

THERAPEUTIC POTENTIALS OF *SYZYGIUM CUMINI* SEED KERNEL EXTRACT IN COMBINATION WITH LEVODOPA THERAPY IN PARKINSON'S DISEASE MICE MODEL

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ABSTRACT

Objectives: Parkinson's disease (PD) is characterized by dopaminergic neuron loss, oxidative stress, and neurotransmitter depletion. Levodopa (L-DOPA) therapy, while effective, is associated with side effects, such as dyskinesia upon long-term use. This study investigated the neuroprotective potential of *Syzygium cumini* (SC) seed kernel methanolic extract, alone or combined with reduced-dose L-DOPA, in a rotenone-induced PD mouse model.

Methods: Adult male Swiss albino mice (n=6/group) were administered rotenone (3 mg/kg i.p., 10 days) to induce PD. Treatment groups received SC extract (100 mg/kg p.o.), L-DOPA (15 mg/kg p.o.), or combination (SC 100 mg/kg+L-DOPA 7.5 mg/kg p.o.) for 28 days. Oxidative stress markers, brain urea and nitric oxide (NO), and neurotransmitters (dopamine, serotonin, and norepinephrine) were assessed. Data were analyzed by one-way analysis of variance with Student-Newman-Keuls *post hoc* test.

Results: Rotenone significantly increased malondialdehyde (p<0.001), urea (p<0.001), and NO (p<0.001), while decreasing glutathione reductase (p<0.001), glutathione peroxidase (GPx) (p<0.001), superoxide dismutase (SOD) (p=0.002), catalase (p=0.038), dopamine (p<0.001), serotonin (p<0.05), and norepinephrine (p<0.001) compared to control. All treatments significantly attenuated these changes (p<0.005–p<0.001 for most parameters). The combination therapy showed superior effects, notably restoring GPx (p<0.001), SOD (p=0.002), and serotonin (p<0.001) closer to control levels.

Conclusion: SC extract, particularly in combination with reduced-dose L-DOPA, effectively mitigates oxidative stress and neurotransmitter deficits in rotenone-induced PD, suggesting its potential as a safe, cost-effective adjuvant therapy.

Keywords: Parkinson's disease, *Syzygium cumini*, Rotenone, Levodopa, Oxidative stress, Neuroprotective, Antioxidant enzymes, Neurotransmitters, Adjuvant therapy, Mouse model.

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INTRODUCTION

Parkinson's disease (PD) is the second most common neurodegenerative disorder affecting aged persons, with a prevalence of about 1% in the population over the age of 60 [1]. Akinesia, bradykinesia, postural instability, rigidity, and tremors are characteristic features of PD. PD is also reported with some cardinal features, such as autonomic disturbances, cognitive impairment, sensory alterations, and sleep disturbances. The reason for the progression of PD is loss of dopaminergic neurons present in substantia nigra pars compacta (SNpc) of the midbrain [2]. Hence, there is a depletion of neurotransmitter dopamine. This is due to the aggregation of α -synuclein protein in the midbrain, known as Lewy bodies (LBs). Due to the abnormal formation of these LBs mitochondrial dysfunction occurs, which in turn causes damage to dopaminergic neurons, resulting in low levels of dopamine [3]. Subsequent changes occur in neuronal activity, which imparts motor dysfunctions. However, apart from the neuronal loss in the SNpc, the neurodegenerative effect in PD affects several other regions of the brain, such as the pedunculopontine nucleus, amygdala, ventral tegmental area, locus coeruleus, raphe nuclei, and the vagal dorsal motor nucleus. This implies that other neurotransmitters associated with the cholinergic, adrenergic, and serotonergic systems, are also involved in the various clinical symptoms in PD [4]. As the mechanism

underlying this neurodegeneration remains unknown, PD is basically incurable at present. There are number of symptomatic therapies developed to improve the PD patient's quality of life, among which, levodopa (L-DOPA) remains the highly prescribed drug since its first introduction as therapy for PD symptoms. A few more therapeutic drugs have also rendered considerable effect in treating PD symptoms, that is, monoamine oxidase type B inhibitor that slows down the breakdown of dopamine, non-dopaminergic agents that act on other neurotransmitter systems involved in PD as mentioned above. The free radical hypothesis in PD is emphasized based on the number of experimental and clinical studies [5]. The role of oxidative stress and its adverse effects on different brain regions in PD has not been fully understood. Moreover, the results of studies on oxidative stress in PD are not homogeneous, and do not specify which antioxidant enzymes are depleted, and which indicators of oxidative stress are essential for PD. Oxidative stress markers, such as malondialdehyde (MDA) (product of lipid peroxidation), superoxide dismutase (SOD), and catalase (CAT) have crucial role on the misfolding of protein to form LBs. These abnormal protein contents can deplete the levels of neurotransmitters, such as dopamine, serotonin, and norepinephrine. They can increase urea and nitric oxide (NO) levels in the brain due to accumulation of α -synuclein and associated pathology [6,7].

L-DOPA induced dyskinesia (LID)

Persistent usage of L-DOPA for PD correlated with the exposure of LIDs, known as abnormal involuntary dyskinetic movements induced by L-DOPA administration. LIDs are common in the late stage of PD, most seen in patients with early onset of disease. Clinical studies have observed that the onset ages of 50–59 years are about 53% of younger onset patients develops dyskinesias within 5 years, when compared with an age of onset at 70–79 years are 16%. There are mutations, such as PARK2 (parkin), PARK6 (pink-1), and PARK7 (DJ-1) that have been associated with a higher risk of L-DOPA-related motor complications [8,9].

To study disease progression and effective and safer treatment protocols, PD-induced animal models provide a better alternative to human participants. Several methods are available for the induction of PD in animal models, such as the 6-hydroxydopamine model, the 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine model, and the rotenone model [10]. Rotenone-induced PD in animal models is more convenient and effective compared to others. Rotenone is a pesticide that belongs to the rotenoid family of neurotoxins. Highly lipophilic, it easily crosses the blood-brain barrier, and after entering the neurons, rotenone blocks the mitochondrial complex I activity, increases reactive oxygen species (ROS), and inhibits proteasome activity, generating proteolytic stress. Rotenone decreases the dopamine (DA) and glutathione levels, and increases lipid peroxidation in DA neurons, resulting in oxidative damage. *In vivo*, chronic systemic (intravenous) exposure rotenone in rat models induces nigrostriatal DAergic neurodegeneration, LB-like cytoplasmic inclusions in the DA neurons, and Parkinsonian features, such as bradykinesia, rigidity, postural instability, unsteady gait, and tremor [11].

Plant-derived extracts inhibit oligomerization and fibrillization of α -syn and serve as a therapeutic target for PD. Several research works have shown the importance of plant extracts and plant-based active compounds as effective remedies for neurological problems, such as PD and Alzheimer's disease [12]. There are studies that support the role of the endocarp of *Mucuna pruriens*, which has compounds that render antiparkinsonian effects as well as enhance L-DOPA's. It has been clinically showed improvements in conditions, such as stiffness, tremor, bradykinesia, and cramp-like pain of lower limbs in PD patients by using *Hyoscyamus reticulatus* seeds with *Withania somnifera* and *Sida cordifolia* roots in a clinical trial involving 18 PD patients in India [13,14]. Other than these plants, such as *Acanthopanax senticosus* (*Eleutherococcus senticosus* [Rupr. and Maxim.] Maxim.), *Bacopa monnieri* (*B. monnieri* [L.] Wettst.), Cinnamon extract precipitate (*Cinnamomum verum* J. Presl), *Centella asiatica* (*C. asiatica* [L.] Urb.), *Panax ginseng* (*P. ginseng* C.A. Mey.), *Polygala tenuifolia* (*Polygala tenuifolia* Willd.), *Rehmannia glutinosa* (*R. glutinosa* [Gaertn.] DC.), *Corema album* (*Trema micranthum* [L.] Blume), *Opuntia ficus-indica* (*O. ficus-indica* [L.] Mill.), *Padina pavonica* (*Sagina japonica* [Sw. ex Steud.] Ohwi), *Carthamus tinctorius* L., and *Crocus sativus* L. are neuroprotective in PD by inhibiting oligomerization, fibrillation, and aggregation of preformed α -syn fibrils.

Syzgium cumini (SC), also known as *Eugenia jambolana*, was employed as a therapeutic agent against rotenone-induced PD in the present study. The prepared SCE was administered by oral route either alone or in combination with L-DOPA to test its potential. Studies have revealed that phytochemicals present in SC are neuroprotective in nature. Seed kernel powder of SC is a rich source of antioxidants and used to treat neurological problems and also acts as a nootropic. SC seeds contain neuroprotective components, such as jambosine, gallic acid, ellagic acid (EA), corilagin, 3,6-hexahydroxy diphenylglucose, 1-galloylglucose, 3-galloylglucose, quercetin, β -sitosterol and 4,6-hexahydroxydiphenylglucose. Therefore, the present study was aimed to evaluate the therapeutic efficacy of the methanolic extract of SC seed kernel powder, either alone or in combination with L-DOPA, in rotenone-induced PD mice. Serum oxidative stress parameters, brain urea levels, and serum neurotransmitter levels were estimated

to assess the protective effect of drugs [15]. We hypothesized that the antioxidant-rich SC extract would mitigate rotenone-induced oxidative stress and, when co-administered, would allow for a reduction in L-DOPA dose while maintaining or enhancing efficacy.

METHODS

Animals

All the experiments were performed after getting approval from the Institutional Animal Ethics Committee (IAEC Approval number- SU/CLAR/RD/001/2015, SU/CLAR/RD/021/2016). Adult male Swiss Albino Mice, weighing 35–50 g were used for the experimentation. Animals were housed three per cage. Animals were distributed 6 animals per group according to the resource equation approach [16]. Standard controlled conditions of natural light/dark, 25±1°C of room temperature, free availability of food and water were to 10 mL. Regular monitoring was done on body weight, food intake, and water intake of the animals for a 28-day period of the experiment. Animals were used to assess their behavioral patterns through different behavioral assessment monitoring apparatus. On completion of 28-day experiment, animals will be sacrificed for the collection of blood and brain samples to assess the oxidative stress parameters, such as MDA, glutathione peroxidase (GPx), glutathione reductase (GR), SOD, and CAT, neurotransmitter levels, neurodegeneration, and other cellular infiltrations in the midbrain region.

Dose fixation of SC (100 mg/kg) has been obtained after the pilot study done in the starting stages of study design by testing 50 mg/kg, 100 mg/kg and 200 mg/kg in rotenone mice and the results have proven that 100 mg dose is more effective than 50 mg/kg and equally potent as 200 mg/kg dose.

Experimental design

1. Control group – Olive oil (0.1 mL, i.p.) for 10 days and dimethyl sulfoxide (DMSO) (P.O) for 28 days
2. Rotenone group – Rotenone (2.5 mg/kg i.p. 0.1 mL) for 10 days and DMSO (P.O) for 28 days
3. R+LD group (L-DOPA treatment group) – Rotenone (2.5 mg/kg i.p. 0.1 mL) for 10 days and L-DOPA (15 mg/kg) as a standard drug (P.O) for 28 days
4. R+SC group (SC treatment group) – Rotenone (2.5 mg/kg i.p. 0.1 mL) for 10 days and SC extract (100 mg/kg P.O, 0.1 mL) for 28 days
5. R+LD+SC (Combination treatment group) – Rotenone (2.5 mg/kg i.p. 0.1 mL) for 10 days and SC extract (100 mg/kg along with 50% reduced dose of L-DOPA 7.5 mg/kg, P.O, 0.1 mL) for 28 days.

Chemicals

Rotenone (>95% pure), L-DOPA was procured from Sigma-Aldrich company. SC seed kernel powder was identified and authenticated by the botanist Dr. Chelladurai, Tirunavelli, Tamil Nadu, India. DMSO from Pure Chemicals, and hydroxypropyl cellulose (HPC) from HIMEDIA (India). All other chemicals and reagents used were of analytical grade.

Preparation of rotenone, embelin, and L-DOPA stock solution

50 mg rotenone was weighed and dissolved in 1 mL of DMSO. From this, an aliquot of 0.2 mL was taken and diluted with 19.8 mL of olive oil. SC seed kernel powder extract and L-DOPA were weighed 15 mg separately and dissolved in 20 mL of 10% of HPC. All the drugs were administered according to the body weight of the animals [17].

Biochemical estimations were performed using standard protocols

MDA

Serum was mixed with 20% trichloroacetic acid and allowed to stand for 10 min. After that, 0.05 M H_2SO_4 and thiobarbituric acid were added. The mixture was mixed and placed in 70°C water baths for 30 min. The resulting chromogen was extracted with n-butanol and centrifuged at 2,000 rpm/min, and measured against a butanol blank at 532 nm excitation and 553 nm emissions by a spectrophotometer [18].

Estimation of GR

GR estimation based on the oxidation of nicotinamide adenine dinucleotide phosphate (NADPH) to NADP⁺ catalyzed by a limiting concentration of GR. One GR activity unit is defined as the amount of enzyme catalyzing the reduction of one micromole of GSSG per minute at pH 7.6 and 25°C. As shown in the above reaction, one molecule of NADPH is consumed for each molecule of GSSG reduced. Therefore, the reduction of GSSG is determined indirectly by the measurement of the consumption of NADPH, as demonstrated by a decrease in absorbance at 340 nm [19].

Estimation of GPx

GPx activity was determined by a modified method of Paglia and Valentine (1967) in both plasma and placental extracts. Briefly, 900 µL of assay mix containing 0.1 mol/L potassium phosphate, pH 7.0; 2 mmol/L ethylenediaminetetraacetic acid; 0.5 U/mL GR; 10 mmol/L glutathione and 0.3 mmol/L reduced NADPH was placed into a WPA Biotech spectrophotometer set at 340 nm. Diluted tissue extracts or plasma (50 µL of 1/10 dilution) were added to the cuvette along with 50 µL of 20 mmol/L tert-butyl hydroperoxide. The decrease in A340 was determined over a 3-min period, and rate calculations were performed. GPx activity was standardized against protein concentrations and expressed as mmol/min/mg protein or mmol/min/mL for plasma samples [19].

Estimation of SOD

SOD was assayed by the method of Hussain *et al.*, based on inhibition of the formation of nicotine amide adenine dinucleotide, phenazine methosulfate, and amino blue tetrazolium formazan. A single unit of enzyme was expressed as 50% inhibition of nitroblue tetrazolium reduction/min/mg protein [20].

Estimation of CAT

CAT was assayed colorimetrically at 620 nm and expressed as µmoles of H₂O₂ consumed/min/mg as described by Sinha (1972). The reaction mixture (1.5 mL, vol) contained 1.0 mL of 0.01 M phosphate buffer (pH 7.0), 0.1 mL of hemolysate, and 0.4 mL of 2 M H₂O₂. The reaction was stopped by the addition of 2.0 mL of dichromate-acetic acid reagent (5% potassium dichromate and glacial acetic acid were mixed in 1:3) [20].

Measurement of NO

Nitrite (NO₂⁻) and nitrate (NO₃⁻) are stable final products of NO metabolism and may be used as indirect markers for the presence of NO. The total NO concentration is commonly determined as a sum of the NO₂⁻ and NO₃⁻ concentrations. In the analyzed samples, was NO₃⁻ reduced to NO₂⁻ in the presence of cadmium and then converted to nitric acid; this process produces a colored product with Griess's reagent (Sigma-Aldrich, Stainheim, Germany). The NO₂⁻ concentration was determined by spectrophotometric analysis at 540 nm [21].

Measurement of brain urea

Brain tissues were immediately washed in ice-cold phosphate-buffered saline and weighed. A 10% (w/v) tissue homogenate was prepared in ice-cold 0.1 M phosphate buffer (pH 7.4). The post-nuclear fraction for the assay was obtained by centrifuging the homogenate at 1,000 rpm for 20 min, at 40°C. Tissue (20 mg) was homogenized in 100 µL urea assay buffer and then centrifuged at 15,000 g for 10 min to remove insoluble materials. In a 96-well plate, a 50 µL reaction mix containing urea assay buffer (42 µL), oxiRed probe (2 µL), enzyme mix (2 µL), developer (2 µL), and converter enzyme (2 µL) was added to each well containing urea standards and test samples. Fifty microliters of the sample control mix was added to the sample control well. The reaction mixture was mixed well and incubated for 60 min at 37°C, protected from light. The absorbance at 570 nm was measured using a spectrophotometer [22] (Elico, B-200).

Estimation of neurotransmitter levels

Dopamine, serotonin, and nor-epinephrine levels were analyzed by using the protocol as described in the enzyme-linked immunosorbent assay kit of E-Lab Biosciences (USA) [23].

Statistical analysis

Results are expressed as mean±standard error of the mean. One-way analysis of variance followed by Bonferroni multiple comparison *post hoc* test was used for comparison between the groups. *p*<0.001 was considered as statistically significant. SigmaPlot 13 (Systat, USA) was used for statistical analysis.

RESULTS

For the estimation of antioxidants, enzymes, the protocols were performed by using serum, neurotransmitters, urea, and NO levels were estimated by using tissue homogenate samples. Those estimated levels of various biochemical parameters were compared between controls, treatment, and Rotenone groups to analyze the statistical significance.

Fig. 1 and Table 1 show the mean±standard error (SE) levels of brain tissue homogenate MDA, GR, and GPx. For MDA, the rotenone group (18.45±1.8) showed significant elevation of MDA levels as compared to the control (6.533±0.65). In comparison with the rotenone group, the SC (9.16±1.94), L-DOPA (8.46 ±0.70), and SC+L-DOPA (6.36±0.60) groups showed a significant (*p*<0.005) decrease in the MDA levels. The GR levels of rotenone group (16.1±1.80) were significantly decreased as compared to the control (25.50±0.75). Whereas in SC, L-DOPA and SC+L-DOPA treatment groups, the levels of GR were found to be 19.53±1.13, 21.13±1.2 and 23.43±0.97, respectively, implicating protective effect with statistical significance (*p*<0.05). The GPx activity of Rotenone group (115.66±15.57) was markedly reduced (*p*-value)

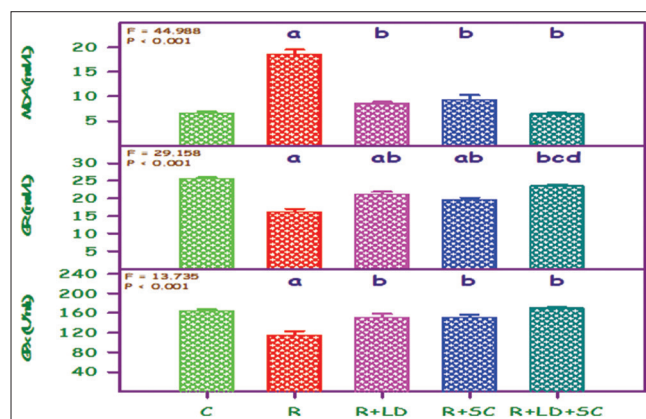


Fig. 1: Protective effect of L-DOPA (LD) *Syzygium cumini* (SC), and combination (LD+SC) in rotenone (R) induced Parkinson's disease model in mice. C: Control, MDA: Malondialdehyde, GR: Glutathione reductase, GPx: Glutathione peroxidase. The "F" and "p" values are by one-way analysis of variance with Student Newman-Keul's multiple comparison test. ^aSignificantly different from the control group. ^bSignificantly different from the rotenone group. ^cSignificantly different from the rotenone+L-DOPA group. ^dSignificantly different from rotenone+group SC

Table 1: Protective effect of SC and SC with L-DOPA combination therapy on rotenone induced biochemical changes in antioxidant enzyme levels in PD mice

Group	MDA (mol/L)	GR (mol/L)	GPx (U/mL)
Control	6.53±0.27	25.50±0.31	164.33±2.25
Rotenone	18.45±0.74	16.10±0.64	114.67±5.95
Rotenone+Levodopa	8.47±0.29	21.13±0.49	151.00±5.12
Rotenone+SC	9.17±0.79	19.53±0.46	151.00±3.89
100 mg/kg			
Rotenone+SC	6.37±0.25	23.43±0.40	170.00±1.63
100 mg/kg+Levodopa			

SC: *Syzygium cumini*, L-DOPA: Levodopa, PD: Parkinson's disease, MDA: Malondialdehyde, GR: glutathione reductase, GPx: Glutathione peroxidase

Table 2: Protective effect of SC and SC with L-DOPA combination therapy on rotenone induced biochemical changes in antioxidant enzyme levels in PD mice

Group	Catalase (U/mL)	SOD (U/mL)
Control	183.00±2.86	6.71±0.20
Rotenone	155.33±2.87	4.60±0.23
Rotenone+Levodopa	166.00±4.26	5.47±0.26
Rotenone+SC 100 mg/kg	165.33±6.11	5.40±0.22
Rotenone+SC 100 mg/kg+Levodopa	178.33±2.72	6.83±0.12

SC: *Syzygium cumini*, L-DOPA: Levodopa, PD: Parkinson's disease, SOD: Superoxide dismutase

Table 3: Protective effect of SC and SC with L-DOPA combination therapy on rotenone induced biochemical changes in Urea and Nitric oxide levels in PD mice

Group	Urea (nmol/mg protein)	Nitric oxide (nmol/mg protein)
Control	152.93±0.79	24.57±1.13
Rotenone	243.57±4.76	56.87±0.80
Rotenone+Levodopa	172.00±2.81	37.13±1.90
Rotenone+SC 100 mg/kg	197.97±1.45	47.87±1.14
Rotenone+SC 100 mg/kg+Levodopa	174.57±3.50	35.83±2.08

SC: *Syzygium cumini*, L-DOPA: Levodopa, PD: Parkinson's disease

Table 4: Protective effect of SC and SC with L-DOPA combination therapy on rotenone induced biochemical changes in Neurotransmitter levels in PD mice

Group	Dopamine (ng/mg protein)	Serotonin (ng/mg protein)	Norepinephrine (ng/mg protein)
Control	118.60±3.34	35.70±2.27	78.90±1.49
Rotenone	81.97±1.80	27.00±1.47	61.50±1.15
Rotenone+Levodopa	97.80±1.21	27.30±0.37	71.50±1.19
Rotenone+SC 100 mg/kg	92.63±1.50	26.73±0.31	71.90±0.96
Rotenone+SC 100 mg/kg+Levodopa	99.60±0.97	33.50±0.90	70.60±0.45

SC: *Syzygium cumini*, L-DOPA: Levodopa, PD: Parkinson's disease

when compared to control (165.33±5.50). However, SC (151±9.53), and L-DOPA (151±12.53) and SC+L-DOPA (170.00±5.00) treatment reverted those changes, and the values were found to be significant ($p < 0.001$). Total increase in the GPx levels showing protection in all three treatment groups. Overall, MDA and GPx activity revealed a similar trend in all the groups, implicating the maximum benefits of SC+L-DOPA combination therapy.

Fig. 2 and Table 2 show the mean±SE levels of brain tissue homogenate SOD and CAT activity. SOD levels in the PD mice group showed a marked decrease (5.59±0.57) as compared to the control (6.70±0.49). Whereas in SC, L-DOPA and SC+L-DOPA treatment groups, the values were 5.40±0.53, 5.47±0.64, and 6.83±0.3, respectively. While all the treatment groups increased the levels of SOD, the SC+L-DOPA group alone showed statistical significance when compared with the Rotenone group. The CAT activity in the PD mice group (155.33±7.02) was significantly decreased ($p < 0.05$) compared with the control (183±7). Whereas the drug-treated groups, such as SC (165.33±15.97), L-DOPA (166±10.44), and SC+L-DOPA (178.33±6.65), did not show a statistical difference when compared with the rotenone group.

Fig. 3 and Table 3 depicts the mean±SE levels of urea and NO content in the brain tissue homogenate samples. Urea levels were significantly ($p < 0.001$) elevated in the Rotenone group (243.56±11.65) compared

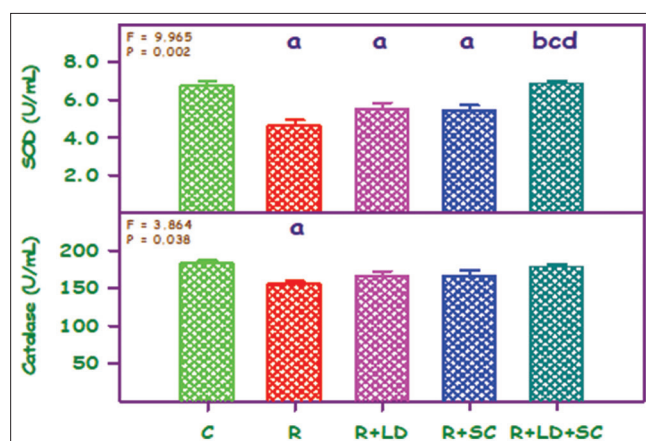


Fig. 2: Protective effect of L-DOPA (LD), *Syzygium cumini* (SC), and combination (LD+SC) in rotenone (R) induced Parkinson's disease model in mice. C: Control, SOD: Superoxide dismutase, Catalase. The "F" and "p" values are by one-way analysis of variance with Student Newman-Keul's multiple comparison test. ^aSignificantly different from the control group. ^bSignificantly different from the rotenone group. ^cSignificantly different from the rotenone+L-DOPA group. ^dSignificantly different from rotenone+SC group

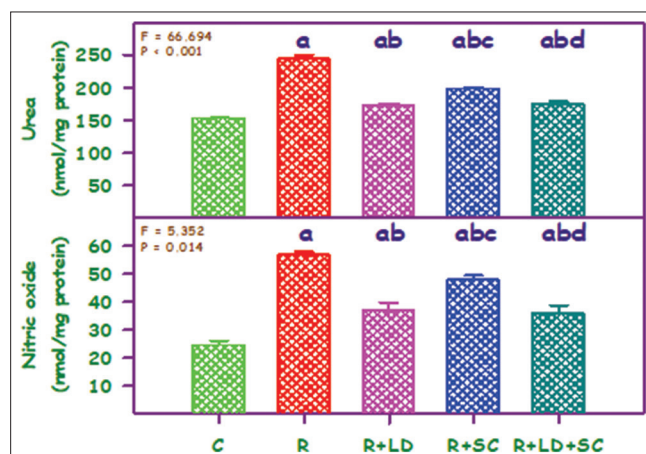


Fig. 3: Protective effect of L-DOPA (LD) *Syzygium cumini* (SC), and combination (LD+SC) in rotenone (R) induced Parkinson's disease model in mice. C: Control, urea, and nitric oxide. The "F" and "p" values are by one-way analysis of variance with Student Newman-Keul's multiple comparison test. ^aSignificantly different from the control group. ^bSignificantly different from the rotenone group. ^cSignificantly different from rotenone+L-DOPA group. ^dSignificantly different from rotenone+SC group

to the control group (152.93±1.94). The therapeutic intervention groups SC (197.96±3.55), L-DOPA (172±6.87), and SC+L-DOPA (175.56±8.56) revealed a significant ($p < 0.001$) decrease in the urea levels when compared with the Rotenone group. The levels of NO were significantly ($p < 0.001$) elevated in the rotenone group (56.86±1.96) compared to the control group (25.56±2.77). The treatment groups, SC (47.86±2.8), L-DOPA (37.13±5.65), and SC+L-DOPA (35.83±5.08) revealed a significant ($p < 0.001$) decrease in the NO levels when compared with the Rotenone group. The decreased urea and NO levels seen in all the drug-treated groups implicate the therapeutic potential of SC extract and SC+L-DOPA therapy. However, the SC+L-DOPA showed better protection as compared to SC only treatment. However, there was no significant difference between the L-DOPA alone group and the SC+L-DOPA group.

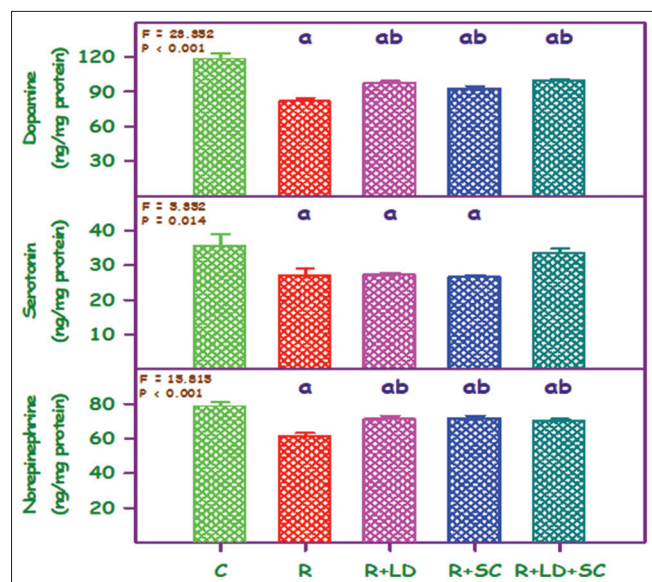


Fig. 4: Protective effect of L-DOPA (LD), *Syzygium cumini* (SC), and combination (LD+SC) in rotenone (R) induced Parkinson's disease model in mice. C: Control; DOPA: Dopamine, serotonin, norepinephrine. The "F" and "p" values are by one-way analysis of variance with Student Newman-Keul's multiple comparison test. ^aSignificantly different from the control group. ^bSignificantly different from the rotenone group. ^cSignificantly different from the rotenone+L-DOPA group. ^dSignificantly different from rotenone+SC group

Fig. 4 and Table 4 show the changes in serum neurotransmitter levels in the samples of various groups. Dopamine levels were significantly ($p < 0.001$) decreased in the Rotenone group (81.96 ± 8.19) compared to the control group (118.6 ± 8.19). The drug treatment groups SC (92.63 ± 3.68), L-DOPA (97.80 ± 2.96), and SC+L-DOPA (99.6 ± 2.34) showed significant protection ($p < 0.001$) by increasing the levels of dopamine as compared with the Rotenone group. The Rotenone group (27 ± 3.6) showed a significant ($p < 0.05$) decrease in serotonin levels when compared with the control (35.7 ± 5.65). The treatment groups SC, L-DOPA, and SC+L-DOPA revealed a significant ($p < 0.001$) increase in the serotonin levels with the values of 26.73 ± 0.76 , 27.30 ± 0.90 , and 33.5 ± 2.2 , respectively, when compared with the Rotenone group. Norepinephrine levels in the Rotenone group (61.5 ± 2.8) were also significantly decreased ($p < 0.001$) compared to the control (78.9 ± 3.65). After treatment with SC (71.9 ± 2.35), L-DOPA (71.5 ± 2.9), and SC+L-DOPA (97.06 ± 1.11), the values were significantly ($p < 0.001$) increased when compared with the Rotenone group.

DISCUSSION

In the present study using PD model mice, we demonstrate that SC extract and a combination of SC and L-DOPA therapy could be a viable therapeutic approach to attenuate oxidative stress and neurotransmitter changes in PD. To date, there are no proven drugs to overcome the toxicity and side effects of L-DOPA in PD. Therefore, we employed half of the standard dose of L-DOPA therapy along with the herbal SC extract to attenuate various biochemical parameters, including brain urea levels. Rotenone-induced PD led to a striking increase in the serum levels of MDA (marker of oxidative stress) with poor antioxidant status (GPx, GR, CAT and SOD). The free radical-associated changes, such as elevation of serum NO was also noticed along with increased brain urea levels [24]. However, treatment of SC and SC plus L-DOPA significantly alleviated those changes. Although L-DOPA standard therapy also revealed such changes, the effect of SC plus L-DOPA showed a better protective effect [25]. Rotenone is well known as an inhibitor of complex I enzymes in the mitochondrial respiratory system. Subsequent injections of rotenone can successfully produce a mice model of Parkinsonism with

degeneration of DA neurons and intracellular Lewy body formation [26]. Although rotenone inhibits mitochondrial respiratory enzyme activity throughout the brain, the selective loss of DA neurons in the substantia nigra and striatal region has attracted much attention. Regarding the possible mechanism, it has been explained that rotenone inhibits mitochondrial respiratory enzyme activity, leading to the production of ROS, which are rich in iron and melanin and damage nigral DA neurons sensitive to oxidative stress [27,28].

Rotenone is highly lipophilic and thus readily gains access to all organs, including the brain and accumulates in subcellular organelles, such as mitochondria to trigger oxidative stress. In mitochondria, rotenone impairs oxidative phosphorylation by inhibiting reduced nicotinamide adenine dinucleotide-ubiquinone reductase activity of the multi-polypeptide enzyme complex I of the electron transport chain [29]. The mechanism by which inhibition of complex I leads to degeneration of dopaminergic neurons, involves activation of mitochondria-dependent apoptotic molecular pathways. It has been reported that neurotoxins inducing Parkinsonian neuropathology, such as rotenone, stimulate superoxide production at complex I of the electron transport chain and stimulate free radical production at proximal redox sites, including mitochondrial matrix dehydrogenases [30,31]. This oxidative stress promotes the expression and/or intracellular distribution of proapoptotic proteins to the mitochondrial outer membrane.

Oxidative stress plays an important role in causing the death of dopamine neurons. In addition, biochemical analyses of postmortem PD brains show enhancements of oxidative stress due to perturbations of the mitochondrial electron transport chain. This may produce an imbalance between the toxic oxidant and the cellular antioxidant defense system [32]. The toxic oxidants are ROS, such as MDA, GR, NO, and other oxidative markers. The endogenous cellular antioxidant defense system comprises of enzymes (SOD and CAT) and GPx. The decrease in the activities of endogenous antioxidant defense systems, such as GPx, SOD, and CAT, has been well documented in PD brains. In the present study, SC and SC plus L-DOPA therapy significantly reduced the rotenone toxicity by restoring the levels/activity of various biochemical enzymes and neuromodulators, implicating those herbal drugs hold potential as an adjuvant therapy for PD [33].

L-DOPA is a well-known standard treatment for PD. L-DOPA treatment in PD is related to decreased levels of the neurotransmitter (DA) in the striatum and the absence of DA transporters on the nerve terminals in the brain. Chronic use of oral L-DOPA is associated with response fluctuations, partially due to the slow rate of gastric emptying. Delayed gastric emptying in PD patients delays the absorption of L-DOPA, leading to lower blood plasma concentrations of the drug and the occurrence of on-off oscillations [34]. These motor fluctuations, together with dyskinesias, are considered as the major side effects of long-term treatment with L-DOPA. Strategies allowing for reducing the dose of L-DOPA could potentially reduce the risk of developing such side effects. Herbal drugs, if used as an adjuvant therapy, may ameliorate the L-DOPA toxicity and associated complications [35].

There are studies which have explored the antioxidant and neuroprotective role of individual phytochemicals, such as quercetin, gallic acid, EA, corilagin, and kaempferol in animal models [36]. However, these phytochemicals are abundantly present in the seed kernel extract of SC. Zhu *et al.*, 2013 explored the molecular mechanism of quercetin-mediated inhibition of α -synuclein fibrillation by various biochemical and biophysical approaches. Quercetin binds covalently with α -synuclein in 1:1 ratio. The formed quercetin- α -synuclein adducts attach to the α -synuclein oligomers or monomers, increasing the surface hydrophilicity and inhibiting further fibrillation. Oxidized by dissolving oxygen, quercetin forms chalcone, benzofuranone, quercetinquinone, and other products [37]. These products react with α -synuclein and elevate polarity and hydrophilicity, making the inhibition more effective. The preformed α -synuclein fibrils may also be disaggregated through the protein/quercetin interaction and

detached into stable oligomers [37]. However, α -synuclein oligomers and amorphous aggregates cannot be further separated by quercetin. Because quercetin can pass through the blood-brain barrier, this inhibition and disaggregation function may imply quercetin as a potential drug for PD [15]. Oxidative stress and inflammation are interrelated pathological events. Inappropriate glial activation contributes to pathological outcomes, such as neurodegenerative disease. Corilagin suppressed the transcription of pro-inflammatory cytokine genes through effects on the DSB-triggered nuclear factor kappa B signaling pathway. Microglia cells play a critical role in the development of an inflammatory response within the brain. Microglia cells are activated in response to ionizing radiation, resulting in the induction of pro-inflammatory cytokines. Pre-treatment of the microglia cells with corilagin inhibited proinflammatory responses and advised for the use of these compounds in brain injury [38]. In a study carried out by Radwan *et al.*, 2023 explained about EA potential effect against PD. As a naturally occurring compound, EA also has various beneficial biological effects on different organ systems through the regulation of oxidative stress and inflammation. Thus, EA may represent a more reasonable natural agent that has potential therapeutic value [39]. Apart from these, there are several studies showing that the antioxidant and anti-inflammatory nature of phytochemicals present in SC seed kernel extract. In the present study, there is a significant lowering of oxidative stress by their biological active phytochemicals of SC seed kernel has been proved for neuroprotection against rotenone-induced PD mice.

CONCLUSION

In our study, it has been proven that combination treatments are a promising approach for diseases, such as PD. Reduced dose of L-DOPA along with SC seed kernel extract has a great therapeutic approach without much side effects, as well as a cost-effective treatment for PD symptoms. Further research should focus on elucidating the precise molecular mechanisms by which these phytochemicals interact synergistically to achieve neuroprotection and to translate these findings into human clinical trials to validate their efficacy and safety in PD patients.

AUTHORS' CONTRIBUTION

All the authors have equal contribution.

CONFLICT OF INTEREST

The authors declare there is no conflict of interest.

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