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Research Article

RUTIN FROM *RUTA CHALEPENSIS* ALLEVIATES ROTENONE-INDUCED DOPAMINE DEPLETION AND MOTOR DEFICITS THROUGH ANTI-OXIDATIVE AND ANTI-APOPTOTIC EFFECTS IN A RAT MODEL OF PARKINSON'S DISEASE

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ABSTRACT

Objectives: Rutin, a flavonol glycoside derived from *Ruta chalepensis*, has shown antioxidant and anti-apoptotic properties in *in vitro* models of Parkinson's disease (PD); however, its neuroprotective mechanisms *in vivo* remain inadequately characterised. The present study aimed to evaluate the neuroprotective and anti-apoptotic effects of rutin in a rotenone-induced rat model of PD.

Methods: Male albino Wistar rats were administered rotenone (2.5 mg/kg, intraperitoneally) for 28 days to induce Parkinsonian features. Rutin was administered orally at doses of 5, 10, and 20 mg/kg to determine the optimal neuroprotective concentration. Motor performance was assessed using behavioral tests, followed by estimation of striatal dopamine levels, oxidative stress markers, antioxidant enzyme activities, and expression of dopaminergic and apoptotic proteins.

Results: Rotenone administration resulted in significant motor impairment, striatal dopamine depletion, increased oxidative stress, reduced expression of tyrosine hydroxylase, dopamine transporter, and vesicular monoamine transporter-2, elevated α-synuclein levels, and activation of apoptotic signaling characterised by increased Bax, cytochrome c, and caspases-3, -8, and -9, along with decreased Bcl-2 expression. Rutin treatment produced a dose-dependent improvement in motor function, with 10 mg/kg identified as the most effective dose. Co-treatment with rutin (10 mg/kg) significantly restored antioxidant enzyme activities, normalized dopaminergic protein expression, reduced α-synuclein accumulation, and suppressed apoptotic pathways.

Conclusion: These findings demonstrate that rutin confers significant neuroprotection against rotenone-induced dopaminergic neurodegeneration by attenuating oxidative stress and apoptosis *in vivo*. Rutin may represent a promising candidate for disease-modifying therapy in PD, warranting further investigation of its mitochondrial and anti-inflammatory mechanisms.

Keywords: Rutin, Nigrostriatal pathway, Oxidative stress, Apoptotic signaling, α-synuclein, Rotenone-induced Parkinsonism.

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INTRODUCTION

Parkinson's disease (PD) is an age-related neurodegenerative disorder marked by progressive motor dysfunction resulting from degeneration of nigrostriatal dopaminergic neurons. Although current pharmacological interventions provide symptomatic relief, they do not halt disease progression [1,2]. Consequently, there is growing interest in identifying disease-modifying agents that target the underlying molecular mechanisms of PD. In recent years, natural products possessing antioxidant and anti-apoptotic activities have gained considerable attention as potential neuroprotective agents in neurodegenerative disorders [3,4]. Among these, flavonoids have been extensively studied for their ability to scavenge free radicals, modulate oxidative stress, and regulate cell-survival signaling pathways.

Rutin is a naturally occurring flavonol glycoside widely recognized for its potent antioxidant, anti-inflammatory, and anti-apoptotic properties [5-11]. *Ruta chalepensis*, a medicinal plant traditionally used in ethnopharmacological practices, is a rich natural source of rutin. While the pharmacological activities of rutin have been well documented, its neuroprotective efficacy in environmentally induced Parkinsonian neurodegeneration remains insufficiently explored. In particular, there is a scarcity of *in vivo* studies evaluating rutin derived from *R. chalepensis* in toxin-based models of PD, such as the rotenone model, which closely mimics mitochondrial dysfunction, oxidative stress, α-synuclein aggregation, and apoptotic neuronal loss observed in human PD.

Given that oxidative stress, α-synuclein accumulation, and apoptosis are central contributors to rotenone-induced dopaminergic degeneration [12-17], the effects of rutin on these pathogenic hallmarks remain largely undefined. Notably, the modulation of α-synuclein expression and intrinsic and extrinsic apoptotic pathways by rutin *in vivo* has not been systematically investigated. Therefore, the present study was designed to address this critical knowledge gap by evaluating the neuroprotective potential of *R. chalepensis*-derived rutin against rotenone-induced dopaminergic dysfunction and motor deficits in rats, with a specific focus on oxidative stress, α-synuclein regulation, and apoptotic signaling mechanisms.

METHODS

Chemicals and reagents

Rotenone was purchased from Sigma-Aldrich (Bangalore, India). Primary antibodies targeting tyrosine hydroxylase (TH; sc-73152), dopamine transporter (DAT; sc-32258), vesicular monoamine transporter 2 (VMAT2; sc-374079), caspase-3 (sc-136219), caspase-8 (sc-81656), caspase-9 (sc-81650), cytochrome-c (Cyt-C; sc-13561), Bcl-2 (#3869), Bax (#2772), α-synuclein (#4179), and β-actin were obtained from Santa Cruz Biotechnology (Santa Cruz, CA, USA).

Experimental animals

Male Wistar rats (225–250 g) were procured from the institutional animal facility and housed under controlled temperature (22±2°C), relative humidity (55±5%), and a 12 h light/dark cycle, with ad libitum

access to standard pellet diet and water. All animals were acclimatized for 7 days before experimentation. Experimental protocols were approved by the Institutional Animal Ethics Committee and conducted in accordance with CPCSEA guidelines [18].

Experiment I: Rutin dose optimization
Dose optimization of rutin was carried out using a rotenone-induced Parkinsonian rat model. Animals were randomly divided into experimental groups (n=6 rats/group): Control (sunflower oil), rotenone alone, rotenone+rutin (5, 10, and 20 mg/kg), and rutin alone. Rutin was administered orally and initiated 1 day before rotenone exposure, followed by daily dosing 2 h before each rotenone injection. Behavioral assessments were performed on day 28, and striatal and substantia nigra (SN) dopamine levels were estimated on day 29.

Dose–response analysis demonstrated a significant, dose-dependent improvement in behavioral performance and dopamine restoration at 5–20 mg/kg. Although both 10 and 20 mg/kg doses showed comparable efficacy, 10 mg/kg was selected for further experiments based on statistical equivalence, reduced drug exposure, and consistency with previously reported effective doses [19,20].

Open field test
Locomotor and exploratory behaviors were assessed using the open field test. Rats were individually placed in the center of the arena and observed for 5 min. Parameters, including locomotion, rearing, and grooming, were recorded. Blinding was implemented by coding treatment groups by an independent investigator, and behavioral scoring was performed by an evaluator unaware of group allocation. The apparatus was cleaned with 10% ethanol between trials to eliminate olfactory cues [21].

Rotarod test
Motor coordination and balance were evaluated using a rotarod apparatus (Harvard Apparatus, Panlab, Barcelona, Spain). Rats underwent training for three consecutive days before testing. During the test session, the rotation speed was gradually increased from 5 rpm to 20 rpm, and latency to fall was recorded for each animal. All assessments were conducted by a blinded observer using coded animal identifiers [22].

Experiment II: Neuroprotective model
Based on dose optimization, rutin at 10 mg/kg was selected for the neuroprotection study. Animals were divided into four experimental groups with n=12 rats per group: Control, rotenone, rotenone+rutin, and rutin alone. Rotenone was administered for 28 days, followed by sacrifice and brain dissection at 4 weeks [23].

No mortality or animal dropouts were observed during the rotenone administration period. All animals completed the study and were included in the final analysis. Animals were monitored daily for body weight, behavioral distress, and general health.

Tissue processing
Following euthanasia, brains were rapidly removed, rinsed in ice-cold saline, and dissected to isolate the SN and striatum (ST). Tissues were processed for dopamine estimation, immunohistochemistry, and protein extraction. Proteins were extracted using radio-immunoprecipitation assay buffer supplemented with protease inhibitors, followed by centrifugation at 13,000 rpm for 15 min at 4°C, and the supernatants were stored at −80°C until analysis. Protein concentrations were determined using standard methods [24,25], and dopamine levels were expressed as ng/mg protein, normalized to total protein content.

Quantification of striatal dopamine
Striatal dopamine levels were quantified using high-performance liquid chromatography (HPLC) coupled with electrochemical detection following perchloric acid extraction and centrifugation and expressed as ng/mg tissue [26].

Oxidative stress and antioxidant enzyme analysis
Oxidative stress parameters were measured in cortical and hippocampal homogenates. Lipid peroxidation was assessed by thiobarbituric acid reactive substances (TBARS) assay, catalase activity by dichromate-based hydrogen peroxide degradation, glutathione peroxidase (GPx) activity by glutathione (GSH) consumption, total GSH by DTNB reaction, and superoxide dismutase (SOD) activity by inhibition of the NADH–PMS–NBT reaction [27,28].

Western blot analysis
Proteins from the SN and ST were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis and transferred onto polyvinylidene fluoride membranes. Immunoreactive bands were detected using enhanced chemiluminescence and quantified using ImageJ software. β-Actin was used as the internal loading control for normalization [29,30].

Immunohistochemistry
Perfusion-fixed brain tissues were sectioned and subjected to antigen retrieval and endogenous peroxidase blocking. Sections were incubated with primary antibodies against TH, followed by appropriate secondary antibodies and DAB visualization. TH-positive neurons in the SN and fibers in the ST were quantified using ImageJ software [31,32].

Statistical analysis
Data were expressed as mean±standard error of the mean. Statistical analysis was performed using one-way analysis of variance followed by Tukey's *post hoc* test. A value of $p<0.05$ was considered statistically significant [33].

RESULTS AND DISCUSSION

Rutin improves motor performance in rotenone-exposed rats
The open field test was employed to assess spontaneous locomotion and exploratory behavior, which serve as indirect indicators of nigrostriatal dopaminergic function. Activity in both the central and peripheral zones of the arena reflects motor performance, curiosity, and adaptation to a novel environment, whereas decreases in rearing and grooming behaviors are associated with stress-related responses. Exposure to rotenone resulted in significant impairments in open field performance, as indicated by reduced central and peripheral entries, rearing, and grooming ($p<0.05$). These deficits were dose-dependently attenuated by rutin treatment, with no behavioral changes observed in the rutin-alone group (Fig. 1a-d).

Rutin improves motor coordination in rotarod testing
The rotarod apparatus was used in the assessment of rats to further assess motor coordination, balance, and neuromuscular function. Rotenone markedly disrupted motor coordination, as reflected by a reduced latency to fall ($p<0.05$). Co-administration of rutin with rotenone produced an enhancement of motor performance in a dose-dependent manner, and no significant differences were found between the rutin-alone group and control group (Fig. 2a-d).

Rutin attenuates rotenone-induced dopamine loss
Rotenone treatment resulted in a significant loss of striatal dopamine, indicative of severe disruption of nigrostriatal dopaminergic neurons. By contrast, animals receiving rutin in addition to rotenone demonstrated a clear, dose-dependent recovery of striatal dopamine content, with 10 and 20 mg/kg yielding substantial recovery towards baseline levels. These data suggest that rutin is effective in protecting dopaminergic neurons from rotenone-induced insult and inhibition of apoptotic cell death. Importantly, rutin treatment alone maintained dopamine levels similar to controls, suggesting that rutin does not interfere with normal dopaminergic function. Together, these data reinforce the potential for rutin to act as a neuroprotectant against rotenone-induced dopaminergic dysfunction (Fig. 3).

Biochemical evaluation of oxidative stress
Rotenone administration significantly increased lipid peroxidation, as evidenced by elevated TBARS levels in both the cortex and

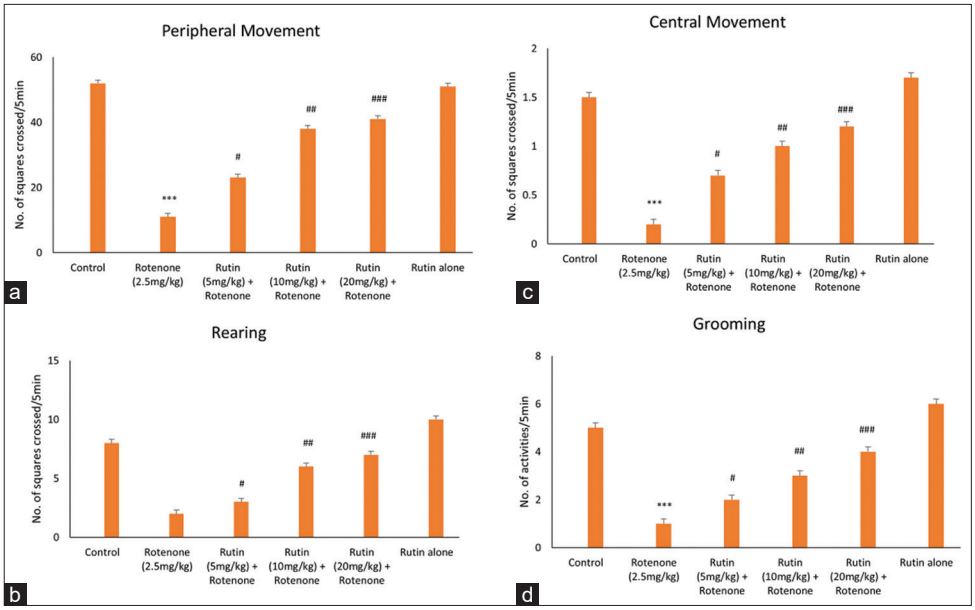

Fig. 1: Open-field behavioral analysis of control and treated rats. Exposure to rotenone produced a marked reduction in peripheral locomotion (a), central zone activity (b), rearing frequency (c), and grooming behavior (d) compared with control animals. Co-treatment with rutin significantly attenuated these behavioral impairments in a dose-dependent manner. Data are presented as mean±standard deviation (n=6). Statistical significance is indicated as ***p<0.001 versus the control group and ###p<0.001, ##p<0.01, or ns (not significant) versus the rotenone-treated group

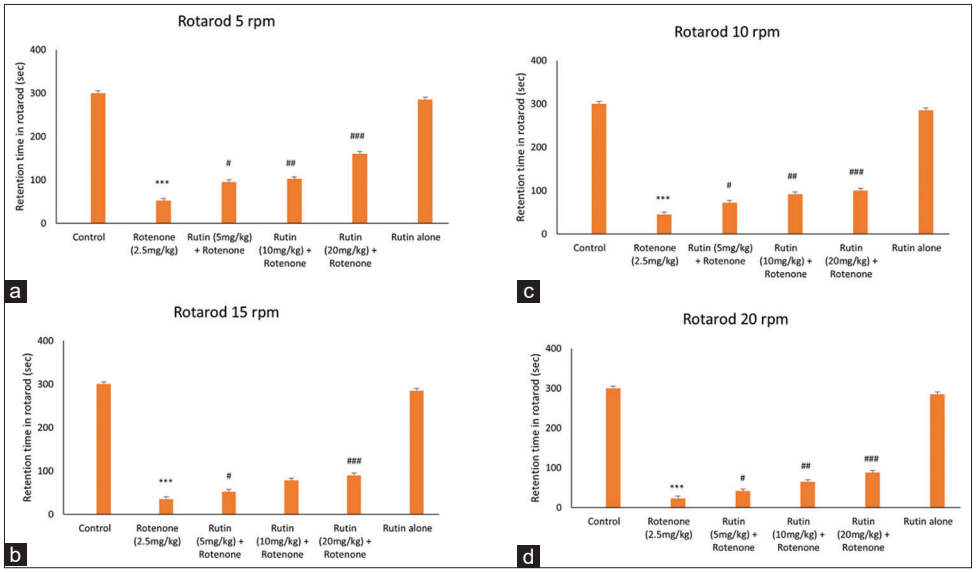

Fig. 2: Rotarod performance following rutin and rotenone treatment. Rats exposed to rotenone exhibited a pronounced reduction in latency to fall from the rotating rod at all tested speeds (A: 5 rpm, B: 10 rpm, C: 15 rpm, and D: 20 rpm) when compared with control animals. Co-treatment with rutin significantly improved retention time in a dose-dependent manner relative to the rotenone-only group. Data are expressed as mean±SD (n=6). Statistical significance is denoted as ***p<0.001 versus the control group, and ###p<0.001, ##p<0.01, #p<0.05, or ns (not significant) versus the rotenone-treated group

hippocampus, indicating enhanced oxidative damage to neuronal membranes Rotenone (2.5 mg/kg) induced oxidative stress in the cortex and hippocampus, as indicated by increased TBARS and decreased antioxidant defense, while rutin co-treatment significantly restored these parameters (Fig. 4a-e; p<0.05). Administration of rutin alone did not produce significant changes in oxidative stress markers or antioxidant enzyme levels compared with controls. These results indicate that rutin effectively mitigates rotenone-induced oxidative damage and strengthens the brain's antioxidant defence system.

Modulation of TH, DAT, and VMAT2 expression by rutin in the SN and ST

TH is the rate-limiting enzyme for dopamine synthesis, while DAT and VMAT2 are critical for dopamine reuptake and vesicular storage, respectively. DAT facilitates the uptake of extracellular dopamine into presynaptic terminals, whereas VMAT2 transports dopamine into synaptic vesicles, preventing cytosolic accumulation. Impairment of VMAT2 function represents an early event in rotenone-induced dopaminergic pathology. In this study, rotenone-treated rats (2.5 mg/kg) displayed a pronounced decrease in TH expression, as shown by western blotting (p<0.001; Fig. 5a and b) and immunohistochemistry (p<0.001;

Fig. 6a-d). Likewise, DAT ($p<0.001$; Fig. 5c and d) and VMAT2 ($p<0.001$; Fig. 5e and f) levels were significantly reduced in both the SN and ST compared with controls, confirming rotenone-induced nigrostriatal neurodegeneration. Co-administration of rutin (10 mg/kg) with rotenone effectively restored TH, DAT, and VMAT2 expression in the SN and ST, as verified by both immunoblot and immunohistochemical analyses ($p<0.001$). Importantly, rutin treatment alone did not significantly alter dopaminergic protein levels relative to control animals, indicating that rutin preserves dopaminergic integrity without causing adverse effects.

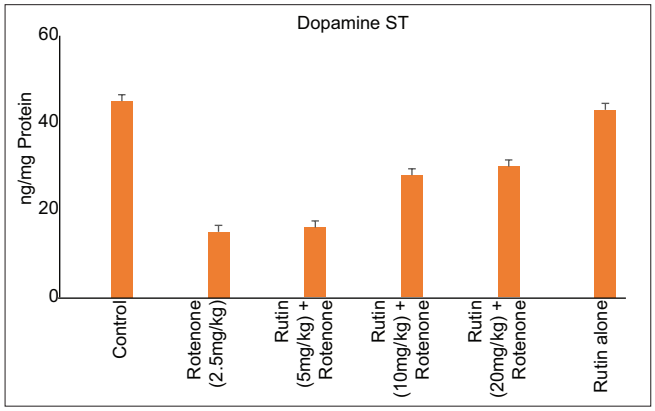

Fig. 3: Effect of rotenone and rutin treatment on striatal dopamine levels. Dopamine concentration is expressed as ng/mg protein, normalized to the total protein content of the striatal tissue homogenate. Striatal dopamine concentrations in control and treated rats. Rotenone exposure resulted in a significant reduction in dopamine levels within the striatum compared with untreated control animals. Co-administration of rutin significantly restored striatal dopamine content in a dose-dependent manner relative to the rotenone-only group. Data are expressed as mean±standard deviation (n=6). Statistical significance is indicated as ***p<0.001 versus the control group and ###p<0.001 or ns (not significant) versus the rotenone-treated group

Rutin attenuates rotenone-induced α-synuclein overexpression

TH is the rate-limiting enzyme for dopamine synthesis, while DAT and VMAT2 are critical for dopamine reuptake and vesicular storage, respectively. DAT facilitates the uptake of extracellular dopamine into presynaptic terminals, whereas VMAT2 transports dopamine into synaptic vesicles, preventing cytosolic accumulation. Impairment of VMAT2 function represents an early event in rotenone-induced dopaminergic pathology. In this study, rotenone-treated rats (2.5 mg/kg) displayed a pronounced decrease in TH expression, as shown by western blotting ($p<0.001$; Fig. 6a and b) and immunohistochemistry ($p<0.001$; Fig. 7a-d). Likewise, DAT ($p<0.001$; Fig. 6c and d) and VMAT2 ($p<0.001$; Fig. 6e and f) levels were significantly reduced in both the SN and ST compared with controls, confirming rotenone-induced nigrostriatal neurodegeneration. Co-administration of rutin (10 mg/kg) with rotenone effectively restored TH, DAT, and VMAT2 expression in the SN and ST, as verified by both immunoblot and immunohistochemical analyses ($p<0.001$). Importantly, rutin treatment alone did not significantly alter dopaminergic protein levels relative to control animals, indicating that rutin preserves dopaminergic integrity without causing adverse effects.

Rutin modulates apoptotic markers in rotenone-exposed rats

Exposure to rotenone (2.5 mg/kg) resulted in a significant increase in pro-apoptotic markers, including Bax, Cyt-C, and caspases-3, -8, and -9, in both the SN and ST compared with control animals ($p<0.001$). In contrast, co-treatment with rutin (10 mg/kg) markedly suppressed the upregulation of these apoptotic proteins in rotenone-exposed rats ($p<0.001$; Figs. 8a-h and 9a-h). Rotenone administration also caused a pronounced downregulation of the anti-apoptotic protein Bcl-2 ($p<0.001$), whereas combined rutin and rotenone treatment significantly restored Bcl-2

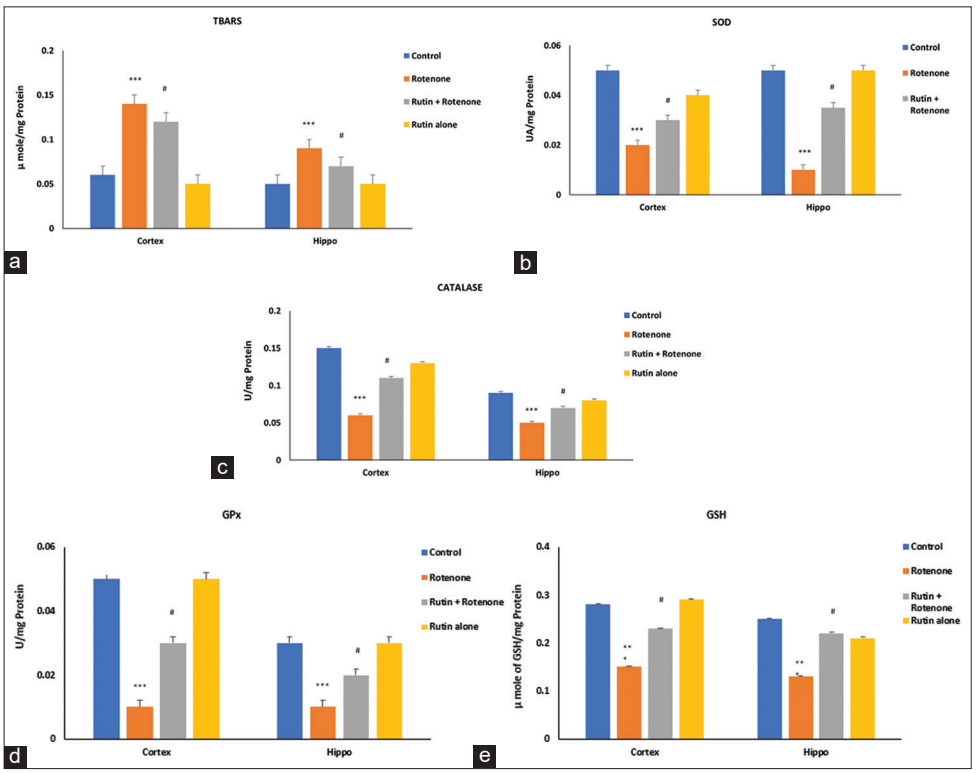

Fig. 4: Influence of rutin on rotenone-induced oxidative stress in experimental and control rats. Graphical representation of lipid peroxidation levels (a) and antioxidant defense markers, including superoxide dismutase (b), catalase (c), glutathione peroxidase (d), and reduced glutathione (e), in the cortex and hippocampus of rats treated with rutin and/or rotenone. Data are expressed as mean±standard deviation (n=6). Statistical significance is denoted as ***p<0.001 versus the control group and ###p<0.001 or ##p<0.01 versus the rotenone-treated group

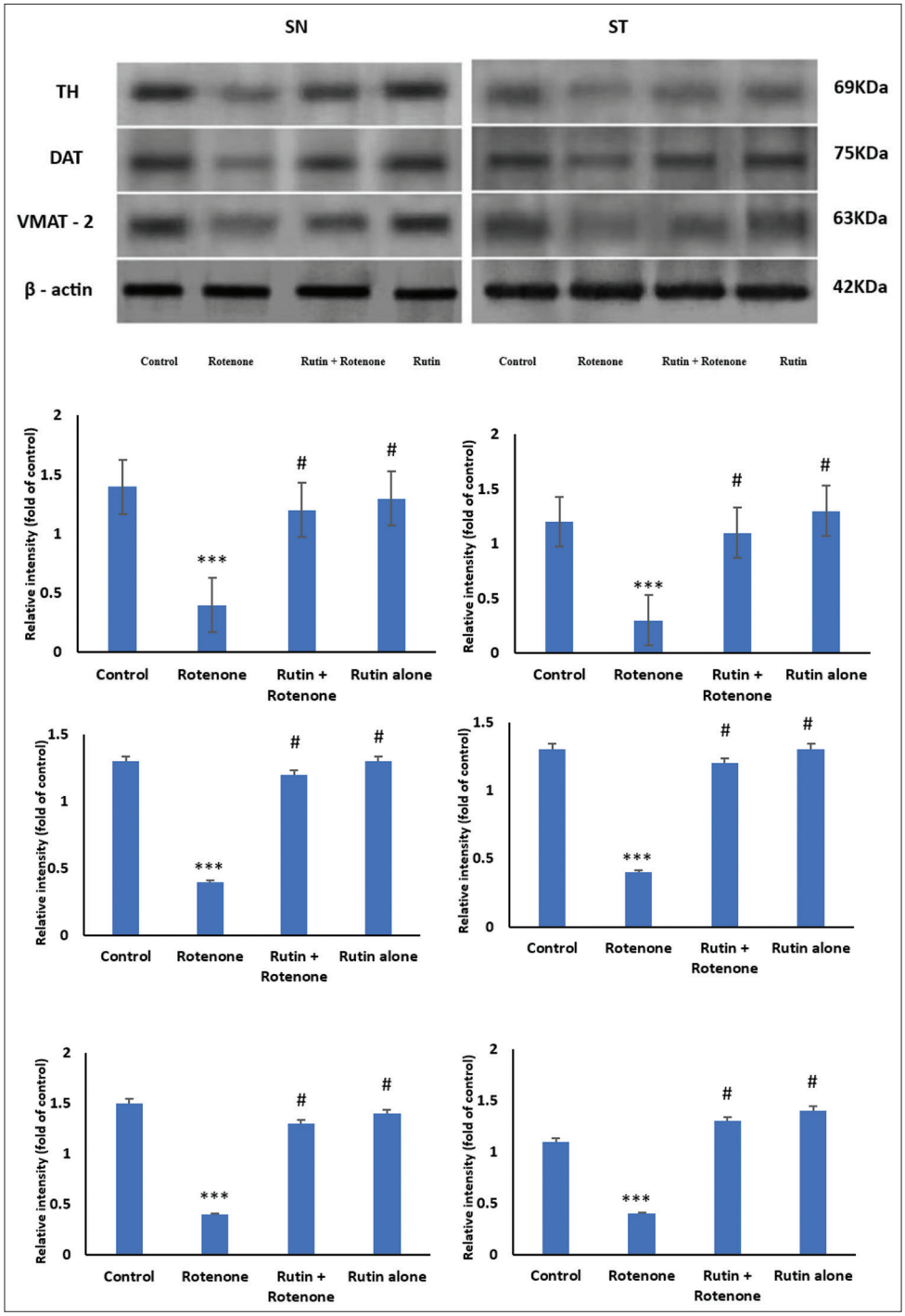

Fig. 5: Western blot analysis of tyrosine hydroxylase (TH), dopamine transporter (DAT), and vesicular monoamine transporter (VMAT2) protein expression in the substantia nigra and striatum of control and experimental rats. Rotenone administration markedly reduced the levels of TH, DAT, and VMAT2 compared with untreated controls. Co-treatment with rutin significantly restored the expression of these proteins relative to the rotenone-only group (a-f). Protein levels were normalized to β-actin. Data are presented as mean±standard deviation (n=3). Statistical significance is indicated as ***p<0.001 versus the control group, and ###p<0.001, ##p<0.01, or #p<0.05 versus the rotenone-treated group

levels in the SN and ST (p<0.01; Figs. 8a,b,e and f). These results indicate that rutin effectively counteracts rotenone-induced apoptotic pathways, promoting neuronal survival in nigrostriatal regions.

DISCUSSION

Rotenone is a well-established neurotoxin that reproduces PD pathology in rodent models, including α-synuclein aggregation, excessive reactive oxygen species (ROS) generation, and mitochondrial dysfunction, ultimately activating caspase-3 and inducing neuronal death. The accumulation of α-synuclein further amplifies apoptotic cascades. Consequently, considerable attention has been directed toward plant-derived antioxidants with neuroprotective properties to counter these pathological mechanisms.

In the present investigation, rutin exhibited pronounced neuroprotective and anti-apoptotic effects against rotenone-induced neurotoxicity in rats. Rutin administration effectively mitigated dopaminergic neuronal degeneration, preserved striatal dopamine levels, and enhanced motor

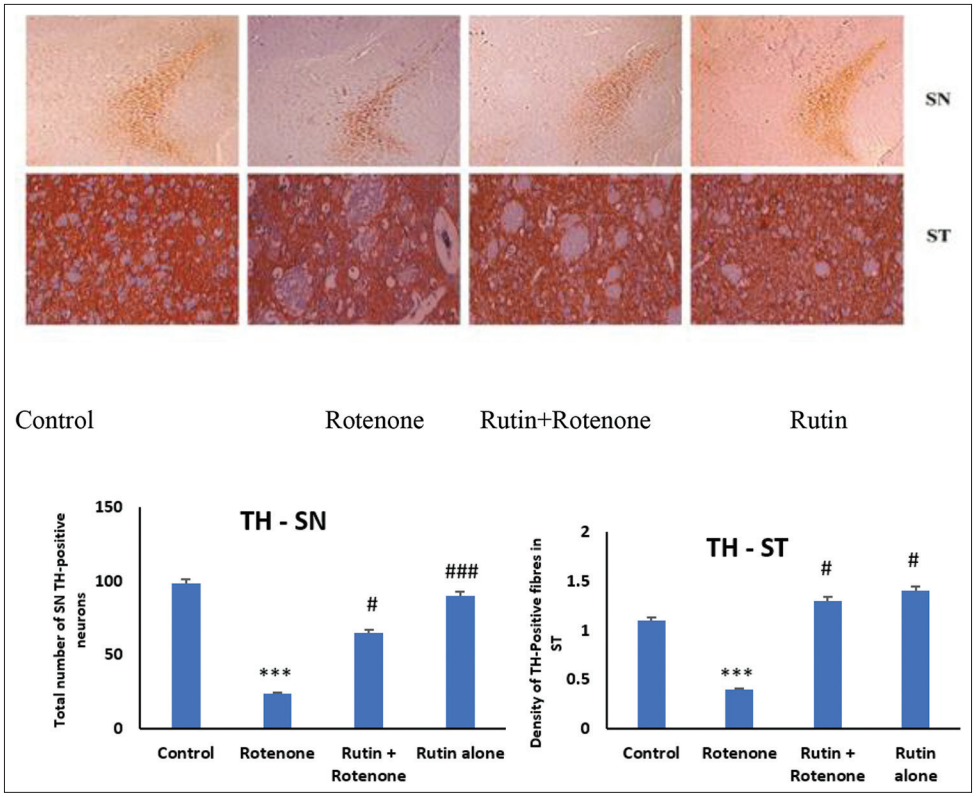

Fig. 6: Neuroprotective effect of rutin on tyrosine hydroxylase (TH)-positive neurons and fibers in the substantia nigra (SN) and striatum (ST) of control and experimental rats. Quantification of TH-positive cell bodies in the SN (a and c) and TH-positive fibers in the ST (b and d) was performed. In the control group, the density of TH-positive fibers in the ST was normalized to 1.00. Rotenone exposure significantly reduced TH immunoreactivity, whereas co-administration of rutin effectively preserved TH-positive cells and fibers. Data are presented as mean±standard deviation. Statistical significance is denoted as ***p<0.001 versus the control group and ###p<0.001 versus the rotenone-only group

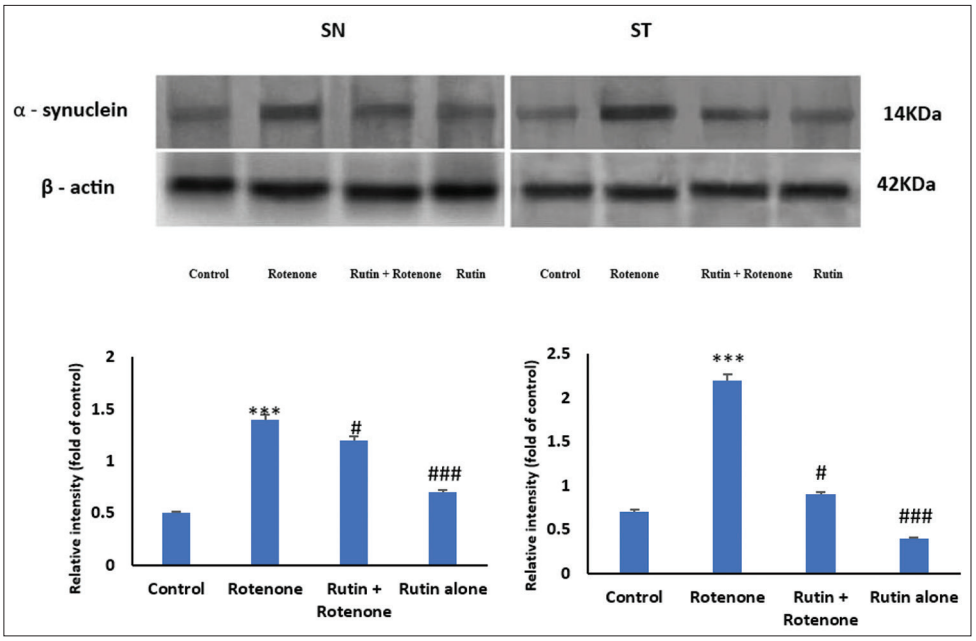

Fig. 7: α-Synuclein protein expression in the substantia nigra and striatum of control and experimental rats. Rotenone administration markedly increased α-synuclein levels compared with control animals, whereas co-treatment with rutin significantly reduced this upregulation (a and b). Protein levels were normalized to β-actin. Data are expressed as mean±standard deviation (n=3). Statistical significance is indicated as ***p<0.001 versus control, and ###p<0.001 or #p<0.05 versus the rotenone-only group

performance in both the open field and rotarod tests, demonstrating its capacity to reverse Parkinsonian behavioral deficits induced by rotenone. Dopamine depletion remains a central contributor to impaired motor function, consistent with observations in rotenone-treated rodent models.

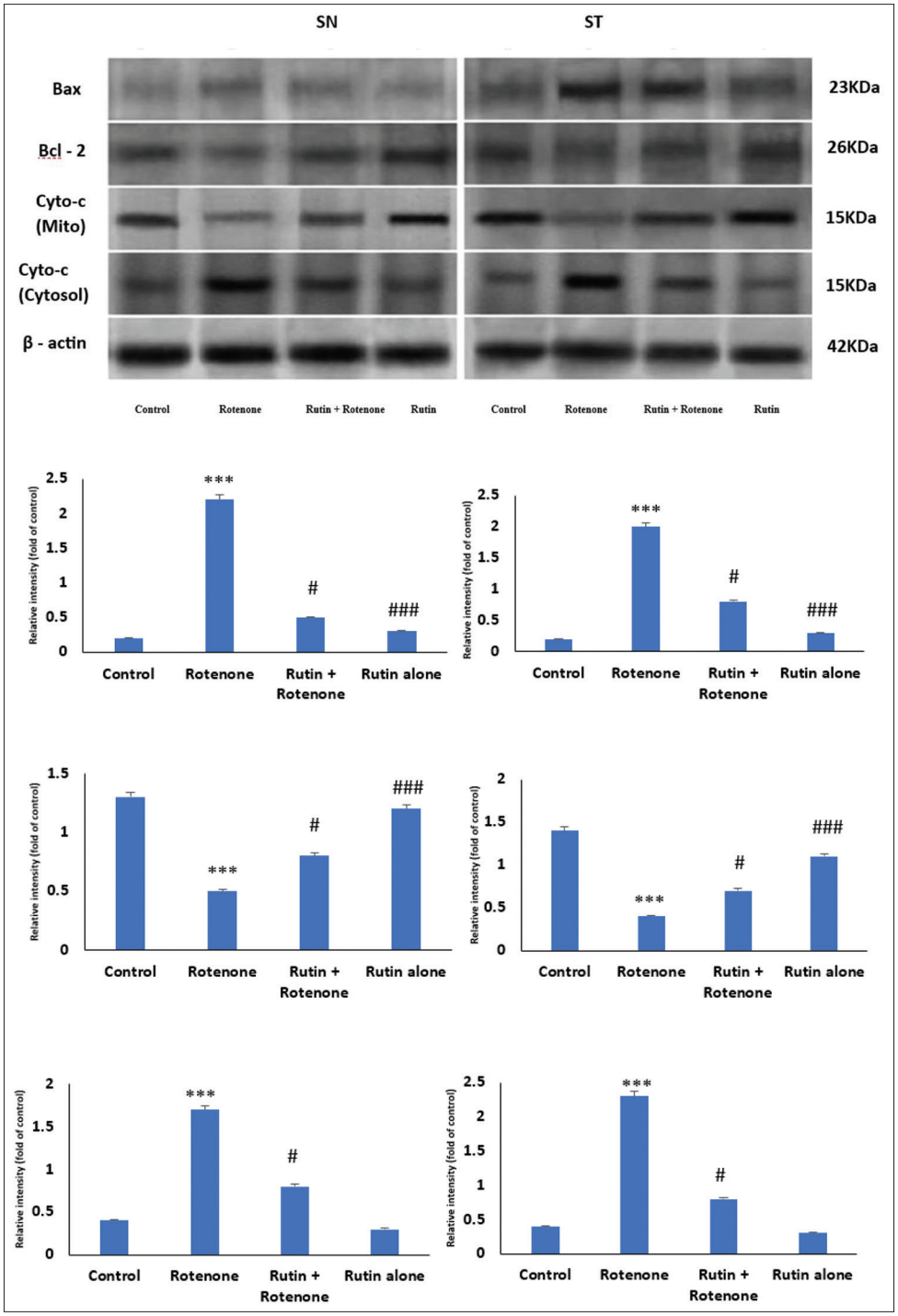

Fig. 8: Expression of Bax, Bcl2, and cytochrome c (Cyt-C) in the substantia nigra and striatum of control and experimental rats. Rotenone administration markedly increased Bax levels while decreasing Bcl2 and mitochondrial Cyt-C expression compared with control animals. Co-treatment with rutin significantly reversed these alterations in protein expression (a and b). Densitometric analysis of the immunoblots is presented in panels (c-h) with β-actin serving as the loading control. Data are expressed as mean±standard deviation (n=3). Statistical significance is denoted as ***p<0.001 versus the control group and ###p<0.001, ##p<0.01, or ns (not significant) versus the rotenone-only group

Rotenone-mediated inhibition of mitochondrial complex I leads to excessive ROS production, oxidative stress, and disrupted dopamine metabolism, creating a self-perpetuating cycle of neuronal injury. In this study, rotenone significantly elevated TBARS levels and reduced the activities of endogenous antioxidant enzymes (CAT, SOD, GPx) and GSH content in the cortex and hippocampus. Co-administration of rutin effectively decreased lipid peroxidation and restored antioxidant enzyme activities and GSH levels, highlighting its potent free radical-scavenging ability and its role in reinforcing endogenous antioxidant defenses. Exposure to rotenone also diminished the expression of TH, DAT, and VMAT2, reflecting dopaminergic neuronal damage. Treatment with rutin prevented the downregulation of these key proteins and reduced α-synuclein accumulation in the SN and ST. These findings are in line with studies demonstrating that other phytochemicals, including piperine, caffeine, and nicotine, similarly enhance TH, DAT, and VMAT2 expression while limiting α-synuclein aggregation in PD models.

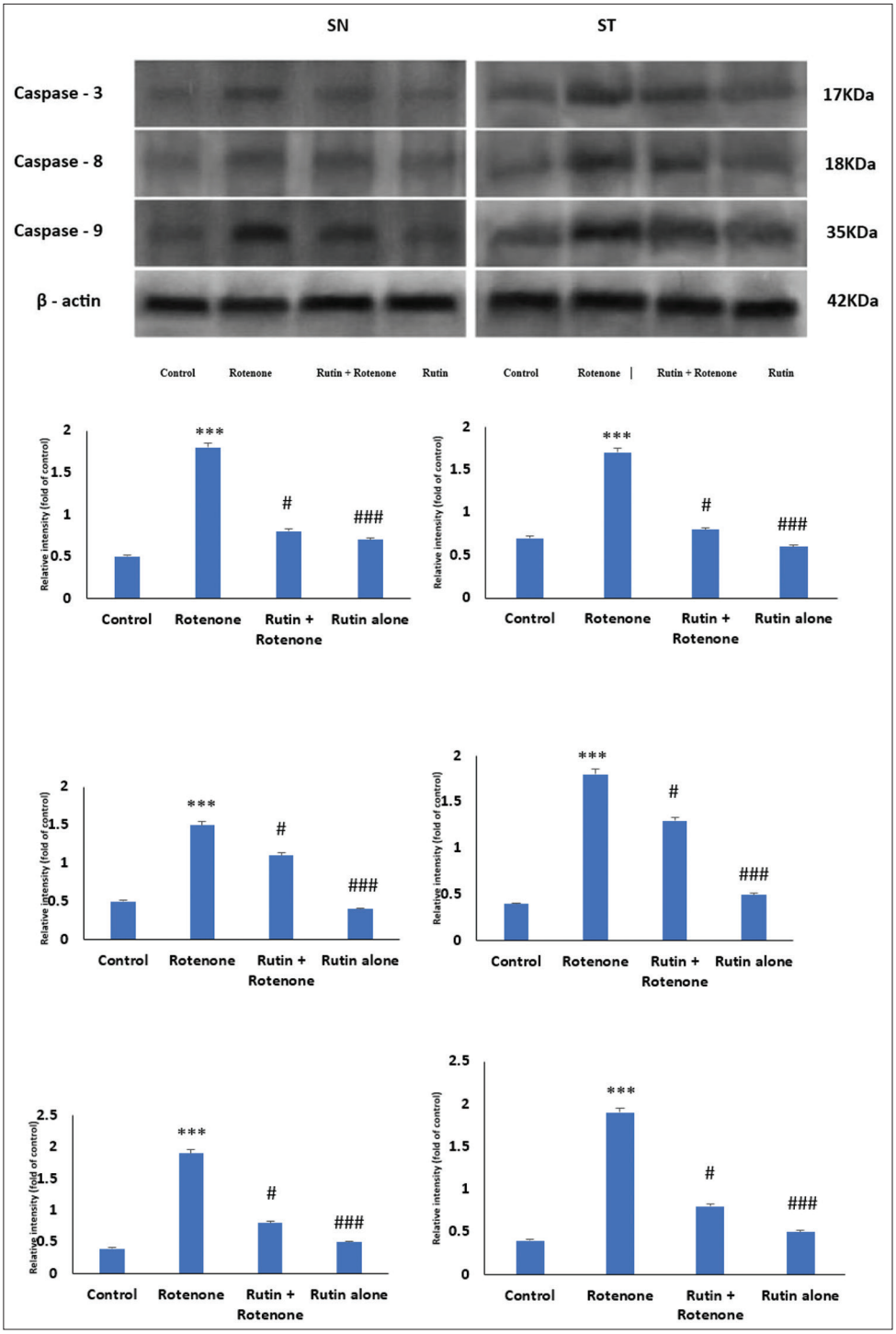

Fig. 9: Caspase-3, -8, and -9 protein expression in the substantia nigra and striatum of control and experimental rats. Rotenone administration significantly elevated the levels of caspases-3, -8, and -9 compared with control animals. Co-treatment with rutin markedly reduced these increases in protein expression (a and b). Densitometric quantification of the immunoblots is shown in panels (c-h) with β-actin used as an internal reference. Data are presented as mean±standard deviation (n=3). Statistical significance is indicated as ***p<0.001 versus control, and ###p<0.001 versus the rotenone-only group

Apoptotic mechanisms are central to dopaminergic neuron loss in PD, involving Cyt-C release, Bax/Bcl2 imbalance, and caspase activation. Rotenone exposure upregulated pro-apoptotic markers, including Bax, Cyt-C, and caspases-3, -8, and -9, while downregulating anti-apoptotic Bcl2. Rutin co-treatment restored Bcl2 expression, suppressed Bax and Cyt-C levels, and inhibited caspase activation, underscoring its anti-apoptotic and neuroprotective effects.

Collectively, these results indicate that rutin mitigates rotenone-induced neurotoxicity through a multifaceted mechanism involving the attenuation of oxidative stress, inhibition of apoptotic signaling, and suppression of α-synuclein accumulation, suggesting its potential as a neuroprotective agent in PD.

CONCLUSION

The present study demonstrates that rutin exerts significant protective effects against rotenone-induced dopaminergic neurodegeneration in rats. Treatment with rutin restored striatal dopamine content, improved behavioral deficits, attenuated oxidative damage, reduced α-synuclein

accumulation, and regulated apoptotic signaling by inhibiting caspase activation and normalizing the Bax/Bcl2 ratio. These findings highlight rutin as a promising candidate for neuroprotective therapy in PD. Future investigations will focus on elucidating its mechanisms of mitochondrial protection and anti-inflammatory activity to further substantiate its therapeutic potential.

ETHICAL STATEMENT

Ethical Number - AKCP/IAEC/2O/23–24 in Arulmigu Kalasalingam College of Pharmacy, Anand Nagar, Tamil Nadu State.

AUTHOR CONTRIBUTIONS

Jayanthi P: Conceptualization, methodology, experimental work, data acquisition, data analysis, and original draft preparation. Shobana C: Study supervision, experimental design, data interpretation, critical revision of the manuscript, and final approval of the version to be published.

CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this manuscript.

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