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Rosa moschata ameliorates haloperidol−induced Parkinson's disease *via* reduction of neurodegeneration and oxidative stress

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ABSTRACT

Objective: To investigate the effect of *Rosa moschata* (*R. moschata*) extract on haloperidol-induced Parkinson's disease (PD) in rats.

Methods: Haloperidol (1 mg/kg) was given to rats intraperitoneally for 3 weeks for induction of PD. *R. moschata* extract (150, 300 and 600 mg/kg) was administered orally for 21 days. The neuroprotective role of *R. moschata* leaf extract in PD was explored by performing neurobehavioral tests and RT-PCR analysis and measuring neurotransmitters and oxidative stress biomarkers.

Results: An improvement in motor functions and muscle strength was observed in PD rats treated with *R. moschata* extract. The levels of dopamine, serotonin, noradrenaline, superoxide dismutase, catalase, glutathione, and superoxide dismutase were significantly increased ($P < 0.001$), whereas acetylcholinesterase and malondialdehyde levels were markedly decreased by treatment with *R. moschata* extract ($P < 0.001$). The extract also markedly downregulated the mRNA expressions of *IL−1β*, *α−synuclein*, *IL−1α*, and *TNF−α* in brain tissue. Moreover, histopathological analysis indicated that neurofibrillary tangles and plaques were noticeably decreased in a dose-dependent manner in PD rats treated with *R. moschata* extract.

Conclusions: *R. moschata* extract alleviates haloperidol-induced PD in rats by reducing oxidative stress and neurodegeneration. It may be used for management and treatment of PD. However, additional studies are required to confirm its efficacy and molecular mechanisms.

KEYWORDS: Parkinson's disease; *Rosa moschata*; Neurotransmitter, α-Synuclein; Haloperidol; Neurodegeneration

Summary

Question: Does *Rosa moschata* ameliorate haloperidol-induced Parkinson's disease?

Findings: *Rosa moschata* extract alleviates haloperidol-induced Parkinson's disease in rats by improving behavioral, biochemical and histopathological parameters by reduction of neurodegeneration and oxidative stress.

Meaning: *Rosa moschata* extract may be used as a natural remedy in the treatment of Parkinson's disease after clinical trials and safety assessment.

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1. Introduction

A wide range of sensory system problems affecting the brain, spinal cord, and peripheral nerves are together referred to as neurological diseases[1]. Proteotoxic stress, oxidative stress, apoptosis, and neuroinflammation are all contributing factors that are usually involved in neuronal degeneration of the sensory system[2]. Studies have shown that a nutrient-deficient diet may also affect the central or peripheral nervous system that could lead to its disturbance[3]. The three main neurodegenerative diseases are dementia, Parkinson's disease (PD), and motor neuron dysfunction. PD is the most complicated neurodegenerative disorder commonly caused by persistent injury to dopamine neurons that extends from the nigrostriatal region of the brain to the neostriatum (hereditary). PD typically develops slowly and results in both motor and non-motor impairment. Bradykinesia, muscle rigidity, resting tremor, and abnormalities in posture and gait are among the primary clinical indicators of the illness. PD which affects 6.1 million individuals globally is the second most common neurological ailment[4]. The presence of Lewy bodies is the main pathological hallmark of PD. These are protein clusters that are immunoreactive to α-synuclein and primarily cause proteolysis. These include the reduced level of dopaminergic neurons in the striatum, which is manifested by decreases in voluntary actions, and ubiquitination. Lewy bodies begin to move into the neocortical and cortical regions as PD worsens[5].

The main pathological mechanisms are mitochondrial damage, protein aggregate accumulation, neuroinflammation, protein reactive strains, reduced protein elimination process, genetic mutations, and excitotoxicity. PD generally causes α-synuclein to change in neurons or the dopamine-containing neurons in the substantia nigra to disappear[6]. The present PD treatment only alleviated symptoms; it neither slows down nor reverses the degeneration of dopaminergic neurons[4]. There are many recommendations for managing PD. Levodopa is used for older patients and dopamine agonists are used for the patients in the initial phase[6]. Treatments such as deep brain stimulation and pharmacological dopamine replacement are extremely effective. Other available therapies include monoamine oxidase B and catechol-*O*-methyltransferase inhibitors[7]. In patients experiencing their initial motor fluctuations, monoamine oxidase B inhibitors are more effective. Similarly, if there are any wearing-off symptoms, catechol-*O*-methyltransferase inhibitors improve levodopa's effectiveness in the therapy[8]. Brain stimulation methods and the replacement of dopamine are effective techniques for improving the patient's life quality[9]. Degradation of dopaminergic neurons starts with oxidative stress and mitochondrial malfunction[10]. Studies have demonstrated natural remedies can be utilized to reduce reactive oxygen species generation and oxidative stress as effective neuroprotective agents[11]. Several plants have been reported for their neuroprotective potential[12]. However, only a few novel treatments have been available to target neuroinflammation, α-synuclein, and improvement of mitochondrial function. Therefore, there is always a need for more advanced treatment methods to successfully reduce symptoms of the disease, enhance patient's quality of life, and stop the course of the disease. Phytochemical-enriched herbal treatments can effectively treat neurological diseases including PD with minimal toxic effects[13].

The plant *Rosa moschata* (*R. moschata*) belongs to the family Rosaceae. The leaves are thought to offer both rubefacient and insecticidal effects. The leaves and roots can cure piles and diarrhea. Flower hydro distillate is used as an antioxidant in the treatment and prevention of cardiovascular disorders[14]. *Rosa* oil is used as a fragrance ingredient in skincare products, including lotions, creams, and ointments[15]. The tea is used to treat eye problems by reducing inflammation. A study evaluated *R. moschata* extract's stress suppressant activity in mice using acute restraint stress[16]. In an *in vitro* assay, methanol extract of *R. moschata* scavenged the free radicals in DPPH and ABTS assays[14]. It also demonstrated strong anticholinesterase potentials against acetylcholinesterase (AChE) and butyrylcholinesterase[17]. Based on the previous *in vitro* anticholinesterase activity, the current study aimed to investigate *R. moschata*'s potential in a haloperidol-induced PD rat model.

2. Materials and methods

2.1. Collection of plant and identification

R. moschata leaves were freshly collected from Shangla, Pakistan, in the spring season 2022. Dr. Muhammad Ajmal Shah, Assistant Professor at Department of Pharmacy, Hazara University, Mansehra, Pakistan did the plant identification (Accession no - RM1911/22). After collection, the leaves were washed to remove any filth and superfluous substances, spread on paper, sheltered from direct sunlight, and left to dry for 8 d. Then dried leaves were crushed into fine powder with an electric blender.

2.2. Chemicals and reagents

Levodopa and carbidopa were obtained from OBS Pharma®, Pakistan. Dimethyl sulfoxide, formalin, chloroform, Benedict's reagent, ferric chloride, lead acetate, Mayer's reagent, Hager's reagent, rutin, gallic acid, sulfuric acid, potassium chloride, sodium hydroxide, *n*-hexane, pyrogallol, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), and hydrochloric acid were bought from Sigma-Aldrich®, USA. Haloperidol (Adamjee Pharmaceutical), DPPH, disodium hydrogen phosphate, phosphate buffer saline (pH 7.4), sodium chloride, trichloroacetic acid (Fisher Chemical®, USA), and thiobarbituric acid (Merck®, USA) were used. All other chemicals used in the study were of laboratory and analytical grade.

2.3. Preparation of extract by microwave–assisted extraction method

A microwave oven (Dawlance DW-142 G, microwave output 1 100 W) with a power of 2 450 MHz was used for the microwave-assisted

extraction. It was done in two cycles. In the first cycle, a Pyrex beaker of 1 000 mL capacity was taken and 50 g of plant powder was added to it, then 700 mL of pure ethanol was poured into it. After that, the resultant suspension was vigorously mixed with the stirrer, and solvent was allowed to penetrate completely into the plant powder. A beaker was placed in a microwave and run for 90 s, then stopped for 30 s, during which the door of oven remained open. Five cycles of the whole process were performed at the power of 800 W. After the filtration, ethanol (500 mL) was poured into residue, and then filtration was done again. In the end, with the help of a rotary evaporator which was set at 40 ℃, an excess solvent was evaporated from the extract[18].

2.4. Characterization of plant extract by high-performance liquid chromatography (HPLC) analysis

HPLC analysis was performed using a Shimadzu chromatograph from Kyoto, Japan, which was equipped with a ternary pump (Shimadzu LC-20AT) and a diode array detector (Shimadzu SPD-M20A). In addition, a C18 guard column (dimensions: 2.0 cm × 4.0 mm; particle size: 5 μm) and an analytical column (Phenomenex® ODS 100 A, dimensions: 250 mm × 4.60 mm) were used in the analysis. Version 1.25 of the LC Solutions software was used for analysis.

Using a gradient chromatography approach, acetonitrile and water served as the mobile phase, flowing at 1 mL/min. A volume of 20 μL was injected into each column whose temperature was set at 25 ℃. In comparison to the initial 2∶8 *v/v* ratio, the final acetonitrile/water ratio was 8∶2. The gas was eliminated by sonicating the mobile phase. The UV spectra observation was made at 450 and 200 nm[19].

2.5. Animal grouping and study design

2.5.1. Ethical approval

The National Institutes of Health's guidelines for the treatment and carefulness of animals were strictly followed in order to complete the current study, with an endorsement from the Institute Review Board of Government College University Faisalabad (reference number: GCUF/ERC/249).

2.5.2. Experimental animals

Young, healthy Wistar rats of both genders weighing 100-120 g were used in the study. They were obtained from animal house and handled in Government College University Faisalabad, Pakistan. The rats were placed under proper conditions (a 12-hour light and dark cycle, room temperature 25 ℃, 30%-60% humidity) with free access to food and water in polypropylene cages one week before experiment to acclimatize to the environment[20].

2.5.3. Experimental design

Rats were randomly divided into six groups with six animals in each group. PD was induced by administration of haloperidol (1 mg/kg) *i.p.* for 21 d in all groups except the control group. Group 1 (control) received only vehicle. Group 2 served as disease control. Group 3 was given orally a standard drug available for PD, *i.e.* levodopa (100 mg/kg) and carbidopa (25 mg/kg). Groups 4-6 were treatment groups and received 150, 300, and 600 mg/kg of *R. moschata* extract orally. The dose was selected according the previous research data[20]. All the groups were treated for consecutive 21 d. Behavioral tests were done on days 1, 14 and 21. After 21 d, the animals were sacrificed by cervical dislocation under mild anesthesia. Isoflurane was used to anesthetize rats at a concentration of 2.5%-5% with a maintenance dose of 1%-3%.

Brain samples from all the groups were collected separately and homogenates were prepared to quantify the neurotransmitters (dopamine, noradrenaline, and serotonin) and oxidative stress biomarkers [catalase (CAT), superoxide dismutase (SOD), glutathione (GSH), malondialdehyde (MDA), and glutathione peroxidase (GPx)].

2.6. Behavioral assessments

2.6.1. Catalepsy investigation

The catalepsy is a condition in which the body becomes rigid and rats cannot move body parts easily and quickly. Briefly, the method is as follows: a wooden block that is 9 cm high and 1 cm in diameter was used in the test on which the forearms of the rats were placed after giving haloperidol injection. A cataleptic marker was identified based on how long it took the rats to move their limbs. The test was completed when the forelimbs of rats touched the floor or climbed up the block. The observations were determined after 30, 60, 90, 120, 150 and 180 min. The point scoring after performing this test was as follows:

(i) if the animals showed normal movement when they were placed on the table, then score = 0;

(ii) if the rats behaved normally when they were pushed or touched, then score = 0.5;

(iii) if the rats could not correct the imposed position in 10 s, then score = 2, and 1 score was allotted for a single paw[20].

2.6.2. Open field test

The open field test was performed to determine the nervousness, gait, and behavioral abnormalities of experimental rats. A wooden square-shaped box that was made of plywood material has 100 cm width, 45 cm height, and 100 cm diameter used for conducting the open field test. The floor of the wooden box was painted with white and black lines that divided the floor into 25 blocks. The whole apparatus was washed with ethanol. The animals were kept in the middle of the container for 5 min and then allowed to move freely in the box. Defecation, freezing, posture changes, number of lines crossed, and horizontal number of squares crossed by rats were noted[21].

2.6.3. Ladder climbing test

This test was performed to ascertain sensorimotor ability of rats to hold the stairs precisely when rats climbed up an inclined ladder.

This test was performed by placing rats on a ladder that was made of wood. The ladder was inclined from the floor at a 45° angle and a 2 cm gap between the two steps of a ladder was present. The movement of rats was observed one by one after placing the rats on the ladder. For every rat, ascending time from one to another site was noted[20].

2.6.4. Wire hanging test

The strength of the neuromuscular area of the rat's forearms was determined by performing a wire-hanging test. The hanging test apparatus had a 90 cm long, 3 mm thick stainless steel wire which was set aside horizontally. The rats were allowed to grasp the wire *via* their forelimbs and fall time from wire to floor in seconds named latency time was noted. The latency time was recorded through a stopwatch. Rats were not allowed to leave the equipment until the latency time became 120 s. This test was conducted three times and three minutes of holding time was given to each consecutive trial[22].

2.6.5. Foot printing test

This test provides quantitative results of gait abnormalities. Before starting the test, with green and blue ink, the back and front forelimbs of rats were colored. A white piece of paper was kept on the floor, then rats were endorsed to walk consecutive 4 steps on the paper. At the beginning and end of the walk, foot patterns were recorded. Then results were evaluated by determining the stride length in centimeters[23].

2.6.6. Forced swimming test

Depression was determined by a forced swimming test. The test was performed in a cylindrical apparatus that had 16 cm height, 40 cm length, and 25 cm width. The apparatus was filled with water and the movement ability of rats was determined. The head of the immovable animal remained above the surface of water. Rats were drowned one by one in the cylinder, which contained 19 cm of water (23 ℃). The test was performed in 8 min for each rat. Swimming behavior of every single rat was examined in the last four minutes, and immobility was also determined[23].

2.7. Quantification of neurotransmitters

2.7.1. Preparation of brain homogenate

One gram of the brain tissue was poured into phosphate buffer saline (10 mL) having pH 7.4 (1∶10) and homogenized in the homogenizer. Then it was centrifuged at 4 000 rpm for 10 min at 4 ℃. The clear layer of supernatant was obtained for measuring the level of neurotransmitters[20].

2.7.2. Preparation of aqueous phase for the quantification of neurotransmitters

Brain homogenate was mixed with HCL-butanol (5 mL) and then centrifuged at 2 000 rpm for 10 min. An upper layer of supernatant was separated and 0.31 mL hydrochloric acid and 2.6 mL heptane were added to this layer. Afterward, the mixture was centrifuged for 10 min at 2 000 rpm. After centrifugation, two films were formed and named organic and aqueous layers. For the determination of neurotransmitter levels, an aqueous layer was used[20].

2.7.3. Measurement of noradrenaline and dopamine

An aqueous phase of homogenate was mixed with EDTA and HCl. For oxidation of this mixture, 0.1 mol/L iodine solution prepared in ethanol was added. Then, 0.1 mL of Na_2SO_3 solution and 0.1 mL of acetic acid were added to stop the reaction. The final solution was heated at 100 ℃ for 6 min and then it was cooled by keeping at room temperature. For dopamine and noradrenaline, the optical densities were measured at 352 nm and 452 nm, respectively[20].

2.7.4. Measurement of serotonin

For serotonin quantification, o-pthaldialdehyde (0.25 mL) was added to 0.2 mL of aqueous layer of homogenate. The solution was heated at 100 ℃ for 10 min and then cooled at room temperature. Absorbance was determined at 440 nm[20].

2.7.5. Measurement of AchE activity

AChE activity was measured in the brain homogenate according to the methods of Saleem *et al.* The aqueous layer of homogenate (0.4 mL) was mixed with 2.6 mL phosphate buffer solution 0.1 mol/L, 0.1 mL of DTNB and 0.2 mL of acetylthiocholine iodide. Yellow color was produced due to the reaction between acetylthiocholine iodide and DTNB. Absorbance was calculated at 412 nm[20].

2.8. Quantification of oxidative stress biomarkers

2.8.1. CAT assay

In brain homogenate (0.05 mL), 50 mmol/L, KH_2PO_4 (1.95 mL) and buffer solution were added. Following that, 30 mM H_2O_2 was poured into the previously prepared solution. A spectrophotometer was used to calculate the optical density at 240 nm[24].

2.8.2. SOD assay

In brief, 0.1 mL of brain homogenate, 2.8 mL of KH_2PO_4, and 0.1 mL of pyrogallol solution were mixed. Absorbance was measured at 312 nm[20].

2.8.3. MDA assay

MDA concentration was measured by estimating lipid peroxidation. It depends upon the thiobarbituric acid mixture. Brain tissue homogenate was centrifuged at 1 500 rpm for 5 min to obtain a clear supernatant layer. Trichloroacetic acid 15%, thiobarbituric acid 0.38%, and 2.5 mL of hydrochloric acid were mixed to prepare a thiobarbituric acid solution. Then, 3 mL of thiobarbituric acid solution was added to 1 mL supernatant. After well mixing, the solution was put in an ice-cold bath. Subsequently, the assay solution was centrifuged at 3 500 rpm for 10 min. The upper layer of solution was obtained and analyzed by a spectrophotometer at 532 nm[20]. The whole procedure was performed in triplicate.

2.8.4. GSH assay

In this assay, 1 mL brain homogenate and 1 mL KCl were added to 4 mL ice-cold water. Then 1 mL trichloroacetic acid was poured into the mixture, and the prepared mixture was centrifuged at 3 000

rpm for half an hour. Subsequently, 0.4 mol/L Tris buffer (4 mL) and 1 mL DTNB were added to 2 mL of supernatant. The absorbance of the sample and blank was noted at 412 nm[22].

2.8.5. GPx assay

The assay solution contained brain homogenate, sodium azide 0.1 mL each, H_2O_2, and EDTA, 0.2 mL individually. Trichloroacetic acid was then added to the solution to block the reaction and centrifugation at 2 000 rpm for 10 min was done to obtain the supernatant layer. The upper layer was isolated and combined with sodium hydrogen phosphate (4 mL) and DTNB (0.5 mL). Absorbance was recorded at 412 nm[22].

2.9. Real-time PCR assay

The expressions of the genes *IL-1α*, *α-synuclein*, *IL-1β*, and *TNF-α* were determined by PCR assay. In brief, RNA from the sample was extracted with triazole and the amount extracted was measured with a NanoDrop spectrophotometer at 260/280 nm. Thermo Scientific's cDNA kit was used to convert RNA into cDNA. For gene expression, *GAPDH* acts as a housekeeping gene. Forward primer 0.5 µL and corresponding reverse primer 0.5 µL were employed for the experiment, and microplate wells were filled with cDNA (5 µL) and 5 µL of K0221/Maxima SYBR® green dye. The microplate was kept at 94 ℃ for 5 min for initial denaturation, followed by annealing at 58 ℃ for 35 s, and at the end, the extension phase was done at 72 ℃ for 45 s (Supplementary Table 1). To visualize the pattern of bands, 5 µL of sample and 5 µL of DNA ladder were pipetted together during gel electrophoresis[25].

2.10. Histopathological analysis of the brain

The histopathological studies were performed in this study. To evaluate the neurodegeneration, the rats were sacrificed by cervical displacement and brain tissue was separated. Isolated brain was conserved in 10% formaldehyde solution and embedded in paraffin. The tissues were sliced into 8 mm segment and stained with haematoxylin-eosin for examination under a light microscope.

2.11. Statistical analysis

The results are expressed as mean ± SEM. One-way ANOVA with Tukey's multiple comparison test was used for statistical analysis. Graph pad prism version 5.0 was applied to evaluate the data. $P < 0.05$ was considered statistically significant.

3. Results

3.1. HPLC results of R. moschata leaf extract

Figure 1 shows the phytoconstituents found in the HPLC analysis, including *p*-coumaric acid, gallic acid, salicylic acid, and vanillic acid. The quantities of identified compounds are demonstrated in Supplementary Table 2.

3.2. Neurobehavioral tests

3.2.1. Catalepsy test

The PD control group showed an increased cataleptic score compared with the normal control group. The cataleptic score was markedly decreased in the PD groups treated with *R. moschata* extract. The extract produced dose-dependent effects, with the most pronounced effect observed at the highest dose. *R. moschata* extract at 600 mg/kg exhibited the maximum reduction in cataleptic score (Figure 2).

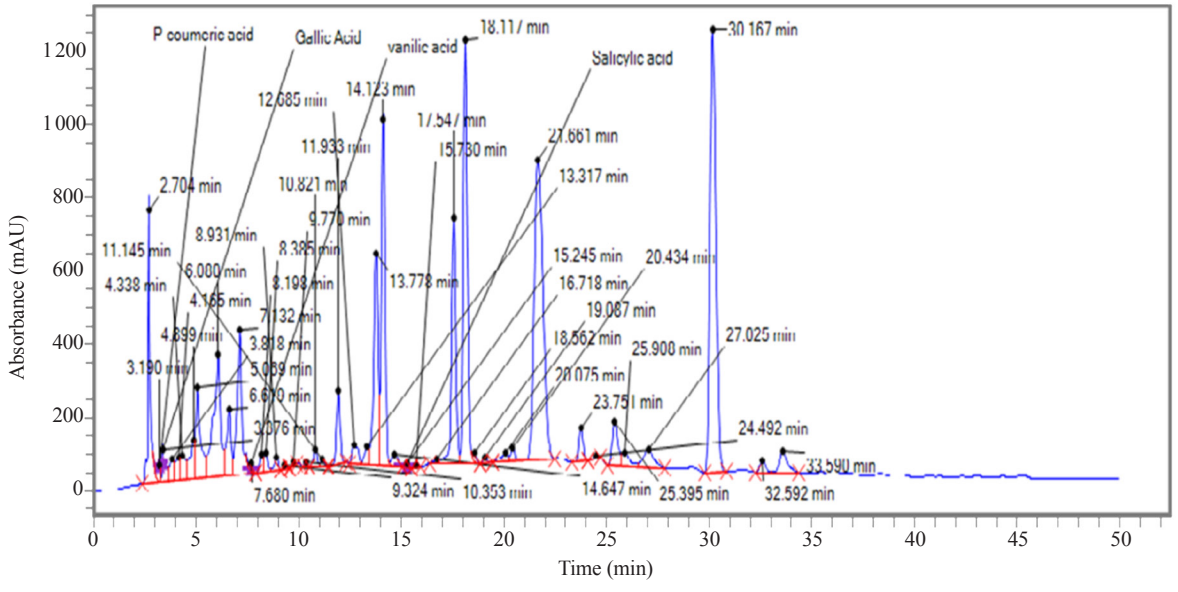

Figure 1. HPLC chromatogram of *Rosa moschata* leaf extract.

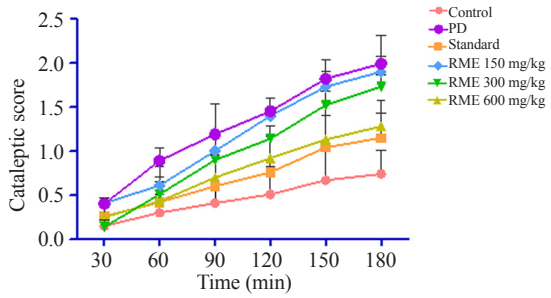

Figure 2. Effect of *Rosa moschata* extract on catalepsy score in rats with Parkinson's disease. Data are given as mean ± SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test.

3.2.2. Open field test

Locomotor action parameters like grooming, rearing, and number of lines crossed were considerably ($P < 0.001$) decreased in the PD group compared with the control group. The administration of all doses of *R. moschata* extract considerably ($P < 0.001$) enhanced locomotor action parameters compared with the PD group (Table 1).

3.2.3. Hanging test

The latency time was considerably ($P < 0.001$) reduced in the PD group in contrast with the control group. Treatment with *R. moschata* extract increased the latency time in rats with PD in a dose-dependent manner ($P < 0.05$) (Figure 3).

3.2.4. Foot printing test

A notable ($P < 0.001$) decline in stride length was noted in the PD group as compared to the control group. However, the extract-treated groups showed a remarkable ($P < 0.001$) increase in stride length in a dose-dependent manner (Figure 3).

3.2.5. Ladder climbing test

A substantial ($P < 0.001$) rise in climbing time was noted in

Table 1. Effect of *Rosa moschata* extract on open field test in rats with Parkinson's disease.

Groups	No. of lines crossed	No. of grooming	No. of rearing
Control	22.00 ± 0.75	8.00 ± 0.43	9.32 ± 0.37
PD	7.12 ± 0.46$^{\#}$	0.00 ± 0.00$^{\#}$	6.12 ± 0.46$^{\#}$
Standard	18.61 ± 0.42**	6.01 ± 0.38**	8.84 ± 0.46**
RME 150 mg/kg	13.48 ± 0.43**	4.20 ± 0.32**	6.75 ± 0.37
RME 300 mg/kg	15.49 ± 0.57**	6.35 ± 0.46**	7.48 ± 0.33*
RME 600 mg/kg	20.33 ± 0.49**	7.45 ± 0.41**	8.32 ± 0.45**

Data are given as mean ± SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}P$<0.001 compared with the control group. $^{*}P$<0.05, $^{**}P$<0.001 compared with the PD group. RME: *Rosa moschata* extract; PD: Parkinson's disease.

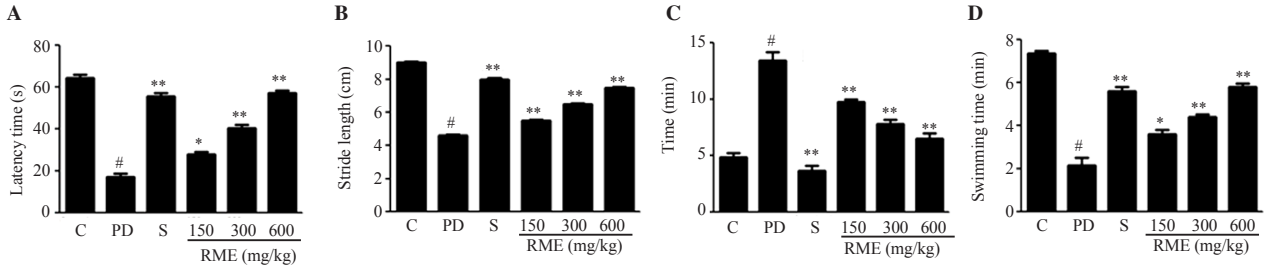

Figure 3. Effect of *Rosa moschata* extract on different behavioral tests (A: hanging test; B: footprinting test; C: ladder climbing test; D: swimming test) in PD rats. Data are given as mean ± SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}P$<0.001 compared with the control group. $^{*}P$<0.05, $^{**}P$<0.001 compared with the PD group. RME: *Rosa moschata* extract; PD: Parkinson's disease; C: control group; S: standard group receiving levodopa (100 mg/kg) and carbidopa (25 mg/kg).

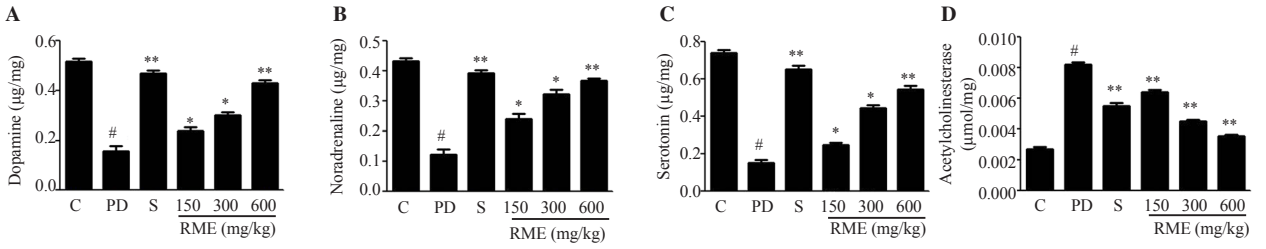

Figure 4. Effect of *Rosa moschata* extract on (A) dopamine, (B) noradrenaline, (C) serotonin, and (D) acetylcholinesterase in PD rats. Data are given as mean ± SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}P$<0.001 compared with the control group. $^{*}P$<0.05, $^{**}P$<0.001 compared with the PD group. RME: *Rosa moschata* extract; PD: Parkinson's disease; C: control group; S: standard group receiving levodopa (100 mg/kg) and carbidopa (25 mg/kg).

PD rats. Treatment with *R. moschata* extract notably ($P < 0.001$) decreased climbing time in a dose-dependent manner (Figure 3).

3.2.6. Swimming test

All treatment groups showed improved behaviors, as evidenced by a substantial ($P < 0.001$) improvement in swimming time. The standard group also presented significant ($P < 0.001$) development in the swimming test (Figure 3).

3.3. Effect of R. moschata extract on neurotransmitters

PD rats demonstrated lower levels of noradrenaline, dopamine, and serotonin, and increased AChE activity ($P < 0.001$) compared with the control rats. The administration of *R. moschata* extract markedly decreased AChE activity while increasing the levels of noradrenaline, dopamine, and serotonin dose-dependently ($P < 0.05$) (Figure 4).

3.4. Effect of R. moschata extract on oxidative stress biomarkers

CAT, GSH, GPx, and SOD activities and protein levels were notably decreased in the PD group with a marked increase in MDA level ($P < 0.001$) compared with the control group. However, *R. moschata* extract reversed PD-induced changes in these biomarkers dose-dependently ($P < 0.05$), indicating oxidative stress was alleviated by treatment with the extract (Table 2).

3.5. Effect of R. moschata extract on the mRNA expression of IL-1β, IL-1α, TNF-α, and α-synuclein

The mRNA expressions of *IL-1β*, *IL-1α*, *TNF-α*, and *α-synuclein* were remarkably upregulated in the PD group ($P < 0.001$). However, *R. moschata* extract significantly downregulated the mRNA expression of these indicators ($P < 0.001$) in a dose-dependent manner (Figure 5).

3.6. Histopathological results

The control group exhibited normal architecture of brain tissues. A reduction in the number of neurons, pigmentation, infiltration of inflammatory cytokines, neurofibrillary tangles, and the production of Lewy clumps were found in the PD group. The plant extract produced dose-dependent recovery and alleviated neurofibrillary tangles, neuronal loss, and neurodegeneration (Supplementary Figure 1).

4. Discussion

Age-related PD is an intricate neuronal system ailment triggered *via* a decrease in dopamine levels[3]. According to medical

Table 2. Effect of *Rosa moschata* extract on oxidative stress biomarkers in brain homogenate of PD rats.

Group	CAT (μmol/mg of protein)	SOD (μg/mg of protein)	GSH (μg/mg of protein)	GPx (μg/mg of protein)	MDA (nmol/mg of protein)	Protein (μg/mg)
Control	2.55 ± 0.02	8.23 ± 0.06	4.47 ± 0.01	6.95 ± 0.02	3.72 ± 0.01	310.7 ± 1.1
PD	1.15 ± 0.01$^{\#}$	3.50 ± 0.10$^{\#}$	2.35 ± 0.20$^{\#}$	4.51 ± 0.03$^{\#}$	5.51 ± 0.04$^{\#}$	213.6 ± 1.4$^{\#}$
Standard	2.45 ± 0.02**	6.45 ± 0.04**	4.07 ± 0.40**	6.89 ± 0.04**	3.62 ± 0.02**	296.8 ± 1.2**
RME 150 mg/kg	2.05 ± 0.01*	5.51 ± 0.80*	3.07 ± 0.10**	4.88 ± 0.01*	4.07 ± 0.01*	239.1 ± 1.6*
RME 300 mg/kg	2.25 ± 0.02**	6.84 ± 0.01**	3.35 ± 0.10**	5.37 ± 0.03*	3.83 ± 0.03**	272.1 ± 1.2**
RME 600 mg/kg	2.35 ± 0.01**	7.44 ± 0.06**	3.75 ± 0.10**	6.65 ± 0.02**	3.70 ± 0.01**	286.6 ± 1.7**

Data are given as mean ± SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}P$<0.001 compared with the control group. $^{*}P$<0.05, $^{**}P$<0.001 compared with the PD group.

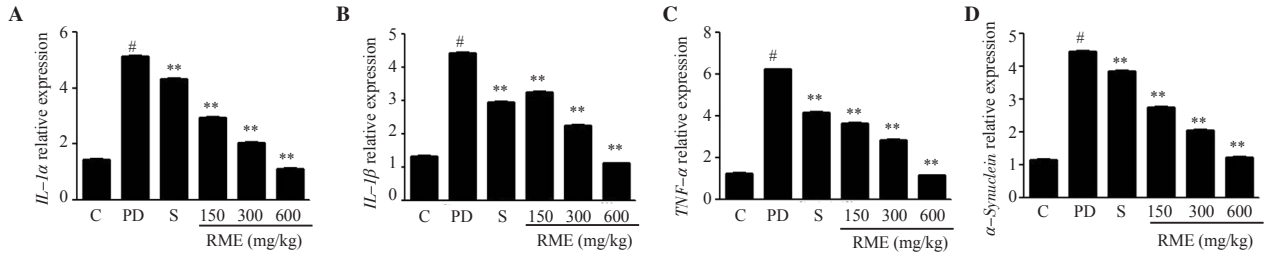

Figure 5. Effect of *Rosa moschata* extract on the mRNA expression of (A) *IL-1α*, (B) *IL-1β*, (C) *TNF-α*, and (D) *α-synuclein* in brain tissue of PD rats. Data are given as mean ± SEM (n=6) and analyzed by one-way ANOVA followed by Tukey's multiple comparison test. $^{\#}P$<0.001 compared with the control group. $^{**}P$<0.001 compared with the PD group. RME: *Rosa moschata* extract; PD: Parkinson's disease; C: control group; S: standard group receiving levodopa (100 mg/kg) and carbidopa (25 mg/kg).

investigations, PD has been widely associated with dementia and motor impairment. Redox imbalance significantly impacts neurodegeneration and neuronal dysfunction which leads to a decline in basal antioxidants[26]. Men are more likely to have PD which mostly occurs at the age of 65-85 years compared with women. Estrogen has a protective neuronal effect in the females[27].

The causative factors of PD are still not fully understood. Mitochondrial damage, protein aggregation, reduced protein clearance pathways, redox imbalance, neuroinflammation, genetic mutations, and excitotoxicity are the foremost compulsive mechanisms[28]. Many efforts are undertaken to identify and characterize the substances that can cure the disease, but these efforts are still limited to managing its symptoms. Dopamine agonists are used for treating PD in children less than 14 years, whereas levodopa is used in adults. Monoamine oxidase B inhibitors are given to patients who suffer from dopaminergic neuron abnormality. Catechol-*O*-methyltransferase inhibitors are the class of drugs used with levodopa if any subsided signs are present. These drugs augmented the action of levodopa[29]. Anti-Parkinson's drugs that were recently present also have many adverse effects, which is why new perspectives have been made for the manufacturing of advanced drugs that will prevent or inhibit the development of PD with high-cost effectiveness. Novel drugs are synthesized from plant origin with fewer adverse effects. Medicinal plants have antioxidant activities, and their components are used for regulating neurological activities[30]. Some medicinal plants include significant amounts of glycosaponins, polyphenols, flavonoids, and alkaloids that are already used for the treatment of neurotoxicity induced by redox imbalance[31]. With the aid of gas chromatography-mass spectrometric analysis, the presence of certain phytoconstituents, for example, unsaturated aldehyde, flavonoids, anthraquinone, tannins, and alcohol (eugenol, linalool) has been recently discovered in the leaves of *R. moschata*[14]. The flavonoid's characteristics have been broadly studied, showing anti-inflammatory and neuron-stabilizing potential. Therefore, they might be used in the prevention and treatment of PD and Alzheimer's disease[21].

Our findings on the antioxidant activity of *R. moschata* extract correlated to previous literature, in which 6-*O*-α-*L* (3"-*O*-*trans*, 4"-*O*-*trans* cinnamoyl)-rhamnopyranosyl catalpol, 3, iridoid glycosides 6-*O*-methyl, 1-glucopyranosyl catalpol, and scropolioside D were isolated from the aerial parts of *Salvia amplexicaulis*. *In vitro* assays indicated that all of the compounds moderately blocked AChE and butyrylcholinesterase[32].

An antipsychotic agent known as haloperidol produces extrapyramidal motor abnormalities like catatonia. In a previous study, haloperidol was injected *i.p.* for 3 weeks to induce catatonia or muscle rigidity. *R. moschata* extract significantly reduced catatonia scores dose-dependently[33]. In an open field test, parameters of locomotor activity were disturbed in the disease control group, but *R. moschata* extract dose-dependently improved locomotor activity[23]. Ladder climbing test, footprinting test, and hanging test were used for assessment of motor synchronization and walking pattern, respectively. Rats treated with haloperidol took more time to climb the ladder and showed low latency time in the hanging test due to weak muscular strength[34–36]. Treatment with *R. moschata* showed improved performance in these behavior tests. Moreover, decreased stride length was observed in rats receiving haloperidol. Rats treated with *R. moschata* extract showed significant improvement in stride length.

Disturbed normal state of cells and provoked oxidative stress can lead to peroxides and free radical production, which cause damage to neurons and cell components including proteins, DNA, and lipids[37]. A major contributing factor in onset and progression of neurodegenerative diseases is increased oxidative stress[38]. Antioxidant enzymes such as CAT, SOD, and GSH act as a defense mechanism against oxidative stress. CAT converts H_2O_2 in inert oxygen and water to neutralize toxic effects. A reduction in CAT level was prompted by haloperidol administration-induced redox imbalance[39]. SOD helps to stop redox imbalance by blocking the damaging properties of free radicals[40]. GSH is an essential enzyme in PD pathogenesis and neuronal loss is associated with depleted GSH levels in the substantia nigra of the midbrain. GSH deficiency reduces neurons' ability to neutralize H_2O_2, which occurs when redox imbalance and lipid peroxidation arise[41]. Lipid peroxidation is an indication of oxidative damage that can be evaluated by determining the amount of thiobarbituric acid. Unsaturated fatty acids and arachidonic acid produced lipid peroxidation when free radicals attacked them and its greater amount were found in substantia nigra of midbrain[42]. In the present study, antioxidants were notably decreased and MDA levels were increased by haloperidol administration. *R. moschata* extract remarkably reversed haloperidol-induced changes in MDA and antioxidants. Two of most important neurotransmitters acetylcholine and dopamine, which exist in the basal ganglia, play a key role in motor functions. The level of acetylcholine is reduced in PD owing to choline acetyltransferase deterioration, which is associated with intellectual impairment. According to this study, *R. moschata* extract has a protective effect on acetylcholine levels by inhibiting AChE, which triggers acetylcholine breakdown[17].

Accumulation of misfolded and aggregated α-synuclein proteins is one of the main contributing factors of PD. Amplification of α-synuclein causes a decrease in neurotransmitter release. Furthermore, pro-inflammatory mediators IL-1α, TNF-α, and IL-1β have been related to neuroinflammation causing neurodegeneration in the PD-induced model. In the present study, the mRNA expressions of IL-1α, TNF-α, IL-1β, and α-synuclein were upregulated in the PD group[20], which were downregulated by *R. moschata* extract. Histopathological studies also revealed that *R. moschata* extract reduced vacuolation, pigmentation, neutrophil infiltration, and neuron shrinkage in PD rats[21].

In conclusion, *R. moschata* is rich in phytoconstituents that may offer neuroprotective benefits. Behavioral assessments showed a

decrease in muscle rigidity in rats receiving *R. moschata* treatment. Furthermore, AChE level was decreased, while the levels of dopamine, noradrenaline, and serotonin were increased. *R. moschata* mitigated oxidative stress by increasing antioxidants and decreasing MDA levels. It also normalized the mRNA expressions of *IL-1β*, *IL-1α*, *TNF-α*, and *α-synuclein*. These findings imply that *R. moschata* alleviated PD *via* neuroprotective and antioxidant effects. However, additional research is needed to validate its therapeutic potential. Futher work on isolation and purification of active phytoconstituents is also needed which can lead to drug discovery and development.

Conflict of interest statement

The authors declare that there is no conflict of interest.

Acknowledgments

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Data availability statement

The data supporting the findings of this study are available from the corresponding authors upon request.

Authors' contributions

BA, MAS, and US supervised the study. BA, IA, AA, MF, ZC and NA did the experimental work. IA, AA, TSA, FAJ, WSA analyzed and interpreted the data results. All authors had equal contribution to drafting and revising the manuscript.

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Publisher's note

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Rosa moschata **ameliorates haloperidol-induced Parkinson's disease *via* reduction of neurodegeneration and oxidative stress**

Running title: *Rosa moschata* alleviates **haloperidol-induced Parkinson's disease**

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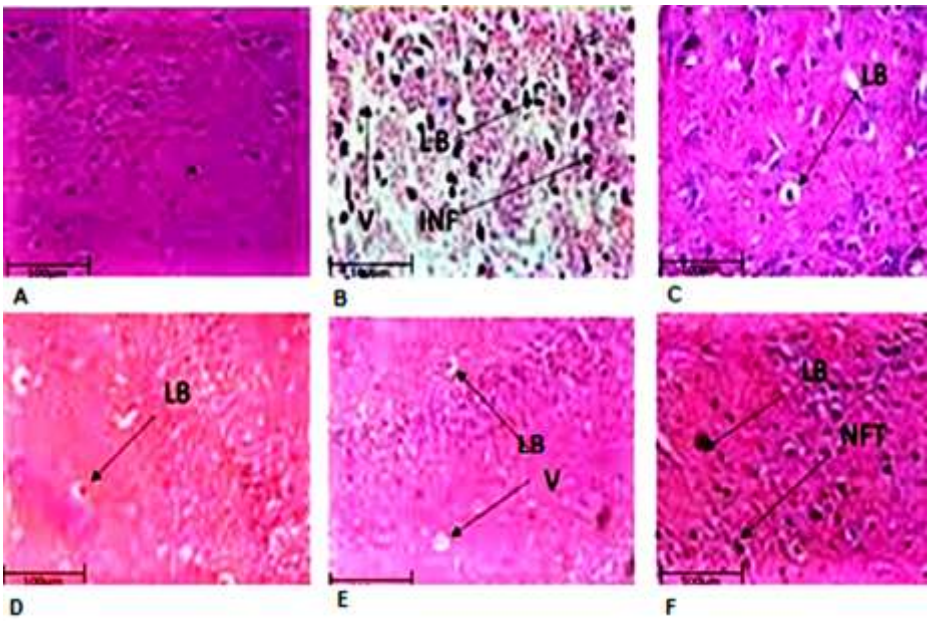

Supplementary Figure 1**.** Histopathological analysis of brain tissues stained with hematoxylin and eosin (magnification: 40×; scale bar: 100 μm). (A) Brain sections of the normal control group display normal histology of brain tissues. (B) Brain tissues from PD rats show numerous Lewy bodies, vacuolization, and infiltration. (C) PD rats treated with levodopa and carbidopa demonstrate reduced number of Lewy bodies. (D) Brain tissues of PD rats treated with 150 mg/kg of *Rosa moschata* show fewer Lewy bodies and reduced abnormal protein aggregates. (E) Brain tissues of PD rats treated with 300 mg/kg *Rosa moschata* show only one Lewy body and no neurofibrillary tangles. (F) PD rats treated with 600 mg/kg *Rosa moschata* exhibit no visible protein aggregates. INF: infiltration; LB: Lewy bodies; V: vacuolization; NFT: neurofibrillary tangles.

Supplementary Table 1. List of primer sequences.

Gene	**Primer sequence (**5'-3'**)**	**Base product size**	**Accession number**
IL-1α	CCTCGTCCTAAGTCACTCGC	102	NM_017019.1
	GGCTGGTTCCACTAGGCTTT		
IL-1β	GACTTCACCATGGAACCCGT	104	NM_031512.2
	GGAGACTGCCCATTCTCGAC		
TNF-α	GGAGGGAGAACAGCAACTCC	168	NM_012675.3
	TCTGCCAGTTCCACATCTCG		
α-Synuclein	TCGAAGCCTGTGCATCCATC	156	XM_017592500.1
	CTCCCTCCTTGGCCTTTGAA		
GADPH	GGAGTCCCCATCCCAACTCA	173	XM_017592435.1
	GCCCATAACCCCCACAACAC		

Supplementary Table 2. HPLC results of *Rosa moschata* extract.

Compound	**Molecular formula**	**Peak area**	**K factor**	**μg/g of dry extract**
p-Coumaric acid	$C_9H_8O_3$	667 017.5	0.000 3	200.10
Gallic acid	$C_7H_6O_5$	1 137 736.8	0.000 079	89.88
Vanilic acid	$C_8H_8O_4$	310 433.6	0.000 044	13.66
Salicylic acid	HOC_6H_4COOH	103 372.8	0.000 377	38.97