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RESEARCH ARTICLE

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Neuroprotective effect of rhaponticin against Parkinson disease: Insights from in vitro BV-2 model and in vivo MPTP-induced mice model

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Abstract

Parkinson's disease (PD) is a complex neurodegenerative illness associated with the loss or damage to neurons of the dopaminergic system in the brain. Few therapeutic approaches and considerable side effects of conventional drugs necessitate a new therapeutic agent to treat patients with PD. Rhaponticin is a natural hydroxystilbene, found in herbal plants such as *Rheum rhaponticum*, and known to have desirable biological activity including anti-inflammatory properties. However, the neuroinflammation on rhaponticin levels has only been investigated partially so far. So, the current study explored whether rhaponticin could ameliorate the pathophysiology observed in both the in vitro microglial BV-2 cells and the in vivo (1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine [MPTP])-mediated PD model. The results show rhaponticin significantly attenuated lipopolysaccharide (LPS)-mediated microglial activation by suppressing nitric oxide synthase in conjunction with abridged reactive oxygen species production together with proinflammatory mediator reduction. In vivo rhaponticin treatment improves motor impairments as well as the loss of dopaminergic neurons in MPTP-treated mice possibly through suppression via mediators of inflammation. Taken together, these results offer evidence that rhaponticin exerts anti-inflammatory effects and neuroprotection in an LPS-induced microglial model and the MPTP-induced mouse models of PD.

KEYWORDS

microglia, neuroinflammation, neurotoxic, Parkinson's disease, proinflammatory cytokines, rhaponticin

1 | INTRODUCTION

Parkinson's disease (PD) is a common neurodegenerative disorder, which is generally defined by four cardinal signs, which are the rest tremor, rigidity, bradykinesia, and postural instability. All these signs excluding tremors are associated with impaired balance control. For patients with PD, impaired balance control greatly influences the life quality of patients.[1,2] PD is the second most prevalent neurodegenerative disease that mainly affects peoples aged more than 65 years.[3] The development of PD is distinguished by neuronal loss in the substantia nigra, intracellular inclusions of alpha-synuclein, and dopamine (DA) signaling deficiency.[4,5] The main pathological pathway observed in PD is due to severe degeneration of DA in the midbrain area and subsequent reduction of DA supply leading to an imbalance of neurotransmitters such as acetylcholine and DA-causing PD symptoms.[6] In general, patients with PD are observed with distinctive symptoms such as myotonia (neuromuscular condition),

bradykinesia (movement impairment), resting tremor (involuntary quivering movement), and postural instability. Furthermore, almost half of patients with PD experience frontostriatal-mediated executive dysfunction, including lack of attention, deficit mental action, communication disturbances, weakening of memory, and impulsive behavior.[7]

Extensive studies have shown that neuroinflammation may be responsible for neurodegenerative events in PD. Microglia, the occupant of immune cells (macrophage) in the brain, is accountable for homeostasis maintenance.[8] In the event of brain damage induced by various factors including inflammation and oxidative stress, microglial cells from ramified cells (resting) become amoeboid microglial cells (activated).[9] Specifically, this activated microglia triggers a cascade of neurotoxic and mediators of proinflammatory including tumor necrosis factor-α (TNF-α), interleukins (ILs), prostaglandin E2 (PGE2), and nitric oxide (NO) via activation of nuclear factor kappa-light-chain-enhancer of activated B cells.[10] As neuroinflammation is the key pathogenic route in PD, thus anti-inflammatory therapeutic strategies may halt disease progression before irreversible damage and clinical symptoms can take place. The activated microglia can release numerous types of proinflammatory mediators like TNF-α, IL-1β, and IL-6 that can lead to accelerating neuronal dysfunction and neuronal degeneration, eventually hastening the progression of PD.[11] However, various studies have demonstrated the links between proinflammatory cytokines and PD progression. These links were unpredictable for the single cytokine.[12-14]

At present, a novel treatment for PD including nonsteroidal anti-inflammatory drugs only relieves clinical symptoms and is accompanied by adverse effects like dyspepsia, headache, gastrointestinal discomfort, and dizziness.[15] These complications, thus, provoke attention on the discovery of new therapeutic agents aiming to reverse the neuroinflammation. *Rheum rhaponticum* L. (rhapontic rhubarb) is a rich hydroxystilbene herbal plant possessing several beneficial properties including estrogenic, antioxidant, antithrombotic, anticancer, and anti-inflammatory effects.[16] A previous finding had demonstrated that the extract from rhubarb root of and its individual constituent's rhaponticin exhibited a promising anti-inflammatory effect.[17] The hypoglycemic activity of rhaponticin has been previously reported.[18] Rhaponticin also possessed the anticancer activity, anti-allergic activity, antimicrobial activity, human cytochrome P450 inhibiting activity, and anti-antihyperlipidemic activity.[19-21] However, the protective properties of rhaponticin in PD remain to be explored. As a follow-up, the antineuroinflammatory activity and the neuroprotective potential of rhaponticin in both animal and cellular neurotoxic settings of PD were investigated.

2 | MATERIALS AND METHODS

2.1 | Reagents

Rhaponticin, dimethyl sulfoxide (DMSO). Dulbecco's modified Eagle's medium (DMEM), penicillin/streptomycin antibiotics, fetal bovine serum, phosphate-buffered saline (PBS), 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT), and lipopolysaccharide (LPS) were acquired from Sigma-Aldrich, St. Louis. All the Enzyme-Linked Immunosorbent Assay (ELISA) Kits were acquired from the Raybiotech and MyBioSource, respectively.

2.2 | BV-2 cell culture collection and maintenance

The immortalized murine BV-2 microglial cells were collected from the ATCC. The BV-2 cells were grown in a complete DMEM medium incorporated with antimycotic mixtures (1%) and sustained in a humidified CO_2 (5%) incubator at 37°C. Cells were cultivated at a confluence of 80%to 90%. Cells were sustained in the same condition and were used for all studies during the log phase of growth only.

2.3 | Cytotoxicity assay

The viable cell was ascertained by 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2-tetrazolium (MTT) assay. BV-2 cells at 10 000 cells/well were seeded in a 96-well plate to permit the cultivation of cells before the onset of treatment with rhaponticin (0-100 μM) for up to 48 hours. Thereafter, the medium was discarded and 10 μL of 500 μg/mL of MTT prepared in PBS solution was added for 4 to 6 hours at 37°C. The resulting formazan salts dissolved are directly proportional to viable cell number. Following solubilization with 50 to 100 μL isopropanol, the absorbance was recorded using a spectrophotometer (563 nm). Cell viability values were calculated as follows:

$$\text{Viable cell (\%)} = (\text{Absorbance of treated cell}) / (\text{Absorbance of untreated cell}) \times 100.$$

2.4 | Nitrite quantification assay

NO secreted from BV-2 cells in the culture medium was converted to nitrite and further investigated using Griess reagent by the colorimetric method. Cells (5×10^5 cells/mL) were plated in 24-well plates in 2 mL of cell culture medium, then followed by pretreatment with the indicated dosage of rhaponticin (10, 25, and 50 μM/mL) for an hour and incubation with LPS (1 μg/mL) for 16 hours. In brief, 50-μL collected culture medium was reacted with an equivalent volume of Griess reagent (0.1% naphthyl ethylene diamine and 1% sulfanilamide in 5% H_3PO_4) in 96-well plates at room temperature for at least 10 minutes in the dark. Standard solutions of sodium nitrite were used to determine the nitrite concentrations in the medium. Furthermore, the absorbance value was recorded at 540 nm by a microplate reader (Tecan Trading AG, Switzerland). Sodium nitrite (0-100 μM) was used to plot the nitrite standard curve.

2.5 | Intracellular reactive oxygen species quantification assay

Intracellular production of reactive oxygen species (ROS) was established using 2′,7′-dichlorodihydrofluorescein-dopamine (H2DCF-DA) staining dye. In brief, BV-2 cells were induced using LPS for 16 hours in the absence and/or presence of rhaponticin followed by staining with 25-μM H2DCF-DA in PBS solution at 37°C for 1 hour. The fluorescence intensity was recorded using a fluorescence reader (485 nm excitation and 535 nm emission).

2.6 | PGE2 quantification assay

After treatment, the cells were detached from the medium and centrifuged for the separation of cells. The cell pellet was homogenized from the buffered saline and the suspension was utilized for the measurement of PGE2 levels. The PGE2 levels in the BV-2 cells were investigated using a commercially procured ELISA Kit by using manufacturer instructions (Raybiotech).

2.7 | Proinflammatory cytokine assay

After the completion of the experiment, the cells were detached from the medium and centrifuged for the separation of cells. The cell homogenate was used to detect the proinflammatory cytokines (IL-1β and IL-6). The commercially acquired ELISA Assay Kits were used for the measurement of proinflammatory cytokines in the BV-2 cells according to the guidelines of the manufacturer (MyBioSource).

2.8 | Experimental animals

Twenty male C57BL/6 mice with body weight between 25 and 30 g were caged in a controlled environment (22-25°C) and permitted acclimatization for 7 days with pellet and water. A 12-hour light-dark cycle was sustained. All the protocols associated with the care of the animals were based on the Institutional Animal Care and Use Committee.

2.9 | Animal experimental protocol

The animals (n = 20) were separated arbitrarily into four groups (n = 5/group). The experimental groups were divided into:

Group I: Control group (excluded from the treatment procedures and fed with regular diet).
Group II: PD induced group (administered with 30 mg/kg body weight of 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine (MPTP) to induce the PD).
Group III: MPTP + rhaponticin-treated group (supplemented with 20 mg/kg body weight of rhaponticin concomitantly with 30 mg/kg body weight of MPTP challenge).
Group IV: Rhaponticin-alone-treated group (supplemented with 20 mg/kg of body weight of rhaponticin alone).

At day 1, MPTP (30 mg/kg) was given intraperitoneally injection daily for 5 consecutive days. The administration of rhaponticin by oral gavage was achieved at the time point of 1 hour after every MPTP injection. Rhaponticin at the dose 20 mg/kg/d was administered for 5 days, respectively. Mice in the vehicle (control) group were administered with an equal volume of 0.9% saline. On day 8, the mice were killed and then serum and brain tissues were collected for further analysis.

2.10 | Motor coordination and grip strength

2.10.1 | Footprint analysis

Footprint studies were done to assess the muscle force at C57BL/6 mice of different groups. Mice were trained in a dark tunnel a day before the test. The mice's forelimbs and hind limbs were immersed in blue ink and red, respectively. Then, the mice were positioned at the dark tunnel entrance. The footprints of the mice were recorded on a clean white paper placed on the floor of the tunnel. The steps carried out only in a straight line were measured. To exclude the variation in the stride length as a result of variation in velocity, only footprints were analyzed. The length of the stride was evaluated by quantifying the distance between every step on the equal side of the body.

2.10.2 | Grip test

The grip test was done to determine the muscle's strength (skeletal) at C57B/6 mice of different groups. The apparatus needed consists of a wire grid connected with an isometric pressure transducer. C57BL/6 mice were captured through tails, which allowed the mice to clutch the grid with the forepaws. Then, the mice were pulled softly by the tail toward the back until the grid was loose. The mean force exerted by the mice was measured. The mean of 10 dimensions for each mouse was recorded, and then the mean force was determined. The muscle strengths were expressed as grams force.

2.10.3 | Rotarod test

The test was executed for the determination of motor deficits in C57BL/6 mice of different groups. The mice were situated on a revolving rod, in the opposite direction for rotation. Then, test was initiated by acceleration lying on the day before scarification. The revolving speed was fixed at 10, 25, and 50 mph. Then, a

chronometer was initiated before the declining times of the rolling rod of mice were estimated.

2.11 | Inflammatory cytokine analysis

The serum samples from all groups were used for multianalyte ELISA and the targeted cytokines (TNF-α, IL-6, and IL-β) levels were measured according to manufacturer's protocol (MyBioSource). Cytokine concentrations were normalized by tissue weight.

2.12 | DA level analysis

The DA levels of the experimental mice were determined from the striatal area. The tissues were homogenized in ice-cold PBS with homogenizer and centrifuged for 15 min at 4°C at 12 000 rpm. The supernatants were obtained and the concentrations of dopamine (3,4-dihydroxyphenylacetic acid) were determined using high-performance liquid chromatography with electrochemical detection.

2.13 | Histopathological analysis

The loss or damage to DA neurons was further justified by histopathology. The striatal sections of 20 μm thickness, next to the autoradiography sections, were also fixed in 10% formalin for 24 hours. The samples were then processed by an automated tissue processing machine and embedded in paraffin wax. The tissue blocks were sectioned with a thickness of 5 μm followed by routine hematoxylin and eosin staining. Sections were imaged through an electric light microscope.

2.14 | Data analysis

Data are recorded as mean ± standard deviation in triplicate data and were estimated using a one-way analysis of variance. The significant differences for comparison among groups were established at $P < .05$.

3 | RESULTS

3.1 | The effect of rhaponticin on the cell viability of BV-2 murine cell line

The murine cell line BV-2 was used to evaluate the cytotoxic effects and effective dose of rhaponticin. Figure 1 shows the dose-dependent growth activity of the rhaponticin on the viability of BV-2 cells. The percentages of cell proliferation at designated increasing concentrations of rhaponticin in BV-2 cells were ascertained as the percentage of treated viable cells in comparison with untreated viable cells (control). At the highest concentration of rhaponticin, the cell viability remains more than 80%; hence, no significant toxicity was exhibited by rhaponticin. On the basis of this preliminary data, the minimum nontoxic doses, that is, 5, 10, and 20 μg/mL were used for further investigation. In addition, the concentration of LPS (1 μg/mL) was used to induce the inflammation.

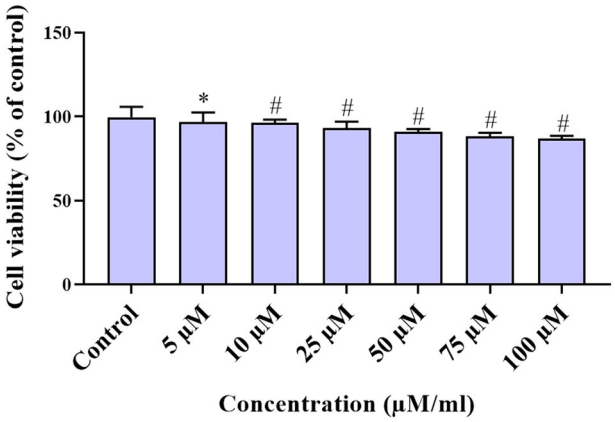

FIGURE 1 Effects of rhaponticin in various concentrations on BV-2 cell viability. Cell cytotoxicity was done by the MTT method. The results are stated in the mean ± SD of five liberated data *($P < .05$; $P < .01$). Significant difference compared to control

3.2 | The effect of rhaponticin on the NO production in the LPS-stimulated BV-2 murine cell line

The anti-inflammatory properties of rhaponticin on the production of NO against the BV-2 cell was investigated by pretreating BV-2 cells with rhaponticin at a low dose (5, 10, and 20 μg/mL) for 2 hours before LPS induction overnight. In BV-2 cells without treatment, LPS significantly induced NO production, identified by the increasing amount of NO from the culture medium. However, with rhaponticin treatment, a dose-dependent NO production inhibition to nearly control levels occurred at the highest concentration of rhaponticin, 20 μg/mL. These inhibitions by rhaponticin were not caused by the cytotoxic effect, as rhaponticin did not indicate any significant reduction in the BV-2 cell viability even at the lowest concentrations (Figure 2).

3.3 | The effect of rhaponticin on the intracellular ROS production in LPS-stimulated BV-2 murine cell line

The production of intracellular ROS in BV-2 cells treated with rhaponticin was assessed using 2′,7′dichlorofluorescein dopamine. The ROS accumulation was observed in LPS-stimulated BV-2 cells (Figure 3). However, treatment with rhaponticin suppressed significantly the ROS production dose dependently. In addition, nearly 50% of ROS was inhibited in the LPS-stimulated BV-2 cells treated

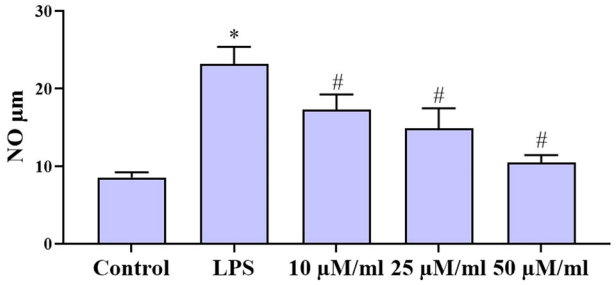

FIGURE 2 Effect of rhaponticin on the nitric oxide (NO) production in lipopolysaccharide (LPS)-stimulated BV-2 cells. The NO production was accessed by the nitrite quantification assay. Outcomes are stated in the mean ± SD of five liberated data ($P < .05$). *Significant difference compared to control and #significant difference compared to LPS induced group

with 20 μM rhaponticin. The fluorescence level justifies the inhibition of ROS production in cells treated with rhaponticin (5, 10, and 20 μg/mL), showing the antioxidant activity.

3.4 | The effect of rhaponticin on the PGE_2, IL-6, and IL-1β production in LPS-stimulated BV-2 murine cell line

The inhibition of proinflammatory mediators including PGE_2, IL-6, and IL-1β in rhaponticin-treated BV-2 cells was investigated. As indicated in Figure 4, the production of PGE_2, IL-6, and IL-1β induced was significantly increased by LPS treatment. When compared to LPS treated rhaponticin BV-2 cells, the release of PGE2, IL-6, and IL-1β proinflammatory mediators was significantly and dose dependently decreased, further alleviating the anti-inflammatory activity of rhaponticin in the cell system.

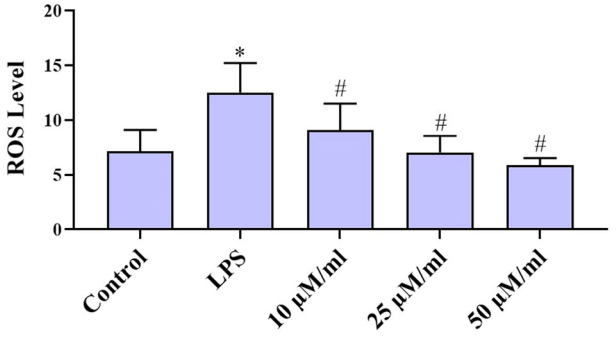

FIGURE 3 Effect of rhaponticin on the intracellular reactive oxygen species (ROS) production in lipopolysaccharide (LPS)-stimulated BV-2 cells. The generation of ROS was accessed by the 2′,7′-dichlorodihydrofluorescein-dopamine staining assay. Outcomes are stated in the mean ± SD of five liberated data ($P < .05$). *Significant difference compared to control and #significant difference compared to LPS induced group

3.5 | The effect of rhaponticin-treated MPTP-induced C57BL/6 mice on motor function

The motor function test was used to determine the strength of muscle's forelimbs, hind limbs as well as the jointed forelimbs. Following MPTP and/or rhaponticin treatment, the motor function of mice was assessed using the footprint test and grip strength test. As predicted, the MPTP groups of C57BL/6 mice exhibited a weak performance in both tests (Figure 5). Remarkably, in rhaponticin -treated groups, both footprint tests (Figure 5A,B) and grip strength test (Figure 5C) performance improved significantly. Therefore, the examination of the motor function activity of rhaponticin-treated MPTP-induced mice reveals improved PD behavioral deficit.

3.6 | The effect of rhaponticin-treated MPTP-induced C57BL/6 mice on motor coordination

The MPTP-mediated motor coordination impairment was assessed by subjecting mice to real run time performance at the range of 10, 25, and 50 rpm (time spent on a rotating rod). The average mean time taken from the mice toward a drop in each group is plotted in opposition to different rpm as shown in Figure 6 showing decreased retention time in MPTP alone received mice when compared to control mice. Rhaponticin administered with MPTP-induced mice exhibited drastically enhanced retention time, which further justifies the fact that the administration of rhaponticin considerably enhanced the motor strength of MPTP-induced PD behavioral deficit mice.

3.7 | Effects rhaponticin-treated MPTP-induced C57BL/6 mice on DA and proinflammatory cytokines levels

The effects of rhaponticin on the concentrations of DA and proinflammatory mediators (TNF-α, IL-1 β, and IL-6) in an MPTP-induced PD mouse model. After 5 days of rhaponticin administration, rhaponticin increased the concentrations of striatal DA when associated to the vehicle group in C57BL/6 mice (Figure 7). Furthermore, rhaponticin treatment as well significantly reduced the expressions of TNF-α, IL-1β, and IL-6 as shown in Figure 7B when compared among control and rhaponticin-alone-treated mice. This result suggests a potent neuroprotective effect of rhaponticin.

3.8 | Effects of rhaponticin on brain damage in MPTP-induced C57BL/6 mice

The effects of rhaponticin on the DA neurons in the mouse of an MPTP-induced PD model were assessed by histopathological assay. After 5 days, rhaponticin administration in the MPTP-induced group, the brains observed were severely injured, related to hemorrhage,

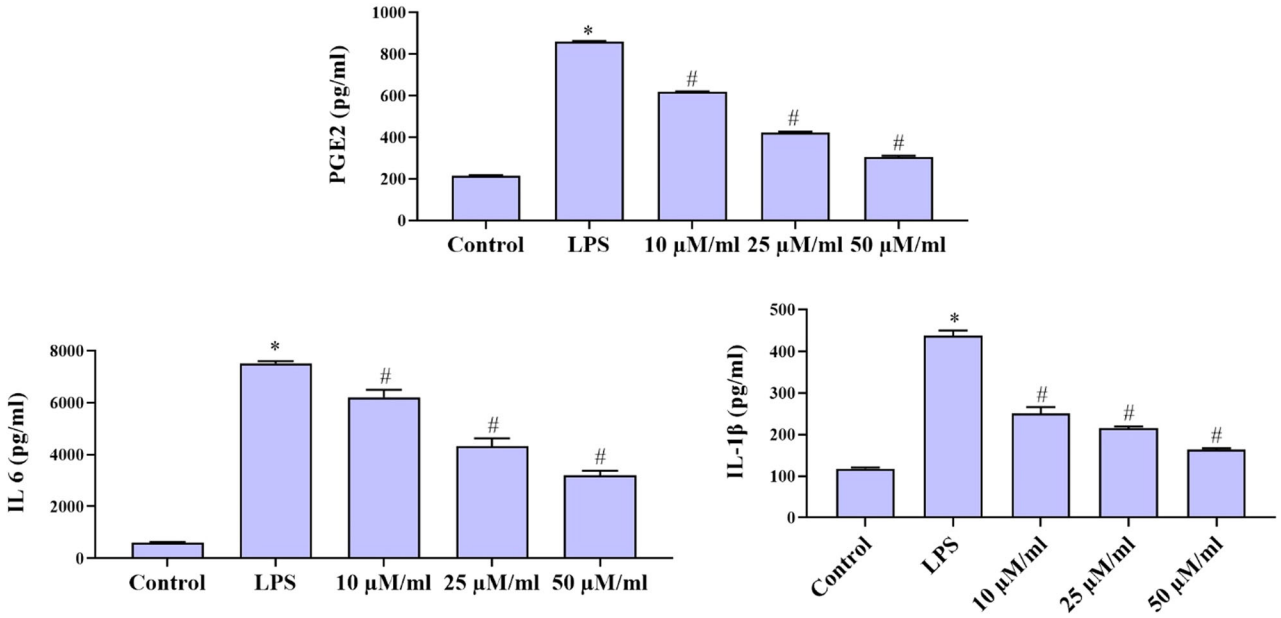

FIGURE 4 Effect of rhaponticin on the secretion of proinflammatory mediators (PGE2, IL-6, and IL-1β) in lipopolysaccharide (LPS)-stimulated BV-2 cells. The production of the proinflammatory mediators was accessed by an Enzyme-Linked Immunosorbent Assay Kit. Outcomes are stated in the mean ± SD of five liberated data ($P < .05$). IL-6, interleukin-6; PGE2, prostaglandin E2. *Significant difference compared to control and #significant difference compared to LPS induced group

and layering in the region of cerebellum compared to the vehicle group in C57BL/6 mice (Figure 8). The rhaponticin at 20-mg/kg restored brain tissue assault, compared to the MPTP group in C57BL/6 mice. These results combined show the comprehensive protection of rhaponticin against brain damage.

4 | DISCUSSION

One of the perilous players in neuroinflammation is elevated microglia activation, which is found homogenously in the central nervous system and is responsible for neuroinflammation

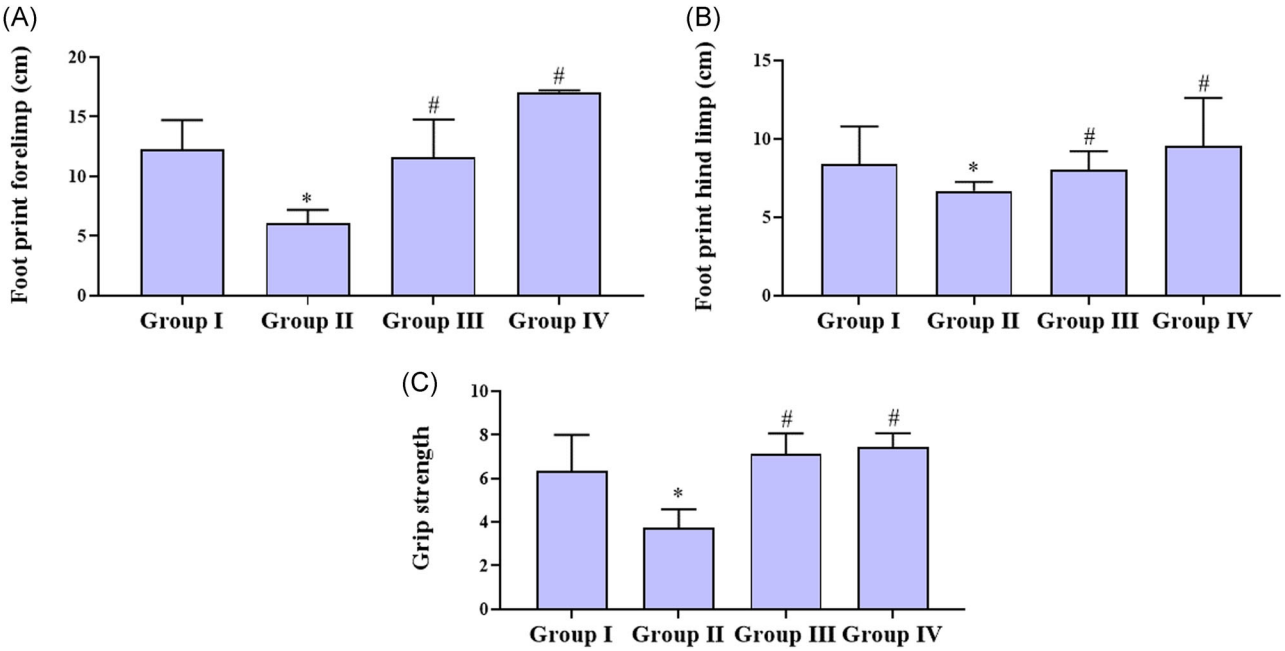

FIGURE 5 Effect of rhaponticin on the motor function in 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine-induced C57BL/6 mice. The motor function was accessed by footprint tests and grip strength tests. Data are of mean ± SD by triplicates ($P < .05$). *Significant difference compared to control and #significant difference compared to disease induced group (Group II)

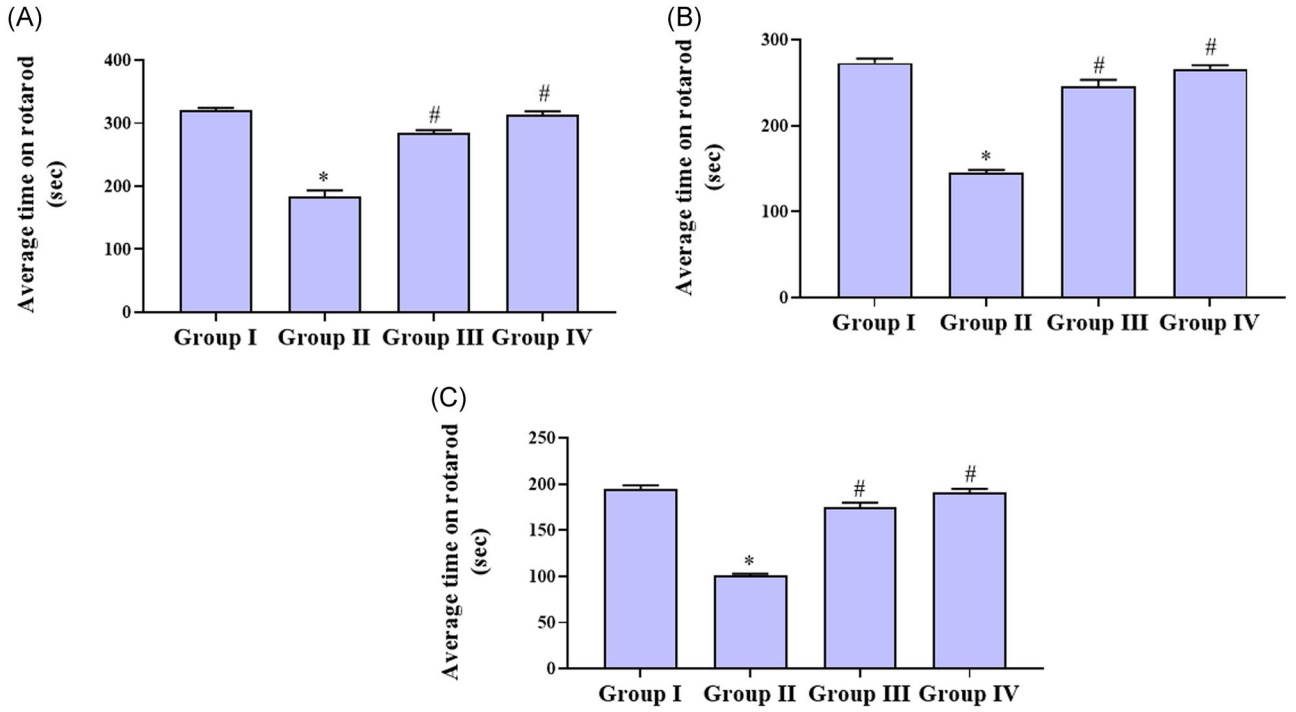

FIGURE 6 Effect of rhaponticin on the motor coordination in 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine-induced C57BL/6 mice. The motor coordination was accessed by rotarod test. (A) 10 rpm, (B) 25 rpm, and (C) 50 rpm. Outcomes are stated in the mean ± SD of five liberated data ($P < .05$). *Significant difference compared to control and #significant difference compared to disease induced group (Group II)

mediated neurodegeneration. Excessive microglial production and activation is the initiation point for numerous possibly neurotoxic molecules including proinflammatory mediators and ROS.[22] It is crucial to halt microglial activation to prevent various neuroinflammatory-related diseases. Findings have evidenced that microglial cells can be induced by LPS in the culture system, evoking the generation of inflammatory mediators. Therefore, in vitro LPS-induced inflammation has been used as the main strategy to investigate potential antineuroinflammatory agents. Recently, many reports have established that microglial activation by LPS can be effectively reversed by bioactive compounds from natural products.[23-25] Thus, in this part of the study, the anti-inflammatory potential of rhaponticin against the LPS-stimulated murine BV-2 microglial was investigated.

Colorimetric assay, methyl tetrazolium assay (MTT assay) was performed to authenticate the experiment by testing the cytotoxicity of rhaponticin in experimental conditions to avoid any misinterpretation of data and the cell viability was measured to optimize the concentration of rhaponticin for further analysis. Results of optimization indicated that rhaponticin did not produce any significant

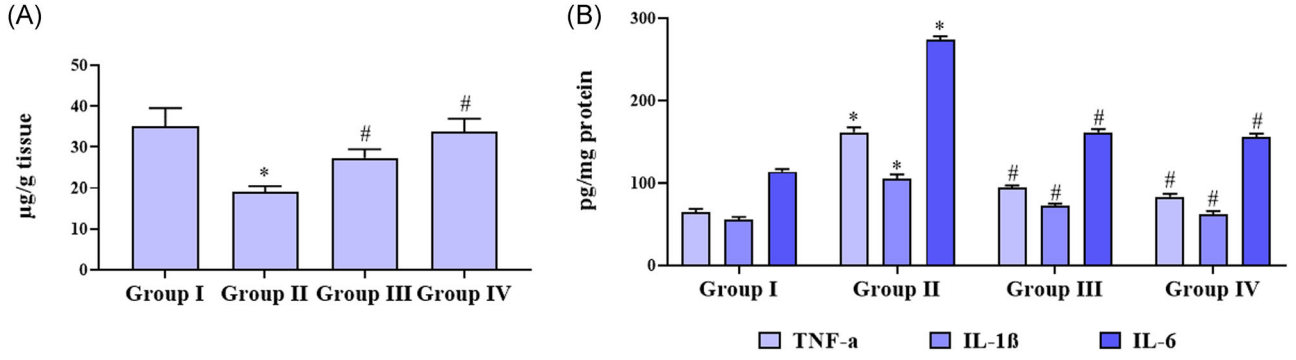

FIGURE 7 Effect of rhaponticin on the dopamine and proinflammatory cytokines levels in 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine-induced C57BL/6 mice. The dopamine and proinflammatory cytokine levels were accessed by an Enzyme-Linked Immunosorbent Assay Kit test. (A) Dopamine level and (B) proinflammatory cytokine levels. Outcomes are stated in the mean ± SD of five liberated data ($P < .05$). *Significant difference compared to control and #significant difference compared to disease induced group (Group II). IL-1β, interleukin-1β; TNF-α, tumor necrosis factor-α

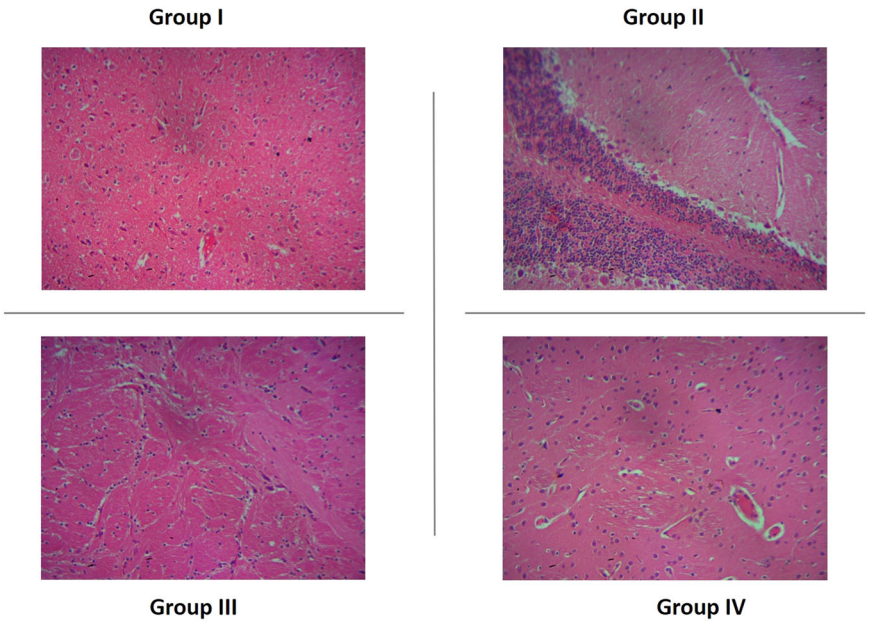

FIGURE 8 Effect of rhaponticin on the neurogenesis in the brain of 1-methyl-4-phenyl-1,2,3,5-tetrahydropyridine-induced C57BL/6 mice. The neurogenesis was accessed by histopathological observations

toxicity as the BV-2 cells remained more than 70% viable even at the highest concentration. As a response to LPS induction by BV-2 cells, the microglia secrete various inflammatory mediators including NO. In the present study, rhaponticin suppressed the production of NO in LPS-stimulated BV-2 cells. Also, considerable evidence exposed that rhaponticin is accountable for NO inhibition and this is not due to rhaponticin cytotoxicity through MTT assay, demonstrating that inhibition of NO is done solely by rhaponticin's bioactive compounds. Collectively, these data propose that rhaponticin may have neuroprotective properties to mitigate LPS-induced neuroinflammatory response in BV-2 cells.[26,27]

Excessive NO production has been associated with ROS production which eventually leads to neuronal damage. Furthermore, excessive generation of ROS might oxidize lipids, proteins, and DNA, and it is thought to be an important mediator in the PD process.[28] In this case, rhaponticin pretreatment presented a substantial protective effect in BV-2 cells by scavenging the accumulation of ROS when compared to the LPS group. Hence, rhaponticin may be able to maintain the cellular redox balance and may prevent the progress of neurogenerative-related diseases such as PD. Other molecules that are released by LPS-induced microglial cells include PGE_2, TNF-α, and IL-6 and IL-β, all of which have a pivotal role in chronic and acute inflammation both directly and indirectly through production of NO.[29] In agreement, the effective concentration (5, 10, and 20 μ/mL) of rhaponticin inhibits the secretion of both proinflammatory prostaglandins and cytokines in a dose-dependent manner. This somewhat illustrates that rhaponticin could halt inflammation caused by unwarranted NO production and that rhaponticin has a wide therapeutic window against neuroinflammation.

A mutual characteristic of any in vitro studies is that the concentration of bioactive compounds assayed cannot transposed from actual animal or human intakes. Hence, further in vivo studies are required to explore the neuroprotective effects of rhaponticin in animals of neurodegenerative models, PD.[30] MPTP is a commonly used synthetic neurotoxin, besides 6-hydroxydopamine and rotenone that induce motor impairment, and neuropathological changes that are comparable to patients with PD, and it is for that reason, MPTP was used to imitate the pathological process and behavioral changes in mice.[31] The aim of this part of the work was to measure the therapeutic activity of rhaponticin in an MPTP-induced C57BL/6 mouse model of PD. Following MPTP injection for 1 week, MPTP roots to behavioral impairment which was demonstrated by grip strength, footprint, and rotarod test. Rhaponticin was found to significantly ameliorate the motor abnormalities in MPTP-induced mice. Similarly, such behavioral findings were in accordance with the earlier reports.[32] These outcomes indicate that rhaponticin exerts a valuable effect on the motor deficits in neurogenerative disease.

The emergence of motor impairment in patients with PD is the consequence of the elongated DA neuron degeneration in the brain. In addition, induction of MPTP in mice will secrete large amounts of proinflammatory cytokines (IL-6, IL-1β, and TNF-α) that may enhance oxidative stress, which is harmful to the survival of DA neurons.[33] Consequently, inhibition of inflammatory response may be a hopeful strategy to protect DA from inflammatory damage in PD. Interestingly, rhaponticin in MPTP-induced mouse brains suppressed the levels of inflammatory cytokines and prevented the striatal DA depletion. Also, another inflammatory signature was observed by histopathology, which recommends that rhaponticin therapeutic activities may be linked to its anti-inflammatory effects. As supporting data, a previous study has revealed a bioactive compound, paeonol exhibit neuroprotective ability through inhibiting inflammation and ROS-induced neuron degeneration.[34]

5 | CONCLUSION

Conclusively, the results of this present study establish rhaponticin's therapeutic effect, which may be associated with the ability to inhibit the overactivation of microglia by reducing oxidative stress along with the secretion of proinflammatory mediators. In addition, rhaponticin guards against motor impairment, DA neuronal damage, and neuroinflammation probably via its bioactive compounds. In summary, rhaponticin may be a promising drug for the treatment of neuroinflammatory disorder in PD. However, additional studies are still needed in the future to understand the exact therapeutic mechanisms of rhaponticin against PD.

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