pm$ SD. Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test. **** $p < 0.0001$ represents statistically significant differences compared to Rotenone-treated group.

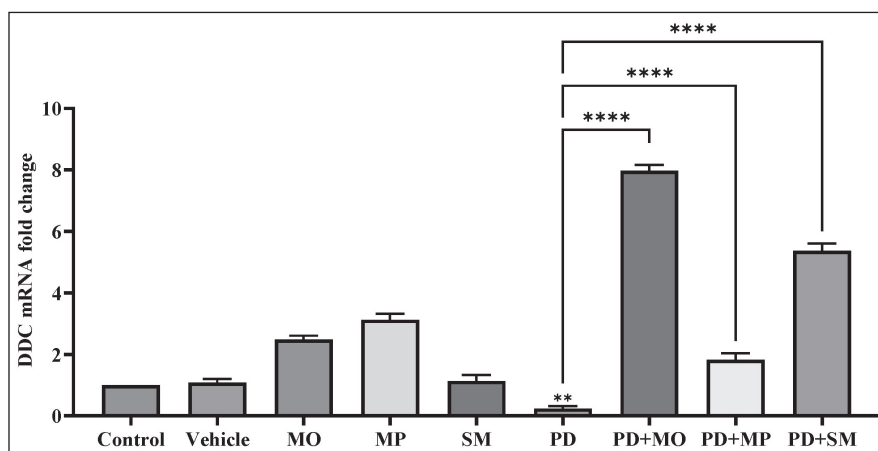


Figure 4. Effects of *Moringa oleifera* Lam. (MO), *Mucuna pruriens* (L.) DC. (MP), and *Silybum marianum* (L.) Gaertn. (SM) on dopa decarboxylase (DDC) gene expression in mice brain tissues. Data are means of 6 mice per group $\pm$ SD. Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test. ** $p < 0.01$ and **** $p < 0.0001$ represent statistically significant differences compared to Rotenone-treated group.

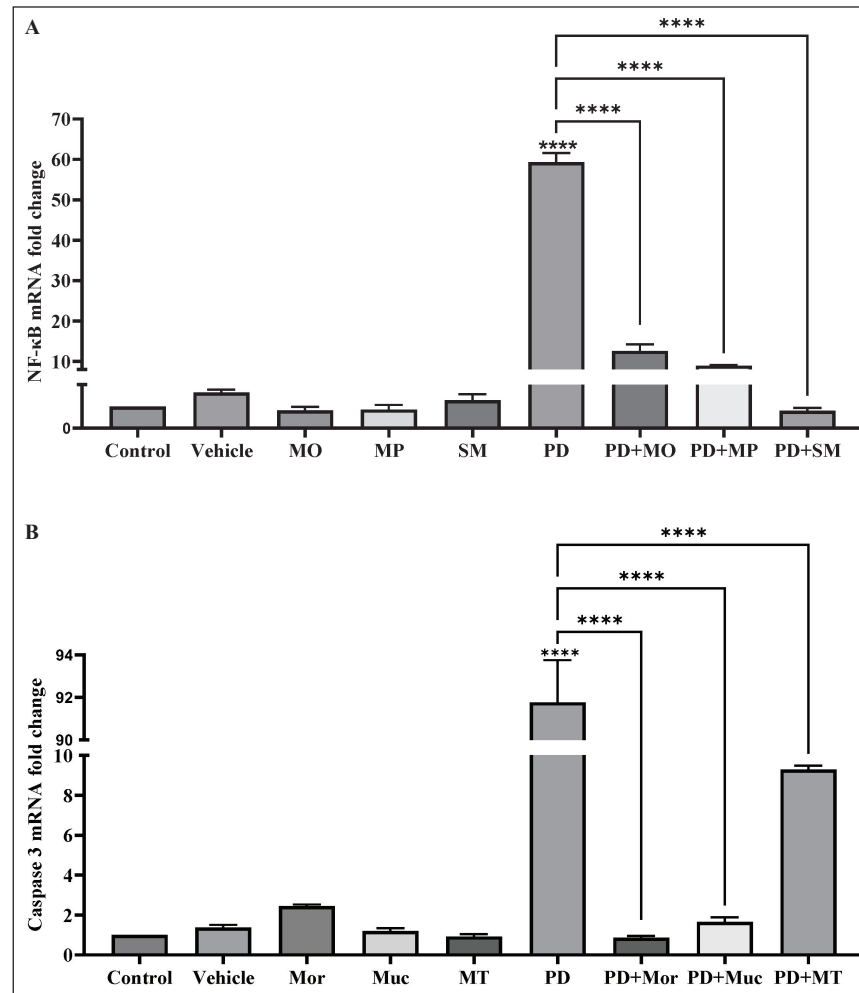


Figure 5. Effects of *Moringa oleifera* Lam. (MO), *Mucuna pruriens* (L.) DC. (MP), and *Silybum marianum* (L.) Gaertn. (SM) on *NF-κB* (A) and caspase-3 (B) gene expression in brain tissues. The results are normalized against GAPDH. Data are means of 6 mice per group $\pm$ SD. Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test. **** p < 0.0001 represents statistically significant differences compared to Rotenone-treated group.

3.5. Effect of plant treatment on NFκB and Caspase-3 gene expression

As shown in Figure 5, no significant changes were observed in *NFκB* and *Caspase-3* gene expression levels in the control, vehicle, MO, MP, and SM groups. PD induction marked upregulation of both genes, with *NFκB* expression increasing by 59.30-fold and *Caspase-3* by 91.77-fold (p < 0.0001), reflecting elevated inflammatory and apoptotic activity associated with PD induction. Co-treatment with MO, MP, and SM extracts effectively normalized these levels (all p < 0.0001). *NFκB* expression was reduced to 21.2% of the PD level with MO, 15.1% with MP, and 1.3% with SM. The magnitude of reduction for *Caspase-3* expression varied, with MO and MP showing pronounced decreases, reducing the level to 0.93% and 1.81% of the PD level, respectively, while SM produced a moderate yet statistically significant decline, reducing the level to 10.1% of the PD level in co-treated mice.

3.6. Plant treatments effect on PI3K/AKT/mTOR pathway

The *PI3K*, *AKT*, and *mTOR* gene expression levels are shown in Figure 6. In the brains of PD mice, the expression levels of the three genes were significantly downregulated—showing reductions of 2.15-fold (p < 0.05), 6.44-fold (p < 0.001), and 6.47-fold (p < 0.0001)—indicating neuronal degeneration compared to the control groups (including control, vehicle, MO, MP, and SM groups). Co-treatment with the plant extracts resulted in a significant upregulation of these genes across all groups. Specifically, *PI3K* expression increased by 14.47-fold with MO (p < 0.001), 12.41-fold with MP (p < 0.0001), and 7.15-fold with SM (p < 0.0001). In the PD mice treated with the three extracts, there was a significant increase in *AKT* expression (p < 0.0001), with MO showing a 6.39-fold increase, MP a 12.07-fold increase, and SM a 29.81-fold increase. Likewise, *mTOR* expression levels significantly increased upon treating PD mice with the aqueous extracts with MO 36.63-fold (p < 0.0001), MP 31.23-fold (p < 0.0001), and SM 5.67-fold (p < 0.01).

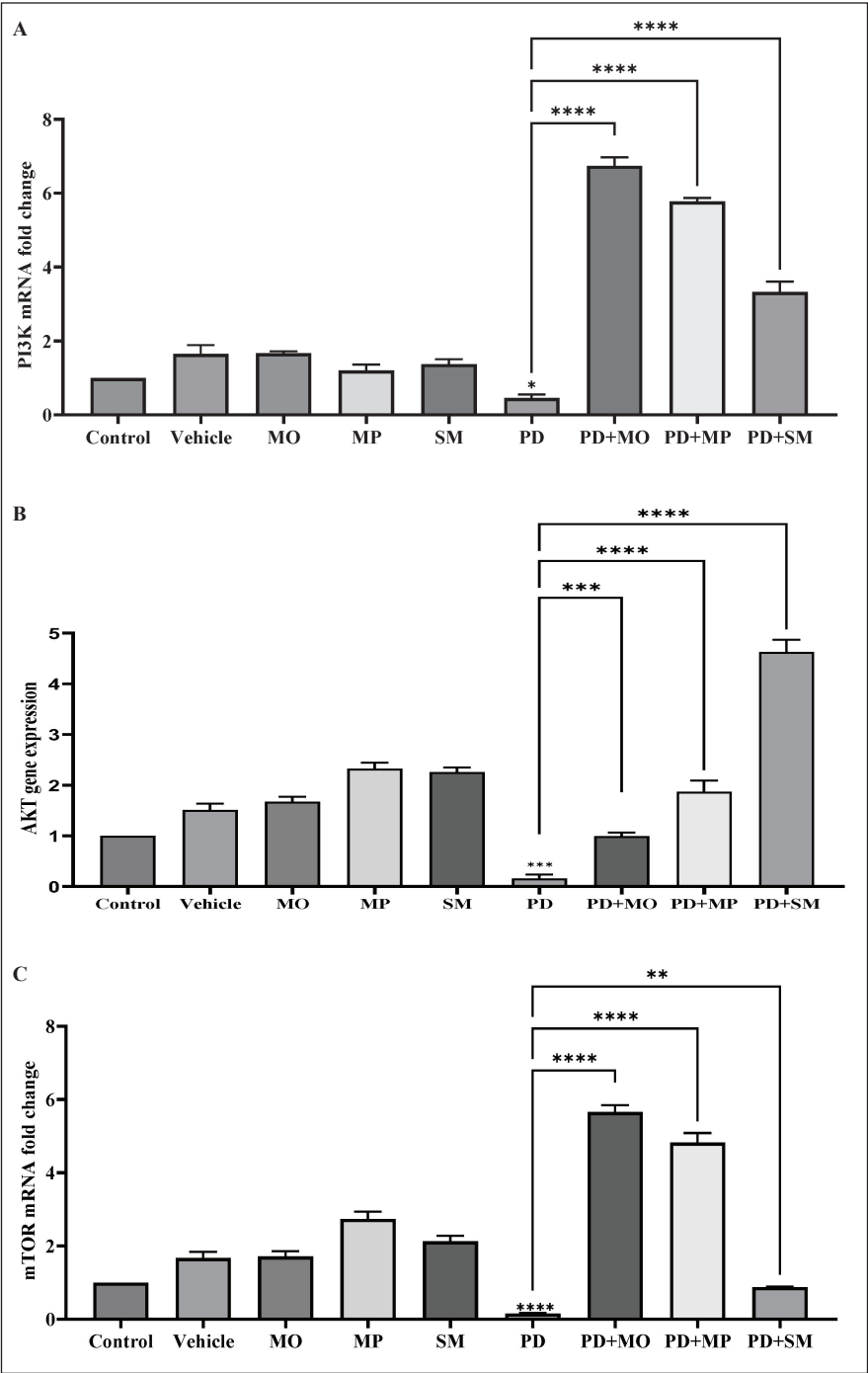


Figure 6. Effects of *Moringa oleifera* Lam. (MO), *Mucuna pruriens* (L.) DC. (MP), and *Silybum marianum* (L.) Gaertn. (SM) on PI3K/AKT/mTOR pathway. Panels (A), (B), and (C) show the gene expression of *PI3K*, *AKT*, and *mTOR*, respectively, in mouse brain tissue. Data are means of 6 mice per group $\pm$ SD. Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ and **** $p < 0.0001$ represent statistically significant differences compared to Rotenone-treated group.

3.7. Plant treatments' effect on Nrf2 gene expression

As presented in Figure 7, Nrf2 gene expression was significantly downregulated following PD induction, showing 66.32-fold decrease ($p < 0.01$) compared to the Control, Vehicle,

MO, MP, and SM. Co-Treatment with MO and SM extracts caused significant upregulation in Nrf2 gene expression, with fold changes of 138.55-fold and 146.97-fold, respectively ($p < 0.0001$). However, treatment with MP extract led to a non-significant 35.93-fold increase.

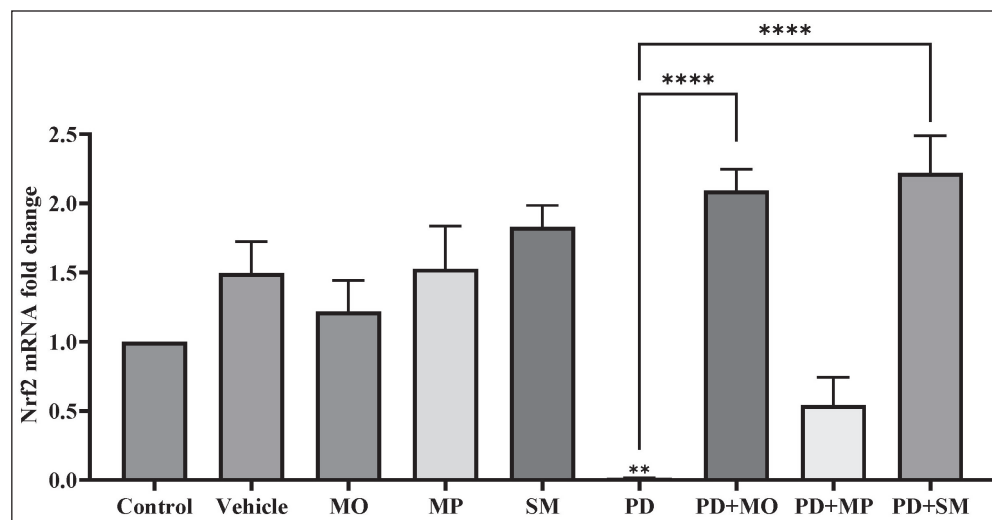


Figure 7. Effects of *Moringa oleifera* Lam. (MO), *Mucuna pruriens* (L.) DC. (MP), and *Silybum marianum* (L.) Gaertn. (SM) on *Nrf2* gene expression in mouse brain tissue. Data are means of 6 mice per group $\pm$ SD. Statistical significance was assessed by one-way ANOVA followed by Tukey's post hoc test. ** $p < 0.01$ and **** $p < 0.0001$ represent statistically significant differences compared to Rotenone-treated group.

4. DISCUSSION

PD is characterized as a progressive neurodegenerative movement disorder with compromised motor function and involuntary movements [1]. Although Parkinson's motor symptoms dominate, the disease increasingly reveals itself as a complex condition featuring impaired neurotransmission, persistent neuroinflammation, oxidative stress, and apoptotic mechanisms [7].

The intricate nature of this disease presents multiple therapeutic options. Phytotherapeutic methods have attracted considerable attention due to their synergistic advantages, little toxicity, and ability to modify disease progression while alleviating symptoms [20]. MP has a high natural L-DOPA concentration, which means it has been shown to restore dopaminergic function [33]. MO has various compounds exhibiting potent anti-oxidative and anti-inflammatory properties [28]. With its main active component, silymarin, SM has potential neuroprotective properties by reversing oxidative damage and regulating the inflammatory cascades [37]. Our main focus in this study was to examine the preventive and neuroprotective potential of MO, MP, and SM when administered during rotenone exposure. While this design does not directly compare the extracts to pharmacological treatments, such as Levodopa, during co-treatment, it provides valuable insight into their ability to protect against PD progression, where co-treatment models emulate real-world scenarios in which individuals are exposed to environmental neurotoxins while taking dietary supplements or nutraceuticals. Future studies may include parallel pharmacological controls to directly benchmark the efficacy of these plant extracts in post treatment models. Although we focused on evaluating each extract independently, several studies have reported enhanced neuroprotection when plant extracts are used in combination or as part of synergistic phytotherapy strategies for PD. For instance, combinations such as *Withania somnifera* with *Centella asiatica*, or *Curcuma*

longa with *M. pruriens* (L.) DC., have demonstrated additive or synergistic effects by simultaneously targeting oxidative stress, mitochondrial dysfunction, and inflammatory pathways [58,59]. Similarly, our previously published work Dakdouk *et al.* [60], evaluated the combination of *Moringa oleifera* Lam., *M. pruriens* (L.) DC., and *S. marianum* (L.) Gaertn. in a rotenone-induced PD model, revealing that co-administration or post ones produced more pronounced behavioral, biochemical, and histopathological improvements compared to individual treatments. These findings support the potential synergistic interaction among the extracts' active phytochemicals.

In the present study, we intentionally examined the extracts separately to isolate and clarify their individual neuroprotective mechanisms. Moreover, due to the high cost of molecular and protein expression analyses, it was not feasible to include a combined-treatment arm within the same experimental dataset. Nevertheless, our results establish a mechanistic foundation for future research to explore combined formulations, which may yield greater therapeutic efficacy through complementary antioxidant, anti-inflammatory, and dopaminergic actions.

Rotenone, a commonly used environmental pesticide, is a well-established agent for inducing parkinsonian pathology in animal models due to its ability to inhibit mitochondrial complex I, leading to oxidative stress, neuroinflammation, and dopaminergic neuron loss in the substantia nigra, ultimately resulting in motor deficits similar to those seen in PD [61] and corresponds with our results shown by various behavioral tests where mice exposed to rotenone exhibited reduced locomotor activity, prolonged descent latency, and impaired motor coordination, consistent with previous findings reporting bradykinesia and rigidity in rotenone-treated models [62]. The behavioral outcomes observed in our study are consistent with previous reports where rotenone administration produced progressive motor impairments reflected by decreased rotarod

latency and Pole climb time delay [63]. Similarly, treatment with MO, MP, and SM markedly improved behavioral performance and mood enhancement, reflecting restored motor coordination and exploratory activity with reduced depressive behavior as reflected by forced swimming and NSS score. These improvements are likely attributed to the extracts' ability to preserve dopaminergic neurons and maintain neurotransmitter balance through antioxidant and anti-inflammatory mechanisms [60]. According to the well-known pathophysiology of PD, the rotenone-induced model demonstrated a decrease in dopamine levels, diminished TH protein expression, and downregulated *DDC* gene expression [62], which corresponds with our results. While our western blot analysis demonstrated a significant reduction in TH in PD group, the corresponding decrease in dopamine levels appeared modest. This can be explained by several compensatory mechanisms [64], including the upregulation of dopamine synthesis in surviving neurons, dopamine turnover from the existing dopamine stores, and the presence of alternative enzymatic pathways contributing to dopamine synthesis [56,57,65]. These findings affirm that compromised dopamine production and signalling substantially contribute to motor impairment in PD. Treatment with MO, MP, and SM extracts substantially reinstated dopamine levels and dramatically enhanced the expression of both TH and *DDC*, indicating a protective influence on dopaminergic neurons and aligns with previous studies highlighting the importance of these plants in modulating the dopaminergic pathway [53,66,67]. As PD progresses, it affects norepinephrine and serotonin levels, resulting in motor impairment, mood disturbances, and autonomic dysfunction [62]. As our results show, norepinephrine and serotonin levels were markedly reduced in the PD group. After the plant extract injections, there was an elevation in norepinephrine and serotonin levels, indicating that the neuroprotective benefits of the therapy extended beyond just alterations in dopaminergic signalling. These results align with other non-Parkinson model studies [68–70], suggesting a more significant phytochemical impact on neurotransmitter modulation.

Neuroinflammation is a primary pathogenic factor in PD. In line with earlier reports, which show that activated microglia produce ROS, NO, and pro-inflammatory cytokines such as TNF- α and IL-6, contributing to a neurotoxic milieu [71,72], our findings demonstrate elevated levels of these mediators in the rotenone-induced model. Caspase may govern apoptotic neuronal demise and central nervous system inflammation [73]. The activation of *caspase-3* constitutes a significant feature of PD [74]. It may provoke neuronal death through apoptosis and activate microglia via inflammation. Consequently, inhibiting the activation of *caspase-3* would produce a synergistic twofold impact in the brain to impede the progression of PD. In our PD model, *caspase-3*, an executioner protease of apoptosis, increased because of rotenone injection [75], demonstrating extensive apoptotic activity [76]. The three extracts inhibited *caspase-3*, showing an anti-apoptotic impact consistent with their maintained neuronal survival. The anti-inflammatory effect of low activated *caspase-3* levels in the brain was confirmed by measuring the pro-inflammatory cytokine TNF- α in mice brain tissues, IL-6, and *NF- κ B*. The

Nrf2 signaling pathway and *NF- κ B* are crucial for regulating the cellular antioxidant defense mechanism. *Nrf2* levels were reduced in PD mice, compromising the antioxidant response and the cells' capacity to withstand oxidative stress [77]. The plant extracts elevated *Nrf2* expression, indicating a potential method for safeguarding neurons by re-establishing redox equilibrium. Although MP showed a numerically large fold-increase (~35.9-fold) in *Nrf2* expression, this change did not reach statistical significance in our dataset. There are several plausible explanations. First, MP's neuroprotective and antioxidant effects are often attributed primarily to its high L-DOPA content and direct radical-scavenging actions, rather than strong activation of transcriptional antioxidant pathways like *Nrf2* [78]. Second, not all phytochemicals can activate *Nrf2* equally. Many *Nrf2* activators are electrophilic polyphenols or flavonoids that modify Keap1–*Nrf2* interactions [79]. MP seed extracts may not contain sufficient bioavailable electrophilic polyphenols compared to MO or SM under the aqueous decoction protocol, thus limiting *Nrf2* induction. Third, they may be rapidly metabolized [80], thus reducing effective cerebral concentration reaching the nigrostriatal pathway; hence, blunting *Nrf2* induction. Finally, MP may exert antioxidant and neuroprotective effects via *Nrf2*-independent mechanisms; for instance, through the enhancement of dopamine turnover, direct ROS scavenging, modulation of mitochondrial enzymes, or upregulation of complementary antioxidant systems. In that sense, the absence of statistical significance in *Nrf2* mRNA induction does not equate to the absence of antioxidant activity. Future work should include quantification of phytochemical profiles, especially electrophilic polyphenols, measurement of *Nrf2* protein activation, determining its nuclear translocation and phosphorylation levels, and measuring the levels of its downstream targets (e.g., HO-1, NQO1). The detrimental cycle involves activated microglia secreting cytokines, resulting in additional neuronal injury and further microglial activation, which may elucidate the progressive loss of dopaminergic neurons observed [71,81,82]. This study suggests that MO, MP, and SM extracts attenuated this feedback loop through *NF- κ B* inhibition and *Nrf2* activation, offering a promising therapeutic strategy for mitigating inflammation-mediated neuronal death.

PI3K/AKT/mTOR pathway plays a central regulatory role in the interplay between neuroinflammation, oxidative stress, and cell survival in the PD context [83]. This pathway often enhances neuronal survival and mitigates excessive inflammatory responses by inhibiting downstream targets such as *GSK-3 β* and *NF- κ B*. Exposure to rotenone has been demonstrated to downregulate *PI3K/AKT/mTOR* signalling [84], resulting in the activation of *caspase-3*, excessive autophagy, and increased oxidative stress and subsequent apoptosis. The dysregulation of this system promotes neuroinflammatory damage due to diminished mTOR-mediated inhibition of pro-inflammatory cytokines, including TNF- α and IL-1 β , together with increased nuclear translocation of *NF- κ B* [85]. In our PD model, *caspase-3* levels were elevated, indicating apoptosis and inflammation-driven neuronal loss. The plant extracts reduced *caspase-3* expression and restored *PI3K/AKT/mTOR* signalling, which may explain both the anti-apoptotic and anti-inflammatory effects observed. The observed

increase in *Nrf2* levels in treated groups may also be regulated through *PI3K*-dependent phosphorylation events since AKT activation enhances *Nrf2* nuclear translocation and antioxidant gene expression while blocking pro-apoptotic signalling [86]. Collectively, the molecular, biochemical, and behavioral data indicate that MO, MP, and SM exert neuroprotective actions through coordinated modulation of oxidative, inflammatory, and apoptotic pathways. The convergence of behavioral recovery with restored *TH*, *DDC*, and *Nrf2* expression strongly supports functional neuroprotection. A central limitation of this study is the absence of quantitative phytochemical standardization of the aqueous extracts, as different extraction conditions can significantly alter compound composition and bioactivity [87]. Although the decoction method and plant authentication are described, we did not quantify marker compounds (e.g., L-DOPA in MP, silymarin constituents in SM, or key flavonoids in MO) using chromatographic techniques. Consequently, the precise dose of active constituents delivered to the animals is unknown, which constrains interpretation in three important ways: [1] it prevents precise dose–response attribution to individual phytochemicals, [2] it limits assurances of batch-to-batch reproducibility, and [3] it prevents direct correlation of specific compounds with the observed molecular and behavioral effects.

5. CONCLUSION

In conclusion, this study proposes that the observed neuroprotective effects of the extracts may involve activation of the *PI3K/AKT/mTOR* axis, leading to enhanced antioxidant response, suppressed apoptosis via reduced caspase-3, and decreased neuroinflammation via inhibition of *NF-κB* and cytokine production. The evaluated extracts may safeguard the brain in PD by equilibrating neurotransmitters, diminishing inflammation, decreasing oxidative stress, averting cell death, and reinstating critical survival mechanisms. This work highlights the significance of environmental exposure to neurotoxins, specifically through the use of rotenone as a modifiable environmental risk factor for PD pathology. These findings underscore the potential of MP, MO, and SM as therapeutic and preventive agents for populations vulnerable to environmental contaminants. Nonetheless, the study is limited by the absence of detailed phytochemical profiling of the extracts. Future analyses employing HPLC or similar advanced techniques are essential to identify and quantify the active constituents responsible for the observed neuroprotective effects, thereby strengthening the mechanistic understanding and reproducibility of these findings.

5.1. Study limitations and future directions

This study provides preliminary evidence that *Moringa oleifera* Lam. (MO), *M. pruriens* (L.) DC. (MP), and *S. marianum* (L.) Gaertn. (SM) may exert neuroprotective effects in a Parkinson's disease model by modulating dopaminergic signaling, reducing inflammation and apoptosis, and enhancing antioxidant pathways. However, these findings should be interpreted cautiously due to variability across molecular markers and functional outcomes. A primary limitation of this work is the absence of phytochemical standardization and chromatographic quantification of the aqueous extracts. Without

LC–MS or HPLC profiling of marker compounds (e.g., L-DOPA, silymarin constituents, and flavonoids), the precise dose of active principles, batch-to-batch reproducibility, and correlation between specific compounds and biological effects cannot be established. Additional limitations included the absence of pharmacokinetic and bioavailability data, and the evaluation was limited to preventive, rather than post-onset, treatment.

Future investigations should incorporate delayed treatment cohorts to assess therapeutic efficacy, extend treatment duration to evaluate long-term effects, and perform comprehensive dose-response, pharmacokinetic, and phytochemical analyses (including LC–MS and HPLC analyses to quantify key bioactive constituents and correlate them with neuroprotective outcomes), to optimize timing, dosing, and mechanistic understanding. Addressing these gaps will strengthen the validation of MO, MP, and SM as neuroprotective agents and provide more robust preclinical evidence for their potential use in Parkinson's disease.

6. AUTHOR CONTRIBUTION

All authors made substantial contributions to conception and design, acquisition of data, or analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work. All the authors are eligible to be author as per the International Committee of Medical Journal Editors (ICMJE) requirements/guidelines.

7. FINANCIAL SUPPORT

There is no funding to report.

8. CONFLICT OF INTEREST

The authors report no financial or any other conflicts of interest in this work.

9. ETHICAL APPROVAL

Ethical approvals details are given in the 'Material and Methods' section.

10. AVAILABILITY OF DATA AND MATERIALS

The data that support the findings of this study are available from the corresponding author upon reasonable request.

11. PUBLISHER'S NOTE

All claims expressed in this article are solely those of the authors and do not necessarily represent those of the publisher, the editors and the reviewers. This journal remains neutral with regard to jurisdictional claims in published institutional affiliation.

12. USE OF ARTIFICIAL INTELLIGENCE (AI)-ASSISTED TECHNOLOGY

The authors declare that they have not used artificial intelligence (AI)-tools for writing of the manuscript, and no images were manipulated using AI.

REFERENCES

- Mhyre TR, Boyd JT, Hamill RW, Maguire-Zeiss KA. Parkinson's Disease. In: Protein aggregation and fibrillogenesis in cerebral and systemic amyloid disease. New York, NY: 2012. 389 p
- Hussein A, Guevara CA, Del Valle P, Gupta S, Benson DL, Huntley GW. Non-Motor Symptoms of Parkinson's Disease: the neurobiology of early psychiatric and cognitive dysfunction. *Neuroscientist*. 2023;29:97–116. doi: <https://doi.org/10.1177/10738584211011979>
- Mahboob A, Ali H, Alnaimi A, Yousef M, Rob M, Al-Muhannadi NA, *et al.* Immunotherapy for Parkinson's disease and Alzheimer's disease: a promising disease-modifying therapy. *Cells*. 2024;13:1527. doi: <https://doi.org/10.3390/cells13181527>
- Bogers JS, Bloem BR, Den Heijer JM. The etiology of Parkinson's disease: new perspectives from gene-environment interactions. *J Parkinsons Dis*. 2023;13:1281–8. doi: <https://doi.org/10.3233/JPD-230250>
- Dash UC, Bhol NK, Swain SK, Samal RR, Nayak PK, Raina V, *et al.* Oxidative stress and inflammation in the pathogenesis of neurological disorders: mechanisms and implications. *Acta Pharm Sin B*. 2025;15:15–34. doi: <https://doi.org/10.1016/j.apsb.2024.10.004>
- Prediger RD, Bortolanza M, de Castro Issy AC, Dos Santos BL, Del Bel E, Raisman-Vozari R. Dopaminergic Neurons in Parkinson's Disease. In: Harris JR, editor. *Handbook of neurotoxicity*. New York, NY: Springer New York; 2014. 753 p doi: https://doi.org/10.1007/978-1-4614-5836-4_7
- Dirkx MF, Bologna M. The pathophysiology of Parkinson's disease tremor. *J Neurol Sci*. 2022;435:120196. doi: <https://doi.org/10.1016/j.jns.2022.120196>
- Paredes-Rodriguez E, Vegas-Suarez S, Morera-Herreras T, De Deurwaerdere P, Miguelez C. The Noradrenergic System in Parkinson's Disease. *Front Pharmacol*. 2020;11:435. doi: <https://doi.org/10.3389/fphar.2020.00435>
- Zhou W, Chu HY. Progressive noradrenergic degeneration and motor cortical dysfunction in Parkinson's disease. *Acta Pharmacol Sin*. 2025;46:829–35. doi: <https://doi.org/10.1038/s41401-024-01428-z>
- Lawana V, Cannon JR. Rotenone neurotoxicity: relevance to Parkinson's disease. In: Richardson JR, Fitsanakis V, Westerink RHS, Kanthasamy AG, editors. *Neurotoxicity of pesticides (advances in neurotoxicology series)*. Amsterdam, Netherlands: Elsevier; 2020. 209 p doi: <https://doi.org/10.1016/bs.ant.2019.11.004>
- Sherer TB, Kim JH, Betarbet R, Greenamyre JT. Subcutaneous rotenone exposure causes highly selective dopaminergic degeneration and α -synuclein aggregation. *Exp Neurol*. 2003;179:9–16. doi: <https://doi.org/10.1006/exnr.2002.8072>
- Peterson LJ, Flood PM. Oxidative stress and microglial cells in Parkinson's Disease. *Mediators Inflamm*. 2012;2012:1–2. doi: <https://doi.org/10.1155/2012/401264>
- Dias V, Junn E, Mouradian MM. The role of oxidative stress in Parkinson's Disease. *J Parkinsons Dis*. 2013;3:461–91. doi: <https://doi.org/10.3233/JPD-130230>
- Jayaram S, Krishnamurthy PT. Role of microgliosis, oxidative stress and associated neuroinflammation in the pathogenesis of Parkinson's disease: the therapeutic role of Nrf2 activators. *Neurochem Int*. 2021;145:105014. doi: <https://doi.org/10.1016/j.neuint.2021.105014>
- Gao C, Jiang J, Tan Y, Chen S. Microglia in neurodegenerative diseases: mechanism and potential therapeutic targets. *Signal Transduct Target Ther*. 2023;8:359. doi: <https://doi.org/10.1038/s41392-023-01588-0>
- Shao QH, Yan WF, Zhang Z, Ma KL, Peng SY, Cao YL, *et al.* Nurrl: a vital participant in the TLR4-NF- κ B signal pathway stimulated by α -synuclein in BV-2 cells. *Neuropharmacology*. 2019;144:388–99. doi: <https://doi.org/10.1016/j.neuropharm.2018.04.008>
- Fellner L, Irschick R, Schanda K, Reindl M, Klimaschewski L, Poewe W, *et al.* Toll-like receptor 4 is required for α -synuclein dependent activation of microglia and astroglia. *Glia*. 2013;61:349–60. doi: <https://doi.org/10.1002/glia.22437>
- Ngo V, Duennwald ML. Nrf2 and oxidative stress: a general overview of mechanisms and implications in human disease. *Antioxidants*. 2022;11:2345. doi: <https://doi.org/10.3390/antiox11122345>
- Maiti P, Manna J, Dunbar GL. Current understanding of the molecular mechanisms in Parkinson's disease: targets for potential treatments. *Transl Neurodegener*. 2017;6:28. doi: <https://doi.org/10.1186/s40035-017-0099-z>
- Nasim N, Sandeep IS, Mohanty S. Plant-derived natural products for drug discovery: current approaches and prospects. *Nucleus*. 2022;65:399–411. doi: <https://doi.org/10.1007/s13237-022-00405-3>
- Pareek A, Pant M, Gupta MM, Kashania P, Ratan Y, Jain V, *et al.* *Moringa oleifera*: an updated comprehensive review of its pharmacological activities, ethnomedicinal, phytopharmaceutical formulation, clinical, phytochemical, and toxicological aspects. *Int J Mol Sci*. 2023;24:2098. doi: <https://doi.org/10.3390/ijms24032098>
- Lampariello LR, Cortelazzo A, Guerranti R, Sticozzi C, Valacchi G. The Magic Velvet Bean of *Mucuna pruriens*. *J Tradit Complement Med*. 2012;2:331–9. doi: [https://doi.org/10.1016/S2225-4110\(16\)30119-5](https://doi.org/10.1016/S2225-4110(16)30119-5)
- Marceddu R, Dinolfo L, Carrubba A, Sarno M, Di Miceli G. Milk thistle (*Silybum marianum* L.) as a Novel Multipurpose Crop for Agriculture in Marginal Environments: a Review. *Agronomy*. 2022;12:729. doi: <https://doi.org/10.3390/agronomy12030729>
- Ahmadu T, Ahmad K, Ismail SI, Rashed O, Asib N, Omar D. Antifungal efficacy of *Moringa oleifera* leaf and seed extracts against *Botrytis cinerea* causing gray mold disease of tomato (*Solanum lycopersicum* L.). *Braz J Biol*. 2021;81:1007–22. doi: <https://doi.org/10.1590/1519-6984.233173>
- Padla EP, Solis LT, Levida RM, Shen CC, Ragasa CY. Antimicrobial isothiocyanates from the seeds of *Moringa oleifera* Lam. *Zeitschrift Für Naturforschung C*. 2012;67:557–64. doi: <https://doi.org/10.1515/znc-2012-11-1205>
- Luetragoon T, Pankla Sranujit R, Noysang C, Thongsri Y, Potup P, Suphrom N, *et al.* Bioactive Compounds in *Moringa oleifera* Lam. leaves inhibit the pro-inflammatory mediators in lipopolysaccharide-induced human monocyte-derived macrophages. *Molecules*. 2020;25:191. doi: <https://doi.org/10.3390/molecules25010191>
- Cuellar-Núñez ML, Gonzalez De Mejia E, Loarca-Piña G. *Moringa oleifera* leaves alleviated inflammation through downregulation of IL-2, IL-6, and TNF- α in a colitis-associated colorectal cancer model. *Food Res Int*. 2021;144:110318. doi: <https://doi.org/10.1016/j.foodres.2021.110318>
- Leone A, Spada A, Battezzati A, Schiraldi A, Aristil J, Bertoli S. Cultivation, Genetic, Ethnopharmacology, Phytochemistry and Pharmacology of *Moringa oleifera* Leaves: an Overview. *Int J Mol Sci*. 2015;16:12791–2835. doi: <https://doi.org/10.3390/ijms160612791>
- Zduńska K, Dana A, Kolodziejczak A, Rotsztein H. Antioxidant properties of ferulic acid and its possible application. *Skin Pharmacol Physiol*. 2018;31:332–6. doi: <https://doi.org/10.1159/000491755>
- Min Zhang. Phytochemicals, antioxidant and antimicrobial activity of *Hibiscus sabdariffa*, *Centella asiatica*, *Moringa oleifera* and *Murraya koenigii* leaves. *J Med Plants Res*. 2011;5:6672–80. doi: <https://doi.org/10.5897/JMPR11.621>
- Asiedu-Gyekye IJ, Frimpong-Manso S, Awortwe C, Antwi DA, Nyarko AK. Micro- and macroelemental composition and safety evaluation of the nutraceutical *Moringa oleifera* leaves. *J Toxicol*. 2014;2014:1–3. doi: <https://doi.org/10.1155/2014/786979>
- Ajibade TO, Arowolo R, Olayemi FO. Phytochemical screening and toxicity studies on the methanol extract of the seeds of *Moringa oleifera*. *Jcim*. 2013;10:11–6. doi: <https://doi.org/10.1515/jcim-2012-0015>
- Katzenschlager R. *Mucuna pruriens* in Parkinson's disease: a double blind clinical and pharmacological study. *J Neurol Neurosurg Psychiatry*. 2004;75:1672–7. doi: <https://doi.org/10.1136/jnnp.2003.028761>
- 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;10(3):261. doi: <https://doi.org/10.1007/s13205-020-02253-x>
35. Misra L, Wagner H. Alkaloidal constituents of *Mucuna pruriens* seeds. *Phytochemistry*. 2004;65:2565–67. doi: <https://doi.org/10.1016/j.phytochem.2004.08.045>
 36. Andrzejewska J, Martinelli T, Sadowska K. *Silybum marianum*: non-medical exploitation of the species. *Ann Appl Biol*. 2015;167:285–97. doi: <https://doi.org/10.1111/aab.12232>
 37. Bhattacharya S. Phytotherapeutic properties of milk thistle seeds: an overview. *J Adv Pharm Educ & Res*. 2011;1:69–79.
 38. Brandon-Warner E, Eheim AL, Foureau DM, Walling TL, Schrum LW, Mckillop IH. Silibinin (Milk Thistle) potentiates ethanol-dependent hepatocellular carcinoma progression in male mice. *Cancer Lett*. 2012;326:88–95. doi: <https://doi.org/10.1016/j.canlet.2012.07.028>
 39. Lee DYW, Liu Y. Molecular structure and stereochemistry of Silybin A, Silybin B, Isosilybin A, and Isosilybin B, Isolated from *Silybum marianum* (Milk Thistle). *J Nat Prod*. 2003;66:1171–74. doi: <https://doi.org/10.1021/np030163b>
 40. Deep G, Agarwal R. Chemopreventive efficacy of silymarin in skin and prostate cancer. *Integr Cancer Ther*. 2007;6:130–45. doi: <https://doi.org/10.1177/1534735407301441>
 41. Abenavoli L, Capasso R, Milic N, Capasso F. Milk thistle in liver diseases: past, present, future. *Phytotherapy Res*. 2010;24:1423–32. doi: <https://doi.org/10.1002/ptr.3207>
 42. Jaffar HM, Al-Asmari F, Khan FA, Rahim MA, Zongo E. Silymarin: unveiling its pharmacological spectrum and therapeutic potential in liver diseases—A comprehensive narrative review. *Food Sci Nutr*. 2024;12:3097–111. doi: <https://doi.org/10.1002/fsn3.4010>
 43. Ansari D, Tingstedt B, Andersson B, Holmquist F, Stureson C, Williamsson C, *et al.* Pancreatic cancer: yesterday, today and tomorrow. *Future Oncol*. 2016;12:1929–46. doi: <https://doi.org/10.2217/fon-2016-0010>
 44. Emadi SA, Ghasemzadeh Rahbardo M, Mehri S, Hosseinzadeh H. A review of therapeutic potentials of milk thistle (*Silybum marianum* L.) and its main constituent, silymarin, on cancer, and their related patents. *Iran J Basic Med Sci*. 2022;25:1166–76. doi: <https://doi.org/10.22038/IJBMS.2022.63200.13961>
 45. Devi KP, Malar DS, Braid N, Nabavi SM, Nabavi SF. A Mini Review on the Chemistry and Neuroprotective Effects of Silymarin. *Curr Drug Targets*. 2017;18:1529–36. doi: <https://doi.org/10.2174/1389450117666161227125121>
 46. Mundkar M, Bijalwan A, Soni D, Kumar P. Neuroprotective potential of *Moringa oleifera* mediated by NF- κ B/Nrf2/HO-1 signaling pathway: a review. *J Food Biochem*. 2022;46:e14451. doi: <https://doi.org/10.1111/jfbc.14451>
 47. Zahra W, Birla H, Singh SS, Rathore AS, Dilnashin H, Singh R, *et al.* Neuroprotection by *Mucuna pruriens* in neurodegenerative diseases. *Neurochem Res*. 2022;47:1816–29. doi: <https://doi.org/10.1007/s11064-022-03591-3>
 48. Rocha SM, Bantle CM, Aboellail T, Chatterjee D, Smeyne RJ, Tjalkens RB. Rotenone induces regionally distinct α -synuclein protein aggregation and activation of glia prior to loss of dopaminergic neurons in C57Bl/6 mice. *Neurobiol Dis*. 2022;167:105685. doi: <https://doi.org/10.1016/j.nbd.2022.105685>
 49. Tanner CM, Kamel F, Ross GW, Hoppin JA, Goldman SM, Korell M, *et al.* Rotenone, Paraquat, and Parkinson's Disease. *Environ Health Perspect*. 2011;119:866–72. doi: <https://doi.org/10.1289/ehp.1002839>
 50. Ashok C, Jeyabalan S, Sugumar R, Jabaris SL, Rajasekaran NK, Bala L, *et al.* Indian medicinal plants as neuroprotective agents in Parkinson's disease: bridging tradition and therapeutics. *Phytomedicine Plus*. 2025;5:100905. doi: <https://doi.org/10.1016/j.phyplu.2025.100905>
 51. Worku B, Tolossa N. A review on the neuroprotective effect of *Moringa oleifera*. *Oxid Med Cell Longev*. 2024;2024:7694516. <https://doi.org/10.1155/2024/7694516>
 52. Yadav SK, Prakash J, Chouhan S, Singh SP. *Mucuna pruriens* seed extract reduces oxidative stress in nigrostriatal tissue and improves neurobehavioral activity in paraquat-induced Parkinsonian mouse model. *Neurochem Int*. 2013;62:1039–47. doi: <https://doi.org/10.1016/j.neuint.2013.03.015>
 53. Borah A, Paul R, Choudhury S, Choudhury A, Bhuyan B, Das Talukdar A, *et al.* Neuroprotective potential of silymarin against u003cscpu003eCNSu003cscpu003e disorders: insight into the pathways and molecular mechanisms of action. *CNS Neurosci Ther*. 2013;19:847–53. doi: <https://doi.org/10.1111/cns.12175>
 54. Mata-Bermudez A, Diaz-Ruiz A, Silva-García LR, Gines-Francisco EM, Noriega-Navarro R, Rios C, *et al.* *Mucuna pruriens*, a possible treatment for depressive disorders. *Neurol Int*. 2024;16:1509–27. doi: <https://doi.org/10.3390/neurolint16060112>
 55. Soleimani V, Delghandi PS, Moallem SA, Karimi G. Safety and toxicity of silymarin, the major constituent of milk thistle extract: an updated review. *Phytotherapy Res*. 2019;33:1627–38. doi: <https://doi.org/10.1002/ptr.6361>
 56. Galvão Silva NR, Costa WK, Assunção Ferreira MR, Breitenbach Barroso Coelho LC, Lira Soares LA, Napoleão TH, *et al.* 13-Week repeated-dose toxicity study of optimized aqueous extract of *Moringa oleifera* leaves in mice. *J Ethnopharmacol*. 2024;335:118637. doi: <https://doi.org/10.1016/j.jep.2024.118637>
 57. Yang PC, Mahmood T. Western blot: technique, theory, and troubleshooting. *N Am J Med Sci*. 2012;4:429. doi: <https://doi.org/10.4103/1947-2714.100998>
 58. Aniruddha K, Manoj T, Meera S. Synergistic effect of *Macuna pruriens* and *Curcuma longa* in rotenone induced Parkinson diseased animal model. *Asian J Pharm Clin Res*. 2025;2025:186–90. doi: <https://doi.org/10.22159/ajpcr.2025v18i6.54421>
 59. Prakash J, Yadav SK, Chouhan S, Prakash S, Singh SP. Synergistic effect of *Mucuna pruriens* and *Withania somnifera* in a paraquat induced Parkinsonian mouse model. *Adv Biosci Biotechnol*. 2013;4:1–9. doi: <https://doi.org/10.4236/abb.2013.411A2001>
 60. Dakdouk H, Housseini I Al, Shaheen M, El Natour H, Borjac J. Comparative neuroprotective effects of *Moringa oleifera*, *Mucuna pruriens*, and milk thistle extracts in a rotenone-induced Parkinson's disease model: Behavioral, biochemical, and histopathological insights. *Edelweiss Appl Sci Technol*. 2025;9:862–86. doi: <https://doi.org/10.55214/25768484.v9i7.8759>
 61. Sherer TB, Betarbet R, Testa CM, Seo BB, Richardson JR, Kim JH, *et al.* Mechanism of Toxicity in Rotenone Models of Parkinson's Disease. *J Neurosci*. 2003;23:10756–64. doi: <https://doi.org/10.1523/JNEUROSCI.23-34-10756.2003>
 62. Khafajah Y, Shaheen M, Natour DE, Merheb M, Matar R, Borjac J. Neuroprotective effects of zinc oxide nanoparticles in a rotenone-induced mouse model of Parkinson's Disease. *Nanotheranostics*. 2024;8:497–505. doi: <https://doi.org/10.7150/ntno.95863>
 63. Prasad EM, Hung SY. Behavioral tests in neurotoxin-induced animal models of Parkinson's Disease. *Antioxidants*. 2020;9:1007. doi: <https://doi.org/10.3390/antiox9101007>
 64. Blesa J, Trigo-Damas I, Dileone M, Del Rey NLG, Hernandez LF, Obeso JA. Compensatory mechanisms in Parkinson's disease: circuits adaptations and role in disease modification. *Exp Neurol*. 2017;298:148–61. doi: <https://doi.org/10.1016/j.expneurol.2017.10.002>
 65. Brotchie J, Fitzer-Attas C. Mechanisms compensating for dopamine loss in early Parkinson disease. *Neurology*. 2009;72:S32–8. doi: <https://doi.org/10.1212/WNL.0b013e318198e0e9>
 66. Singh S, Keshri PK, Mishra VN, Singh SP. *Moringa oleifera* Modulates MPTP-induced mitochondrial dysfunction in parkinson's mouse model: an *in silico* and *in vivo* analysis. *J Pharmacol Pharmacother*. 2023;14:187–200. doi: <https://doi.org/10.1177/0976500X231220085>

67. Luthra PM, Singh S. Identification and optimization of tyrosine hydroxylase activity in *Mucuna pruriens* DC. var. utilis. *Planta*. 2010;231:1361–9. doi: <https://doi.org/10.1007/s00425-010-1140-y>
68. Patel JS, Galani VJ. Investigation of noradrenaline and serotonin mediated antidepressant action of *Mucuna pruriens* (L) D.C seeds using various experimental models. *Orient Pharm Exp Med*. 2013;13:143–8. doi: <https://doi.org/10.1007/s13596-012-0089-8>
69. Kosari-Nasab M, Shokouhi G, Ghorbanihaghjo A, Abbasi MM, Salari AA. Anxiolytic- and antidepressant-like effects of Silymarin compared to diazepam and fluoxetine in a mouse model of mild traumatic brain injury. *Toxicol Appl Pharmacol*. 2018;338:159–73. doi: <https://doi.org/10.1016/j.taap.2017.11.012>
70. Thakare VN, Patil RR, Oswal RJ, Dhakane VD, Aswar MK, Patel BM. Therapeutic potential of silymarin in chronic unpredictable mild stress induced depressive-like behavior in mice. *J Psychopharmacology*. 2018;32:223–35. doi: <https://doi.org/10.1177/0269881117742666>
71. Block ML, Zecca L, Hong JS. Microglia-mediated neurotoxicity: uncovering the molecular mechanisms. *Nat Rev Neurosci*. 2007;8:57–69. doi: <https://doi.org/10.1038/nrn2038>
72. Chéret C, Gervais A, Lelli A, Colin C, Amar L, Ravassard P, *et al.* Neurotoxic activation of microglia is promoted by a Nox1-dependent NADPH oxidase. *J Neurosci*. 2008;28:12039–51. doi: <https://doi.org/10.1523/JNEUROSCI.3568-08.2008>
73. Nguyen TTM, Gillet G, Popgeorgiev N. Caspases in the developing central nervous system: apoptosis and beyond. *Front Cell Dev Biol*. 2021;9:702404. doi: <https://doi.org/10.3389/fcell.2021.702404>
74. Hartmann A, Hunot S, Michel PP, Muriel MP, Vyas S, Faucheux BA, *et al.* Caspase-3: a vulnerability factor and final effector in apoptotic death of dopaminergic neurons in Parkinson's disease. *Proc Natl Acad Sci*. 2000;97:2875–80. doi: <https://doi.org/10.1073/pnas.040556597>
75. Liu Y, Guo Y, An S, Kuang Y, He X, Ma H, *et al.* Targeting caspase-3 as dual therapeutic benefits by RNAi facilitating brain-targeted nanoparticles in a rat model of Parkinson's Disease. *PLoS One*. 2013;8:e62905. doi: <https://doi.org/10.1371/journal.pone.0062905>
76. D'Amelio M, Cavallucci V, Cecconi F. Neuronal caspase-3 signaling: not only cell death. *Cell Death Differ*. 2010;17:1104–14. doi: <https://doi.org/10.1038/cdd.2009.180>
77. Anandhan A, Chen W, Nguyen N, Madhavan L, Dodson M, Zhang DD. A-Syn overexpression, NRF2 suppression, and enhanced ferroptosis create a vicious cycle of neuronal loss in Parkinson's disease. *Free Radic Biol Med*. 2022;192:130–40. doi: <https://doi.org/10.1016/j.freeradbiomed.2022.09.015>
78. Cacciatore I, Marinelli L, Di Stefano A, Di Marco V, Orlando G, Gabriele M, *et al.* Chelating and antioxidant properties of L-Dopa containing tetrapeptide for the treatment of neurodegenerative diseases. *Neuropeptides*. 2018;71:11–20. doi: <https://doi.org/10.1016/j.npep.2018.06.002>
79. Huenchuguala S, Segura-Aguilar J. Natural compounds that activate the KEAP1/Nrf2 signaling pathway as potential new drugs in the treatment of idiopathic Parkinson's disease. *Antioxidants*. 2024;13:1125. doi: <https://doi.org/10.3390/antiox13091125>
80. Alavijeh MS, Chishty M, Qaiser MZ, Palmer AM. Drug metabolism and pharmacokinetics, the blood-brain barrier, and central nervous system drug discovery. *NeuroRx*. 2005;2:554–71. doi: <https://doi.org/10.1602/neurorx.2.4.554>
81. Gao HM, Hong JS. Why neurodegenerative diseases are progressive: uncontrolled inflammation drives disease progression. *Trends Immunol*. 2008;29:357–65. doi: <https://doi.org/10.1016/j.it.2008.05.002>
82. Gao HM, Zhou H, Zhang F, Wilson BC, Kam W, Hong JS. HMGB1 acts on microglia mac1 to mediate chronic neuroinflammation that drives progressive neurodegeneration. *J Neurosci*. 2011;31:1081–92. doi: <https://doi.org/10.1523/JNEUROSCI.3732-10.2011>
83. Goyal A, Agrawal A, Verma A, Dubey N. The PI3K-AKT pathway: a plausible therapeutic target in Parkinson's disease. *Exp Mol Pathol*. 2023;129:104846. doi: <https://doi.org/10.1016/j.yexmp.2022.104846>
84. Zhang Y, Guo H, Guo X, Ge D, Shi Y, Lu X, *et al.* Involvement of Akt/mTOR in the neurotoxicity of rotenone-induced Parkinson's disease models. *Int J Environ Res Public Health*. 2019;16:3811. doi: <https://doi.org/10.3390/ijerph16203811>
85. Han CJ, Zheng JY, Sun L, Yang HC, Cao ZQ, Zhang XH, *et al.* The oncometabolite 2-hydroxyglutarate inhibits microglial activation via the AMPK/mTOR/NF-κB pathway. *Acta Pharmacol Sin*. 2019;40:1292–302. doi: <https://doi.org/10.1038/s41401-019-0225-9>
86. Lee JA, Son HJ, Choi JW, Kim J, Han SH, Shin N, *et al.* Activation of the Nrf2 signaling pathway and neuroprotection of nigral dopaminergic neurons by a novel synthetic compound KMS99220. *Neurochem Int*. 2018;112:96–107. doi: <https://doi.org/10.1016/j.neuint.2017.11.006>
87. Noviana E, Indrayanto G, Rohman A. Advances in fingerprint analysis for standardization and quality control of herbal medicines. *Front Pharmacol*. 2022;13:853023. doi: <https://doi.org/10.3389/fphar.2022.853023>

How to cite this article:

Dakdouk H, Al Housseini I, Hachem S, Borjac J. Neuroprotective effects of *Moringa oleifera*, *Mucuna pruriens*, and *Silybum marianum* in a rotenone-induced Parkinson's disease model. *J Appl Pharm Sci*. 2026;16(03):421-436. DOI: 10.7324/JAPS.2026.278673