

***Sarcocephalus latifolius* Ethanol Leaf Extract Attenuates Rotenone-Induced Behavioural Deficits, Oxidative Stress and Neuroinflammation in a Rat Model of Parkinsonism**

Akinyinka Oyedolapo Alabi^{1*}, Stella Afolakemi Adeyemo^{1,2}, Paul A. Adeleke², Joseph Chimezie², Ibronke Oluwafunmilayo Oyenuga², Luqman Opeoluwa Ogunjimi¹, Abdullah Akanji Muritala¹, Elijah Oladapo Oyinloye³, Abayomi Mayowa Ajayi².

¹Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria

²Department of Pharmacology and Therapeutics, Faculty of Basic Medical Sciences, University of Ibadan, Ibadan, Oyo State, Nigeria.

³Department of Pharmacology and Toxicology, Faculty of Pharmacy, Olabisi Onabanjo University, Sagamu, Ogun State, Nigeria

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*Corresponding Author:

Dr. Akinyinka Oyedolapo Alabi
Email: alabi.akinyinka@oouagoiwoye.edu.ng
Tel: +234 706 505 9677

ABSTRACT

Background and Aim: *Sarcocephalus latifolius* (Sm.) E.A. Bruce is a medicinal plant with recognized antimicrobial, antioxidant, anti-inflammatory, and antidiabetic properties. This study evaluated the protective effects of *Sarcocephalus latifolius* ethanol leaf extract (SLEE) against rotenone-induced neurotoxicity, oxidative stress, and neuroinflammation in rat brain regions.

Materials and methods: Male Wistar rats were divided into four groups (n = 5). Group 1 received control treatment (1% ethanol in sunflower oil, i.p.) for 28 days, while group 2 was administered rotenone (2.5 mg/kg, i.p.) on 14 alternate days during the 28-day experimental period. Group 3 received SLEE (400 mg/kg, p.o.) alongside rotenone (2.5 mg/kg, i.p.), and group 4 received Levodopa/Carbidopa (25 mg/kg, p.o.) plus rotenone (2.5 mg/kg, i.p.) for 28 days. Behavioural assessments including catalepsy, negative geotaxis, Y-maze, elevated plus maze, and sucrose splash tests were conducted on day 29. Biochemical assessments of MDA, nitrite, GSH, SOD, GST, proinflammatory cytokines (TNF- α and IL-6), AChE activity, and dopamine concentration were performed in the prefrontal cortex (PFC), striatum (STR), cerebellum (CER), and hippocampus (HPC).

Results: Rotenone significantly increased catalepsy duration, negative geotaxis latency, oxidative stress markers (MDA and nitrite), AChE activity, and pro-inflammatory cytokines (TNF- α and IL-6), while reducing Y-maze alternation, grooming duration, antioxidant status (GSH, SOD, GST, and CAT), and dopamine levels compared with vehicle controls ($p < 0.05$). It also impaired elevated plus maze performance by reducing open arm exploration ($p < 0.05$). SLEE treatment significantly improved negative geotaxis performance [$F(3,16)=39.10$, $p<0.0001$] and spontaneous alternation [$F(3,16)=37.98$, $p<0.0001$] compared with rotenone-treated rats, although effects on catalepsy, grooming duration and open arm behaviour were not significant. SLEE significantly attenuated rotenone-induced alterations compared with the rotenone group, with significant treatment effects observed for oxidative stress markers ($p<0.05$), dopamine concentration [$F(3,48)=497.3$, $p<0.0001$], AChE activity [$F(3,64)=43.10$, $p<0.0001$], TNF- α [$F(3,48)=124.7$, $p<0.0001$], and IL-6 [$F(3,48)=50.53$, $p<0.0001$].

Conclusion: SLEE treatment demonstrated a potential to reverse oxidative stress and modulate neuroinflammation induced by rotenone. These effects on antioxidant enzyme activities and proinflammatory cytokines suggest that SLEE may offer ameliorative benefits in neurodegenerative conditions.

1.0: INTRODUCTION

Parkinson's disease (PD) is a chronic, progressive neurodegenerative disorder that affects the elderly population. It is the second most prevalent neurodegenerative disorder following Alzheimer's disease, affecting around 10 million people globally^[1]. Its prevalence increases with age, affecting 1% of individuals aged 60–64 and 4% of those aged 85 and above^[2]. As a result, PD significantly contributes to the societal and economic challenges posed by aging populations globally. Clinically, PD is defined by motor symptoms including bradykinesia, resting tremor, stiffness, and postural instability, alongside non-motor symptoms such as cognitive impairment, melancholy, anxiety, dysphagia, hallucinations, sleep problems, and olfactory dysfunction^[3]. These manifestations collectively contribute to the overall disease burden and diminish the quality of life for affected individuals. PD is characterized neuropathologically by the progressive degeneration of dopaminergic neurons in the substantia nigra pars compacta (SNpc), resulting in diminished dopamine levels in the striatum, caudate nucleus, and putamen^[4]. This depletion affects the circuitry of the basal ganglia, leading to the characteristic movement abnormalities seen in patients with PD^[5]. In addition to neuronal loss, the accumulation of misfolded α -synuclein aggregates, known as Lewy bodies and Lewy neurites, is a defining feature of the disease^[6].

Despite comprehensive research, the exact etiology of PD continues to be unclear. PD development is believed to result from a combination of genetic and environmental factors, with familial cases constituting about 10% of all occurrences^[7]. Multiple pathogenic pathways have been proposed in Parkinson's disease, including oxidative stress, dopaminergic dysfunction, mitochondrial dysfunction, neuroinflammation, and apoptosis^[8]. Oxidative stress and mitochondrial dysfunction are considered to be of utmost importance to the neurodegenerative processes associated with PD. In support of this idea, several research have demonstrated that mitochondrial complex I inhibitors, such as rotenone, induce Parkinson's disease-like pathologies by impairing the electron transport chain, resulting in ATP deficiency and increased production of reactive oxygen species (ROS)^[9]. The excessive generation of ROS, intensified by the actions of enzymes such as monoamine oxidase and tyrosine hydroxylase, renders dopaminergic neurons especially susceptible to oxidative injury^[10]. Compounding this process is a marked reduction in endogenous antioxidant defenses, including decreased levels of glutathione, and enzymatic antioxidants such as catalase, and superoxide dismutase^[11]. Moreover, the

activation of microglia, the overexpression of inflammatory mediators, such as tumor necrosis factor- α , interleukin-1 β , inducible nitric oxide synthase, and diminished glutathione in the SNpc intensify oxidative damage^[12] thus, establishing a self-reinforcing cycle of neuronal injury and degeneration.

Current treatments for PD remain primarily focused on symptomatic management rather than modifying or halting disease progression. Dopaminergic therapy serves as the cornerstone of treatment, with levodopa (L-3,4-dihydroxyphenylalanine) being the gold standard^[13]. Levodopa, often combined with decarboxylase inhibitors alleviates motor symptoms by increasing dopamine levels in the striatum^[14]. However, the long-term efficacy of levodopa therapy diminishes due to disease progression, leading to complications such as motor fluctuations and dyskinesias^[15]. Dopamine agonists alongside monoamine oxidase-B (MAO-B) inhibitors and catechol-O-methyltransferase (COMT) inhibitors, are commonly employed as adjuncts to levodopa therapy to enhance its efficacy or extend dopamine activity. Although these treatments effectively alleviate motor symptoms, their long-term use is often accompanied by severe adverse effects, including impulse control disorders, dopamine dysregulation syndrome, and cognitive impairments, which complicate disease management^[16]. Deep Brain Stimulation, stem cell, gene therapies, adenosine A2A receptor antagonists, antioxidants and complementary therapies such as yoga, acupuncture, and Tai Chi may have the potential to improve patient outcomes due to their perceived safety and benefit in managing PD symptoms, however, their long-term efficacy is limited^[17]. Despite these advances, the need for neuroprotective therapies that can alter PD progression by targeting multiple underlying pathophysiological mechanisms remains a critical area of research.

Sarcocephalus latifolius (Sm.) E.A. Bruce, commonly known as African peach, is a flowering plant of the Rubiaceae family, widely distributed across Africa and Asia^[18]. Ecologically, it is typically found in forested areas, often in association with other tropical plants^[19]. *Sarcocephalus latifolius* was formerly known as *Nauclea latifolia*. *Sarcocephalus latifolius* is a widely used traditional remedy for conditions such as malaria and diabetes, toothache, dental caries, septic mouth, diarrhea, dysentery, hypertension, and infections, tumors and liver-related disorders^[20,21,22].

Numerous bioactive chemicals are found in different parts of *Sarcocephalus latifolius*, according to phytochemical investigations. The leaves and stem bark contain decanoic acid, benzene carboxylic acid, and terpenes^[23]. The root

bark and leaves contain 10-Hydroxystictosamide, which shows potent α -amylase inhibitory properties^[24]. The bark extract demonstrates strong antioxidant activity attributed to compounds like vitamin E and caffeic acid^[25]. Additionally, its fruits contain essential nutrients such as copper, iron, cobalt, calcium, magnesium, zinc, phosphorus, and vitamins A, B1, B2, C, and E^[26]. Different parts of *Sarcocephalus latifolius*, including the roots, root bark, stem bark, and leaves, have been reported to contain diverse phytoconstituents, including indole and indole-quinolizidine alkaloids such as nauclefine and nauclefine, triterpenoid glycosides, sterols, saponins, tannins, and flavonoids^[18,27,28]. Pharmacological studies have demonstrated diverse effects of *Sarcocephalus latifolius*, including antimicrobial^[29], aphrodisiac^[30], antioxidant^[31], hepatoprotective^[32], anti-inflammatory^[33], antidiabetic^[34], antinociceptive, antipyretic, anxiolytic, and anticonvulsant activities^[35]. Root bark extracts of *Sarcocephalus latifolius* have demonstrated cytotoxic and growth-inhibitory effects in tadpole and seed-radicle bioassays, suggesting the presence of biologically active constituents with potential cellular effects^[36]. Despite the extensive pharmacological activities reported for *Sarcocephalus latifolius*, research on its neuroprotective potential remains limited. This study, therefore, aimed to explore the ameliorative effects of *Sarcocephalus latifolius* on neurobehavioral impairments and biochemical changes in a rotenone-induced Parkinson's disease rat model.

2.0: MATERIALS AND METHODS

2.1: Plant Materials

Fresh leaves of *Sarcocephalus latifolius* were collected from Ikenne-Ilishan Road, Ogun State, Nigeria (approximately 6.8813° N, 3.7022° E), in June 2025, coinciding with the onset of the rainy season. The plant was subsequently identified and authenticated at the Department of Pharmacognosy, Faculty of Pharmacy, Olabisi Onabanjo University, Nigeria, with the reference number PHS/OOU/436.

2.2: Preparation of *Sarcocephalus latifolius* Leaves Ethanol Extract (SLEE)

The leaves of *Sarcocephalus latifolius* were dried, pulverized into powder, and subjected to maceration. Approximately 1500 g of the powdered leaves was extracted with 5 L of 70% ethanol (ethanol:water, 70:30 v/v) for 72 hours, yielding a solvent-to-material ratio of 3.33 mL/g. The extract was filtered, and the filtrate was concentrated using a rotary evaporator at 40°C. The concentrated crude extract was further dried to constant weight in an oven and subsequently ground using a

grinding machine to obtain a dry extract powder, yielding 225 g of crude ethanol extract, giving a percentage extraction yield of 15% w/w (weight of dried extract relative to the initial weight of powdered plant material $\times$ 100). The extract powder was stored under refrigerated conditions (4°C) prior to use. For animal administration, 400 mg of SLEE was dissolved in 10 mL distilled water to obtain a working concentration of 40 mg/mL. The extract was administered at a dose of 400 mg/kg body weight, with the dosing volume adjusted according to each animal's body weight (equivalent to 10 mL/kg).

2.3: GC-MS: Phytochemical analysis of volatile constituents using Gas-Chromatography Mass Spectrophotometry

The chemical composition of fatty acids present in *S. latifolius* ethanol extract was analyzed using gas chromatography-mass spectrometry (GC-MS) (Agilent Technologies system) equipped with a 5975C inert mass spectrometer and an electron impact ionization source. Volatile constituents were separated using an HP-5 capillary column coated with 5% phenyl methyl siloxane. Helium served as the carrier gas at a constant flow rate of 1.573 mL/min and a pressure of 1.9514 psi. The temperature was programmed to increase from 225°C to 260°C throughout the analysis. The mass spectrophotometer operated in electron-impact ionization mode at 70 eV. Compound identification was performed by scanning and comparing mass spectral data with the NIST 14 Mass Spectral Library. The GC-MS data were quantified as relative percentages using the total sum normalization method (Noonan et al., 2018). Identified compounds were verified and compared with synonyms using online literature, specifically the PubChem database from the National Library of Medicine <https://pubchem.ncbi.nlm.nih.gov/compound/>.

$$\% \text{inhibition} = [(Ac - At) / Ac] * 100$$

Where Ac – Control reaction absorbance, At – test compound absorbance

2.4: Experimental Animals

Male Wistar rats (150 g –200 g) were obtained from the laboratory animal facility at the University of Ibadan. The animals were housed in standard cages under controlled environmental conditions of temperature (20–24°C) and relative humidity (50–60%), with a 12-hour light/dark cycle, and fed standard pelletized chow from Vital Feeds Ltd. (Jos, Nigeria) with water provided ad libitum. Animals were acclimated for one week in the Pharmacology laboratory before experiments. All experimental

procedures were conducted in strict accordance with national and institutional guidelines for the care and use of laboratory animals, adhering to the principles of the 3Rs (Replacement, Reduction, and Refinement). The study protocol was reviewed and approved by the College of Medicine, University of Lagos Animal Care and Use Research Ethics Committee (CMUL/ACUREC), with ethical approval number CMUL/ACUREC/08/25/2131. All procedures complied with the WMA Statement on Animal Use in Biomedical Research.

2.5: Acute toxicity study.

Male Swiss mice, with a body weight range of 18 g to 22 g were selected to assess the acute toxicity of the ethanol extract of *Sarcocephalus latifolius* leaves (SLEE) in accordance with the OECD 423 guidelines. The mice were divided into two groups, each consisting of three animals. Group 1 received a vehicle (distilled water) at a dose of 10 mL/kg, while Group 2 was administered 2000 mg/kg of the ethanol extract. The mice were carefully monitored for signs of acute toxicity during the first four hours post-treatment, followed by daily observations for 14 days to detect any delayed toxic effects.

2.6: Experimental Design and Treatment

Following a 14-day acclimatization period in the laboratory animal facility, twenty male (20) Wistar rats ($n = 5$ per group) were randomly assigned into four experimental groups and individually marked for identification. Group I served as the control group and received vehicle treatment of 1% ethanol in sunflower oil (10 mL/kg, i.p.). Group II served as the rotenone-induced Parkinsonian group and received rotenone (2.5 mg/kg, i.p.) prepared in sunflower oil^[37]. Group III served as the SLEE-treated group and received *Sarcocephalus latifolius* ethanol extract (SLEE, 400 mg/kg, p.o.) followed by rotenone administration. Group IV served as the positive control group and received Levodopa/Carbidopa (25 mg/kg, p.o.) followed by rotenone administration. Rotenone was freshly prepared by dissolving rotenone in DMSO to prepare a stock solution, which was subsequently diluted in sunflower oil prior to intraperitoneal administration at a dose of 2.5 mg/kg every 48 hours for 28 days. On rotenone administration days, SLEE and Levodopa/Carbidopa were administered 1 hour before rotenone injection, while SLEE and Levodopa/Carbidopa were administered daily throughout the 28-day treatment period. Following completion of the treatment regimen, behavioural assessments were performed, including the catalepsy test, negative geotaxis test, Y-maze test, elevated plus maze test, and sucrose splash test.

2.7.0: Behavioral Assessments

Behavioral assessments were conducted on day 29, 24 hours after the final treatment administration. All tests were performed in the morning (09:00 h–12:00 h) in the following sequence: catalepsy test to assess motor rigidity, negative geotaxis test to evaluate motor coordination, Y-maze test to assess spatial working memory, elevated plus maze test to evaluate anxiety-like behaviour, and sucrose splash test to assess grooming behaviour. Each test was completed before proceeding to the next, with a 5-minute rest interval between consecutive assessments. Apparatuses were cleaned with 70% ethanol between sessions to prevent bias from olfactory cues.

2.7.1: Catalepsy Test

Cataleptic rigidity and immobility were assessed by gently placing both forepaws of each rat on a horizontal bar elevated 9 cm from the floor. The time taken for the animal to move at least one paw was recorded (maximum cutoff time = 2mins).

2.7.2: Negative Geotaxis Test

Reaction to gravity and thigmotactic tendencies (wall hugging) was evaluated using Geotaxis test apparatus, which is a wooden inclined platform. Each rat was placed facing downward on a 45° inclined plane. The time required to turn 180° and face upward was recorded as a measure of vestibular and motor coordination.

2.7.3: Y-Maze Test

Cognitive function, particularly the spatial working memory was evaluated using a Y-shaped maze with three identical arms (40 × 10 × 12 cm). Each rat was placed at the center and allowed to explore the arms freely for 5 min. Spontaneous alternation percentage (SAP) was calculated as: $SAP = \frac{\text{Number of triads with entries into all three arms}}{\text{Total number of arm entries} - 2} \times 100$

2.7.4: Elevated Plus Maze (EPM)

Anxiety-like behavior was evaluated using an elevated plus-shaped maze with two opposed-open and two opposed-closed arms (10 × 40 × 50cm) with each arm facing the central platform. Each rat was placed at the center of the maze facing an open arm and allowed to explore for 5 min. The time spent and also the number of entries into open versus closed arms were recorded.

2.7.5: Sucrose Splash Test (SST)

Grooming behavior was evaluated using a sucrose splash test. A 10% sucrose solution was sprayed onto the dorsal coat of each rat, followed by a 2-minute accommodation

period, after which total grooming time was recorded over a 4-minute observation window. Reduced grooming time was interpreted as a proxy for diminished self-care and motivational drive.

2.8.0: Biochemical Assays

Twenty-four hours after completion of the behavioural assessments on day 29, the animals were anesthetized with ether and sacrificed by decapitation for biochemical analyses. The prefrontal cortex, cerebellum, striatum, and hippocampus were rapidly dissected on ice to preserve tissue integrity. Each region was homogenized at 10% (w/v) in cold phosphate buffer (0.1 M, pH 7.4) and centrifuged at 10,000 rpm for 10 minutes at 4 °C. The resulting supernatants were carefully collected, divided into smaller portions, and stored at -20 °C for subsequent biochemical analyses. For colorimetric assays (MDA, nitrite, GSH, SOD, GST), all five animals per group (n = 5) were analyzed. For ELISA-based assays (TNF- α , IL-6, dopamine), four animals per group (n = 4) were analyzed.

2.8.1: Oxidative Stress Markers

Reduced glutathione (GSH) levels in brain tissues were determined using Ellman's reagent. Brain supernatants were deproteinized with trichloroacetic acid, centrifuged, and the resulting supernatant was reacted with 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), and absorbance measured at 405 nm using a microplate reader. Lipid peroxidation was assessed as malondialdehyde (MDA) at 532 nm according to the thiobarbituric acid-reactive substances (TBARS) assay described by Nagababu et al^[38]. Brain nitrite levels, a marker of nitrenergic stress, were determined by mixing equal volumes of the supernatant with Griess reagent [1% sulfanilamide in 5% phosphoric acid and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride (NED)], with absorbance measured at 540 nm^[39]. Catalase activity in the supernatant was measured using a colorimetric assay based on the formation of a yellow complex with molybdate and H₂O₂^[40]. Superoxide dismutase (SOD) activity was evaluated using the adrenaline auto-oxidation method^[41] while glutathione-S-transferase (GST) activity was monitored kinetically as described by Habig et al^[42].

2.8.2: Pro-Inflammatory Cytokines

Pro-inflammatory cytokines TNF- α and IL-6 were quantified in brain region supernatants using rat-specific ELISA kits (Biolegend, USA) according to the manufacturer's protocol. Absorbance was read at 450 nm using a microplate reader, and concentrations were expressed in pg/mg protein. All the measurements were

carried out at room temperature. The concentration of TNF- α and IL-6 from the tissues were extrapolated from the standard curve in the assay kit.

2.8.3: Dopamine Estimation

Dopamine levels in the prefrontal cortex, cerebellum, striatum, and hippocampus were determined using a competitive ELISA kit (Elabscience, China). Tissue samples and standards were processed according to the manufacturer's instructions, and absorbance was measured at 450 nm. Dopamine concentrations were expressed as pg/mg protein.

2.9: Statistical Analysis

Statistical analyses were performed using GraphPad Prism version 10. All data were expressed as mean $\pm$ standard error of the mean (SEM). Comparisons involving a single experimental factor (treatment groups) were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's multiple comparisons test. Biochemical parameters assessed across different brain regions were analyzed using two-way ANOVA with treatment and brain region as factors, including the treatment $\times$ brain region interaction, followed by Tukey's multiple comparisons test. Statistical significance was set at $p < 0.05$.

3.0: Result

3.1: GC-MS Analysis

GC-MS analysis of *Sarcocephalus latifolius* leaf extract identified several compounds, with Benzoic acid, methyl ester (7.63%) and Cyclopentanetridecanoic acid, methyl ester (4.80%) present in the highest concentrations. Other compounds detected in smaller quantities included 2-Hydroxy-3-thiophen-2-ylmethyl-5-methoxy-1,4-benzoquinone and 6,8-Dichloro-2-trifluoromethyl-4-quinolinol (Table 1, Figure 18).

Volatile constituents in *S. latifolius* extract

Retention Time (min)	Compound Name	Area (%)
4.764	25H-Furanone, 3-phenoxy-carbonylamino-4-	0.27
5.210	Benzoic acid, methyl ester	7.63
6.291	2-Hydroxy-3-thiophen-2-ylmethyl-5-methoxy-1,4-benzoquinone	1.07
6.463	3,5,6-Trimethyl-p-quinone, 2-2,5-dioxotetrahydrofuran-3-ylthio-	0.47
8.512	6,8-Dichloro-2-trifluoromethyl-4-quinolinol	1.95
8.655	1-2-Adamantylidenesemicarbazide	0.35
9.055	Phenol, 4-2-amino-5-nitrophenyliminomethyl-2-methoxy-	0.48
9.130	3-Methyl-2-nitrophenol	0.28
10.194	Phenol, 2,5-bis(1,1-dimethylethyl)-	2.92
10.675	2-Thiazolylazo-p-cresol	0.71
11.796	2,6-Diisopropyl-naphthalene	2.38
14.079	Cyclopentanetridecanoic acid, methyl ester	4.80
15.550	2,4,5-Trichlorophenyl propenoate	2.17
16.013	1-Dodecanol, 2-hexyl-	2.27

Table 1: GC-MS chromatogram of ethanol extract of *Sarcocephalus latifolius* leaves, illustrating the retention times and peak areas of identified compounds, along with their proposed biological activities.

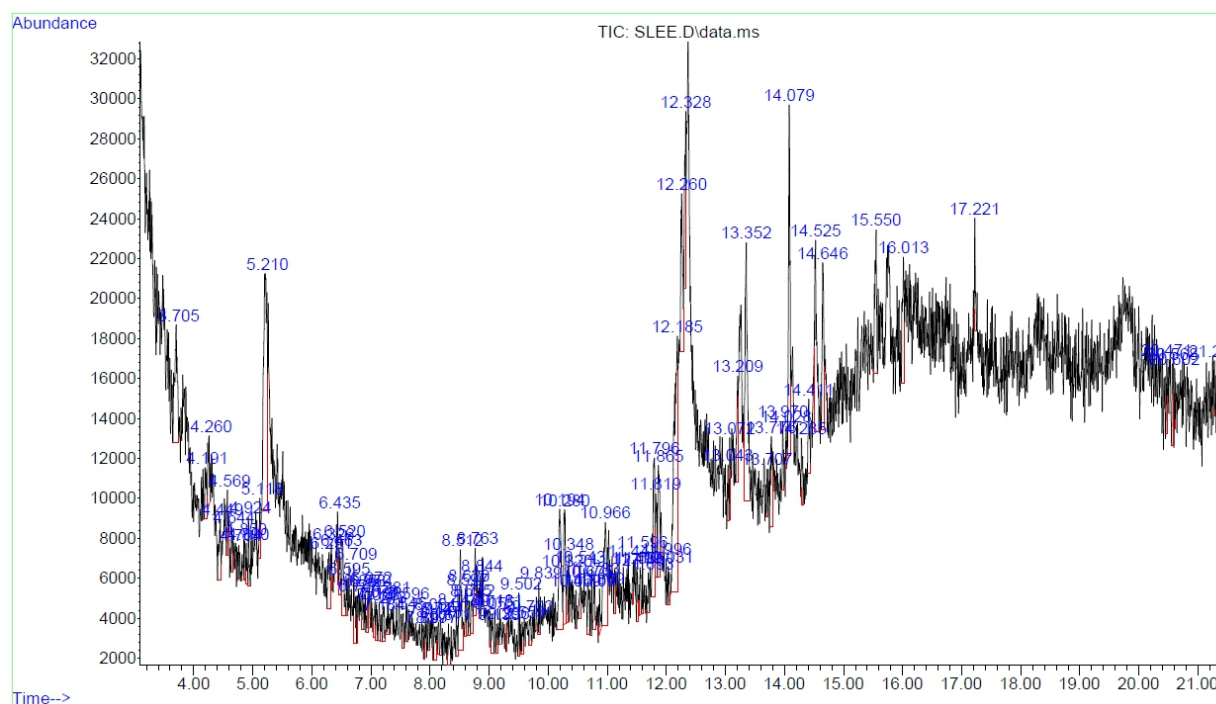


Table 2: Acute Oral Toxicity Assessment of SLEE (OECD 423)

Group	Dose (mg/kg)	Animals (n)	Signs of toxicity			Mortality
			Seizures	Coma	Agitation	
Control (Distilled water)	10mL/kg	3	Absent	Absent	Absent	0/3
SLEE-treated	2000	3	Absent	Absent	Absent	0/3

3.3: Effect of *Sarcocephalus latifolius* Ethanol Extract on Motor Coordination in Rotenone-Induced Parkinsonian Rats

Fig. 1 illustrates the effects of *Sarcocephalus latifolius* ethanol extract (SLEE) on catalepsy latency in a rotenone-induced model of Parkinsonism. Rats in the rotenone-treated (negative control) group exhibited a significant increase in catalepsy latency (132.50 ± 17.64 s) compared with the vehicle control group (4.90 ± 0.40 s), indicating increased motor rigidity following rotenone administration ($p < 0.0001$). Treatment with SLEE (400 mg/kg) reduced catalepsy latency (93.00 ± 16.95 s) compared with the rotenone group; however, this reduction was not statistically significant ($p = 0.1568$). In contrast, the Levodopa/Carbidopa-treated group showed a marked reduction in catalepsy latency (24.00 ± 5.25 s) compared with the rotenone group ($p < 0.0001$). Furthermore, Levodopa/Carbidopa treatment produced a significantly lower catalepsy latency compared with SLEE treatment ($p = 0.0063$), indicating a greater improvement in motor rigidity. One-way ANOVA revealed a significant difference among the treatment groups [$F(3,16) = 22.62, p < 0.0001$].

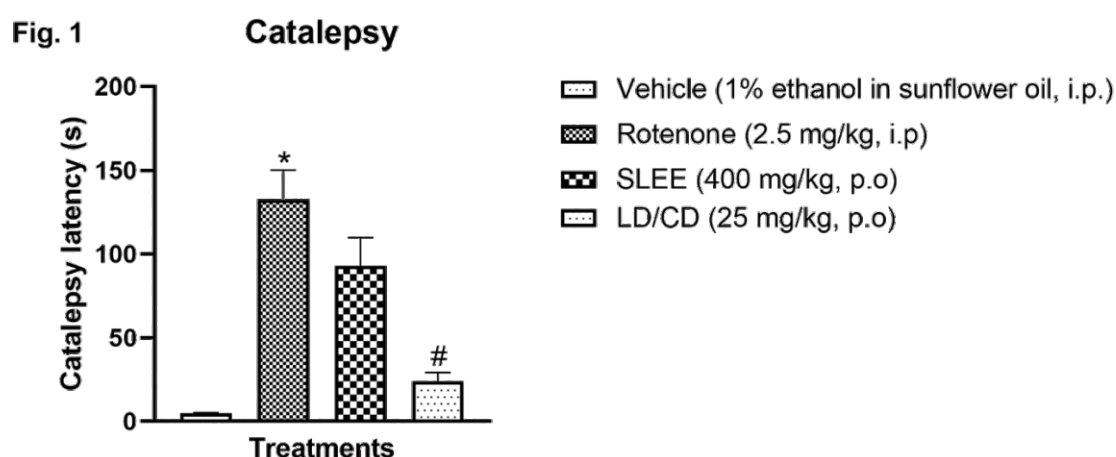


Figure 1: Effect of *Sarcocephalus latifolius* leaves ethanol extract on the catalepsy test in rotenone-induced Parkinsonian rats.

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using one-way ANOVA, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

As regards the negative geotaxis performance test (fig. 2), administration of rotenone produced a significant increase ($p < 0.0001$) in the time taken to reorient on the inclined plane (49.50 ± 5.49 s) when compared with the vehicle control group (3.11 ± 0.29 s), indicating impaired postural reflex and motor coordination. Treatment with *Sarcocephalus latifolius* ethanol extract (SLEE, 400 mg/kg) significantly reduced the reorientation time (20.17 ± 1.49 s) ($p < 0.0001$) relative to the rotenone group, suggesting an improvement in balance and motor performance. Rats treated with Levodopa/Carbidopa (12.44 ± 2.94 s) showed a more pronounced reduction, exhibiting near-normal reflex recovery comparable to the control group ($p < 0.0001$). Statistical analysis by one-way ANOVA confirmed a significant difference among the groups [$F(3, 16) = 39.10, p < 0.0001$].

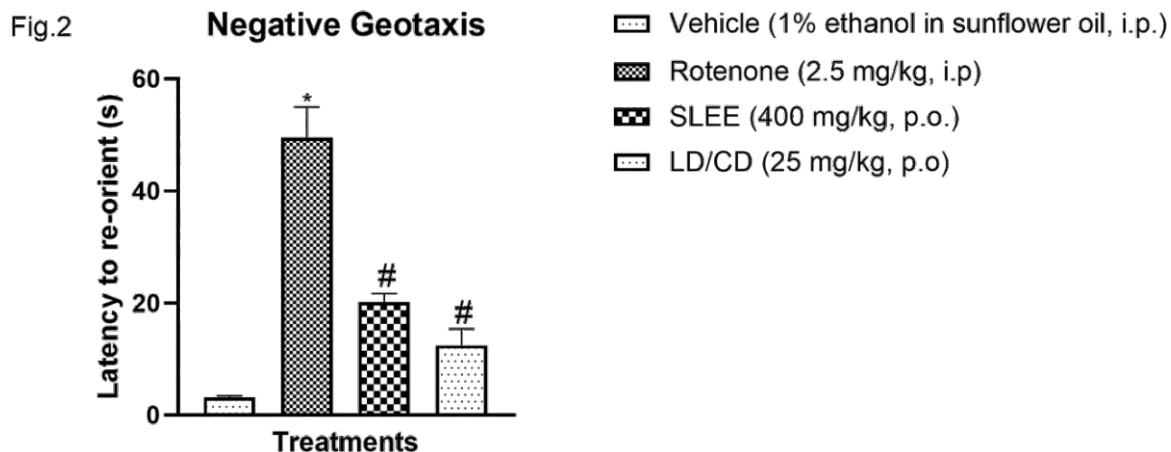


Figure 2: Effect of *Sarcocephalus latifolius* leaves ethanol extract on negative geotaxis test in rotenone-induced Parkinsonian rats.

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using one-way ANOVA, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

3.4: Effect of *Sarcocephalus latifolius* Ethanol Extract on Spatial Working Memory in Rotenone-Treated Rats (Y-Maze Test)

Fig. 3 presents the effect of *Sarcocephalus latifolius* ethanol extract (SLEE) on total arm entries in the Y-maze test following rotenone administration. The rotenone-treated group showed a higher number of total arm entries (9.00 ± 0.32) compared with the vehicle control group (6.60 ± 0.51), with a significant difference observed between both groups ($p = 0.0145$). SLEE treatment (400 mg/kg) significantly reduced the number of total arm entries (6.50 ± 0.67) compared with the rotenone group ($p = 0.0108$), whereas the Levodopa/Carbidopa-treated group showed a comparable number of arm entries (9.75 ± 0.37) to the rotenone group ($p = 0.7006$), although this was not statistically significant. A significant difference was observed between the SLEE-treated and Levodopa/Carbidopa-treated groups ($p = 0.0012$). Overall, one-way ANOVA revealed a significant effect of treatment on total arm entries [$F(3,16) = 11.63$, $p = 0.0003$], indicating differences in exploratory activity among the treatment groups.

Administration of rotenone produced a marked and significant reduction ($p < 0.0001$) in spontaneous alternation behavior ($13.99 \pm 4.88\%$) compared with the vehicle control group ($80.28 \pm 5.11\%$), indicating severe impairment in spatial working memory (Fig 4). Treatment with *Sarcocephalus latifolius* ethanol extract (SLEE, 400 mg/kg) moderately improved alternation behavior ($32.50 \pm 3.35\%$) relative to the rotenone group ($p = 0.0475$). Similarly, rats treated with Levodopa/Carbidopa ($41.25 \pm 4.58\%$) showed a significant improvement in performance when compared with the rotenone group ($p = 0.0031$). One-way ANOVA confirmed a significant overall difference among the groups [$F(3,16) = 37.98$, $p < 0.0001$].

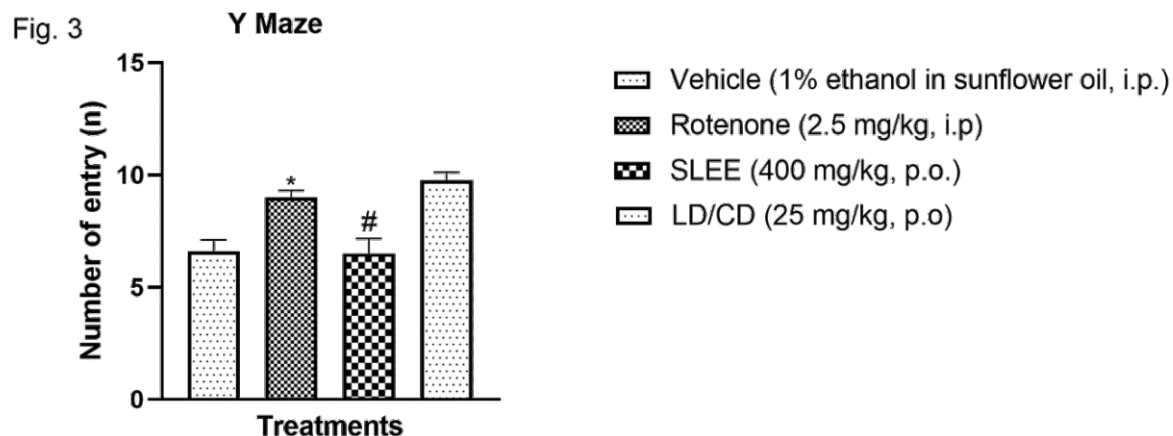


Figure 3: Effect of *Sarcocephalus latifolius* leaves ethanol extract on number of entries in the Y maze test.

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using one-way ANOVA, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

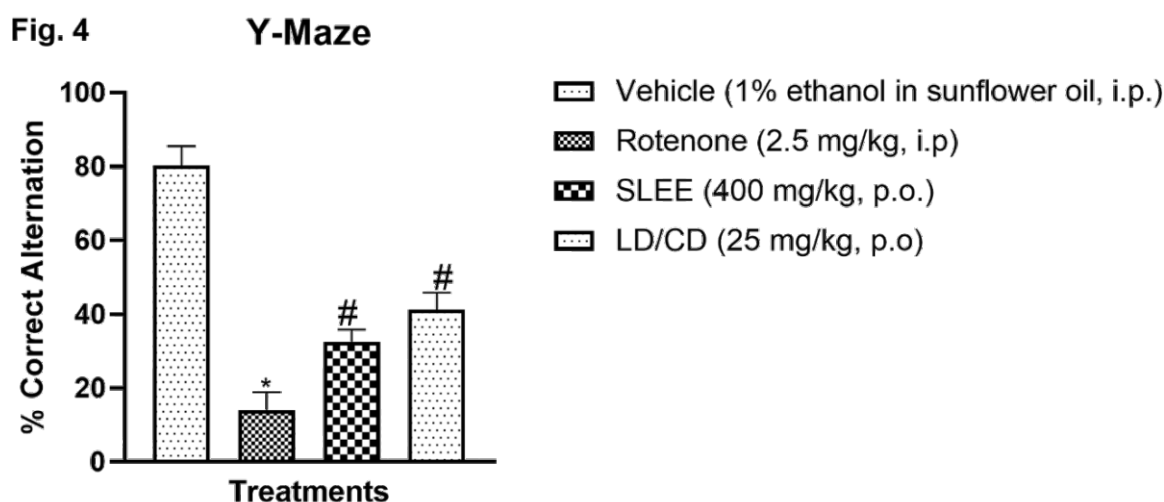


Figure 4: Effect of *Sarcocephalus latifolius* leaves ethanol extract in the on percentage correct alternation in rotenone-induced Parkinsonian rats.

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using one-way ANOVA, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

3.5: Effect of SLEE on Anxiety-like Behaviour in Rotenone-Treated Rats

As regards the **number of open arm entries**, administration of rotenone significantly ($p = 0.0086$) reduced open-arm exploration (0.50 ± 0.22) in rats compared to the negative control group (2.20 ± 0.40) (fig 5). Treatment with *Sarcocephalus latifolius* ethanol extract (SLEE) produced a mild, non-significant improvement (1.25 ± 0.19) relative to the rotenone group ($p = 0.3797$). However, rats treated with the standard drug, carbidopa-levodopa, showed a significant increase (2.60 ± 0.40) in open arm entries compared to the rotenone group ($p = 0.0014$). Statistical analysis using one-way ANOVA confirmed overall group differences [$F(3,16) = 8.685, p = 0.0012$].

With respect to the **duration spent in the open arms** (fig 6), rotenone administration markedly decreased (5.50 ± 1.20) the time rats spent exploring the open arms ($p < 0.0001$) relative to the vehicle group (29.28 ± 1.93). Treatment with SLEE led to

a non-significant increase (9.25 ± 1.77) in open arm duration compared with the rotenone group ($p = 0.3275$), suggesting a limited effect on open-arm exploration at the administered dose. Similarly, carbidopa-levodopa produced an increase (14.08 ± 0.87) in open arm duration that reach statistical significance when compared to the rotenone group ($p = 0.0049$). Nonetheless, the overall group effect was statistically significant [$F(3,16) = 48.02, p < 0.0001$], as revealed by one-way ANOVA.

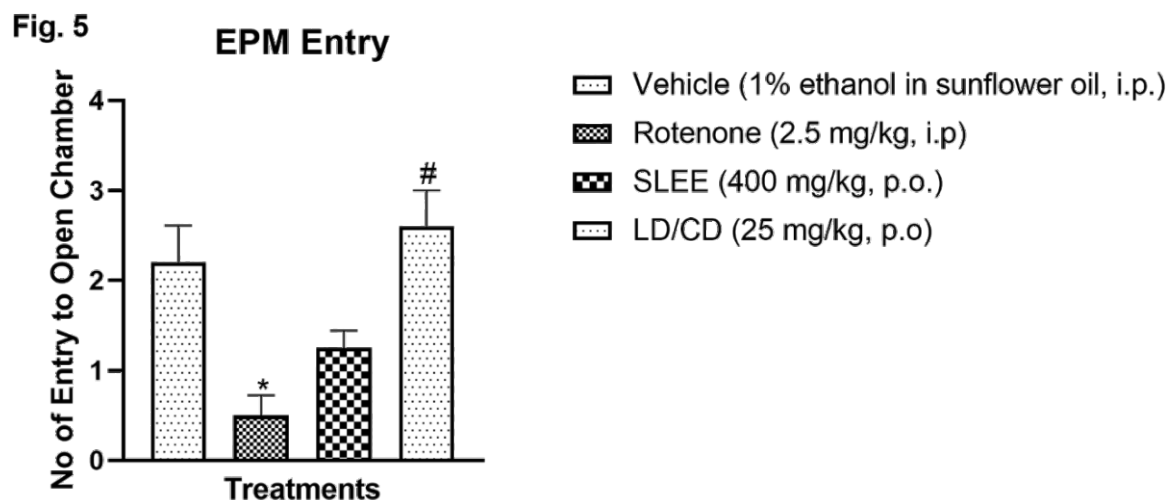


Figure 5: Effect of *Sarcocephalus latifolius* Ethanol Extract on Number of Open Arm Entry in the Elevated Plus Maze in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using one-way ANOVA, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

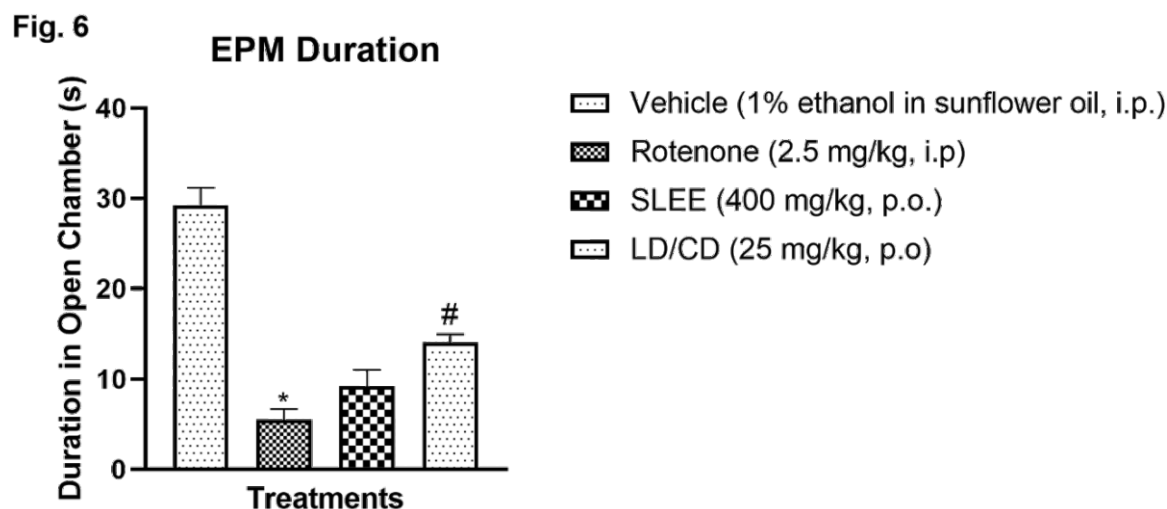


Figure 6: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on Duration of Open Arm Entry in the Elevated Plus Maze in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using one-way ANOVA, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

3.6: Effect of SLEE on Grooming Behaviour in Rotenone-Treated Rats

Administration of rotenone produced a significant reduction ($p < 0.0001$) in grooming duration (3.6 ± 1.03 s) compared with the negative control group (151.8 ± 8.28 s) (Fig. 7). Treatment with *Sarcocephalus latifolius* ethanol extract (SLEE) elicited a modest increase in grooming time (14.08 ± 1.57 s) relative to the rotenone group, though the difference did not attain statistical significance ($p = 0.3603$). In contrast, carbidopa–levodopa–treated rats exhibited a more pronounced improvement in grooming activity (25.75 ± 2.870 s) when compared with the rotenone group ($p = 0.0133$) (Fig 7). One-way ANOVA confirmed significant differences among the experimental groups [$F(3, 16) = 238.9, P < 0.0001$].

Fig. 7 Sucrose Splash Test

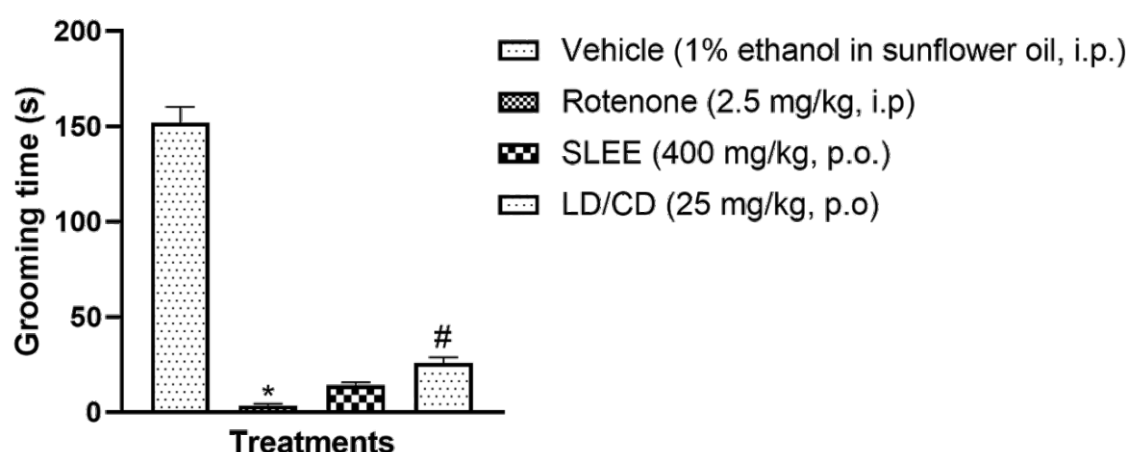


Figure 7: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on Sucrose Splash Test Performance in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using one-way ANOVA, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

3.7: Effects of SLEE on MDA and Nitrite Levels in Rotenone-Induced Oxidative Stress Across Brain Regions

As shown in Fig. 8, MDA levels were significantly elevated in the rotenone-treated group compared to controls across all four regions (PFC, CER, STR, HPC; $p < 0.0001$). Treatment with SLEE significantly reduced MDA levels relative to rotenone in the CER ($p < 0.0001$), STR ($p = 0.0013$), and HPC ($p = 0.0017$), but not in the PFC ($p = 0.0879$). Levodopa/Carbidopa treatment significantly reduced MDA levels compared with the rotenone group in the PFC ($p = 0.0073$), CER ($p = 0.0180$), STR ($p < 0.0001$), and HPC ($p < 0.0001$). Comparisons between SLEE and Levodopa/Carbidopa showed no significant difference in the PFC, CER, and HPC, whereas a significant difference was observed in the STR ($p = 0.0287$). Two-way ANOVA revealed significant effects of treatment ($F(3,64) = 256.3, p < 0.0001$), brain region ($F(3,64) = 56.70, p < 0.0001$), and treatment $\times$ brain region interaction ($F(9,64) = 2.446, p = 0.0184$).

Nitrite levels were significantly elevated in the rotenone-treated group compared with the control group in the CER ($p < 0.0001$), STR ($p < 0.0001$), and HPC ($p < 0.0001$), but not in the PFC ($p = 0.6350$). Treatment with SLEE (400 mg/kg) significantly reduced nitrite levels relative to the rotenone group in the CER ($p = 0.0002$), STR ($p = 0.0036$), and HPC ($p = 0.0101$), but not in the PFC ($p = 0.8206$). Levodopa/Carbidopa treatment significantly reduced nitrite levels compared with the rotenone group in the PFC ($p = 0.0002$), CER ($p = 0.0016$), STR ($p = 0.0005$), and HPC ($p < 0.0001$). Comparison between SLEE and Levodopa/Carbidopa showed no significant difference in the CER ($p = 0.9215$) and STR ($p = 0.9271$), while significant differences were observed in the PFC ($p = 0.0035$) and HPC ($p < 0.0001$). Two-way ANOVA revealed significant effects of treatment [$F(3,64) = 18.85, p < 0.0001$], brain region [$F(3,64) = 50.49, p < 0.0001$], and treatment $\times$ brain region interaction [$F(9,64) = 6.868, p < 0.0001$] (Fig. 9).

Fig. 8

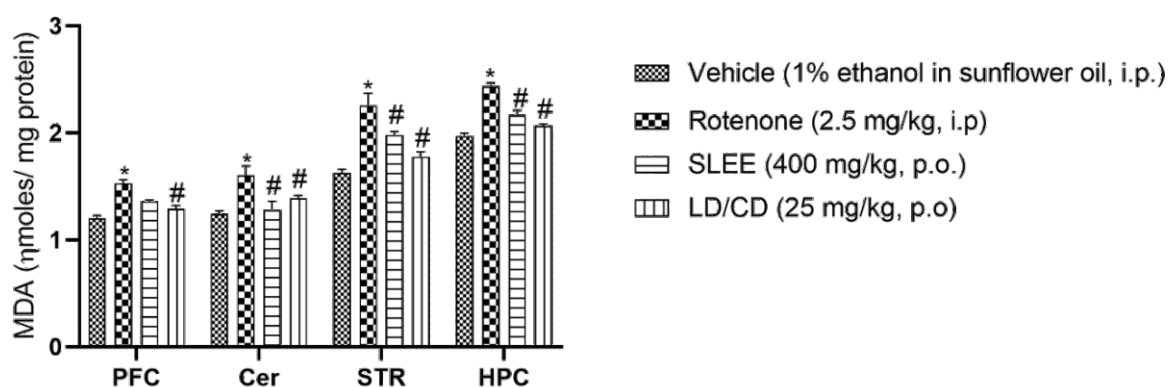


Figure 8: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on Malondialdehyde (MDA) Levels in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

Fig. 9

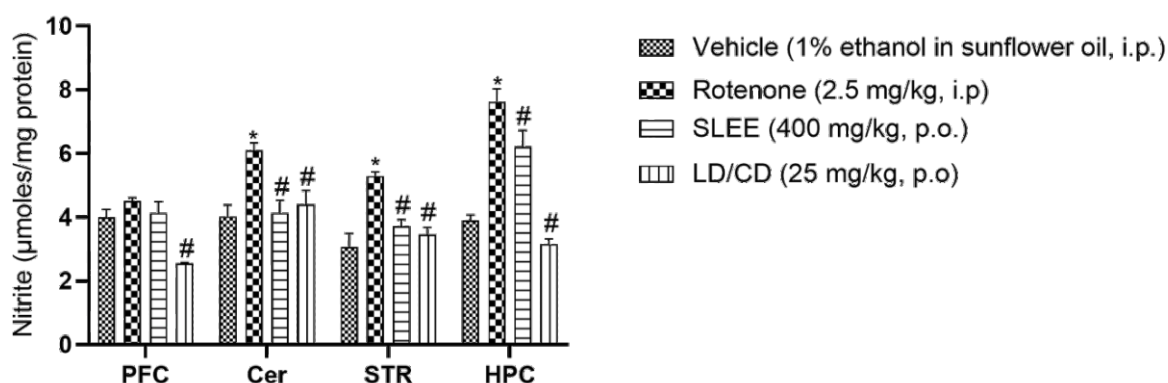


Figure 9: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on Nitrite Levels in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

3.8: Effects of SLEE on Antioxidant Status in Rotenone-Induced Oxidative Stress Across Brain Regions

GSH levels (Fig. 10) were significantly lower in the rotenone-treated group compared to the control group in the PFC ($p=0.0004$), CER ($p=0.0198$), STR ($p<0.0001$), and HPC ($p<0.0001$). Treatment with SLEE significantly increased GSH levels relative to rotenone in the PFC ($p=0.0034$), STR ($p<0.0001$), and HPC ($p=0.0056$), but not in the CER ($p=0.9992$). Similarly, levodopa carbidopa group showed a significant difference in the PFC ($p<0.0001$) and CER ($p<0.0001$) when compared to the rotenone treated group, However, no significant difference was found in the STR ($p = 0.687$) and the HPC ($P= 0.4704$). Two-way ANOVA revealed significant effects of treatment ($F(3,64)=84.97$, $p<0.0001$), brain region ($F(3,64)=40.52$, $p<0.0001$), and treatment $\times$ brain region interaction ($F(9,64)=8.519$, $p<0.0001$).

SOD activity was significantly reduced by rotenone relative to control in the PFC ($p < 0.0001$), CER ($p < 0.0001$), STR ($p = 0.0067$), and HPC ($p < 0.0001$). SLEE significantly increased SOD activity relative to rotenone in the PFC ($p = 0.0012$) and HPC ($p < 0.0001$), but not in the CER ($p = 0.0981$) or STR ($p = 0.0575$). However, the Levodopa/Carbidopa-treated group showed significant differences compared with the rotenone group in the prefrontal cortex ($p < 0.0001$) and cerebellum ($p = 0.0003$), whereas no significant differences were observed in the striatum ($p = 0.1850$) and hippocampus ($p = 0.7705$). Two-way ANOVA revealed significant effects of treatment ($F(3,64) = 127.0$, $p < 0.0001$), brain region ($F(3,64) = 39.30$, $p < 0.0001$), and treatment $\times$ brain region interaction ($F(9,64) = 8.781$, $p < 0.0001$) (Fig. 11).

GST activity was significantly reduced by rotenone relative to control in the PFC ($p < 0.0001$), CER ($p < 0.0001$), STR ($p = 0.0433$), and HPC ($p = 0.0096$) (Fig. 12). SLEE significantly increased GST activity relative to rotenone in the PFC ($p < 0.0001$) and STR ($p = 0.0018$), but not in the CER ($p = 0.9966$) or HPC ($p = 0.8960$). However, the Levodopa/Carbidopa-treated group showed significant differences compared with the rotenone group across all examined brain regions, including the prefrontal cortex ($p < 0.0001$), cerebellum ($p < 0.0001$), striatum ($p < 0.0001$), and hippocampus ($p < 0.0001$). Two-way ANOVA revealed significant effects of treatment ($F(3,64) = 70.84$, $p < 0.0001$), brain region ($F(3,64) = 86.41$, $p < 0.0001$), and treatment $\times$ brain region interaction ($F(9,64) = 11.55$, $p < 0.0001$).

Catalase (CAT) activity (Fig. 13) was significantly reduced by rotenone relative to control in the PFC ($p = 0.0003$), CER ($p < 0.0001$), and STR ($p < 0.0001$), but not in the HPC ($p = 0.8704$). Treatment with SLEE significantly increased CAT activity relative to rotenone in the CER ($p = 0.0013$), STR ($p < 0.0001$), and HPC ($p = 0.0002$), but not in the PFC ($p = 0.1273$). Compared with the rotenone group, Levodopa/Carbidopa treatment produced significant changes in the cerebellum ($p = 0.0110$), striatum ($p < 0.0001$), and hippocampus ($p < 0.0001$), but not in the prefrontal cortex ($p = 0.0541$). Two-way ANOVA revealed significant effects of treatment ($F(3,64) = 28.75$, $p < 0.0001$), brain region ($F(3,64) = 36.80$, $p < 0.0001$), and treatment $\times$ brain region interaction ($F(9,64) = 5.560$, $p < 0.0001$).

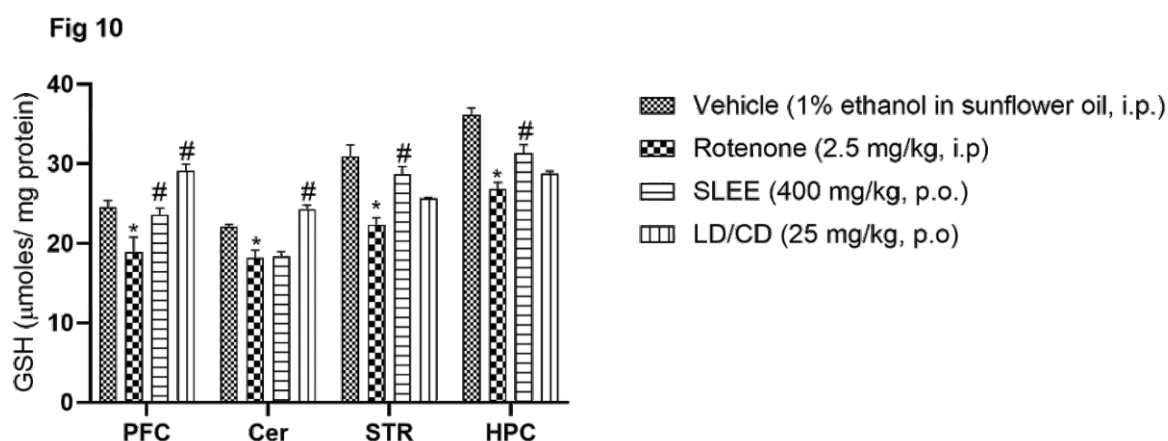


Figure 10: Effect of *Sarcocephalus latifolius* Ethanol Extract on Glutathione (GSH) Levels in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

Fig 11

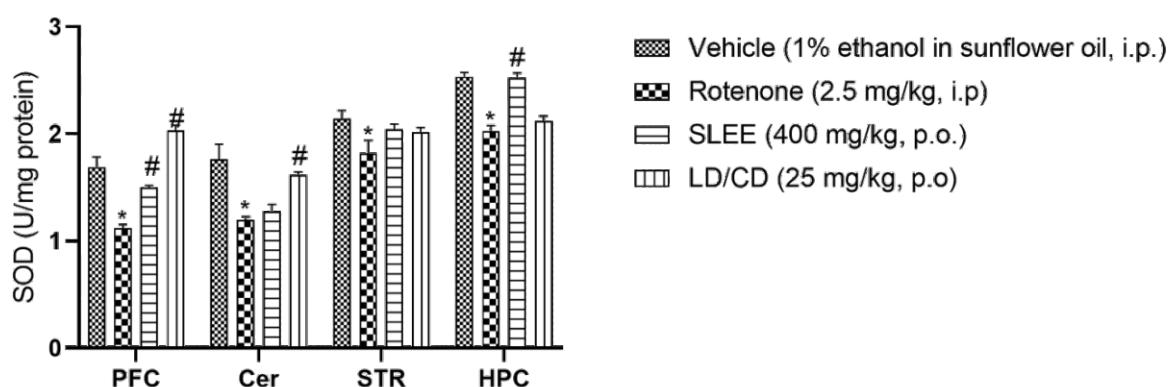


Figure 11: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on Superoxide Dismutase (SOD) Activity in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

Fig 12

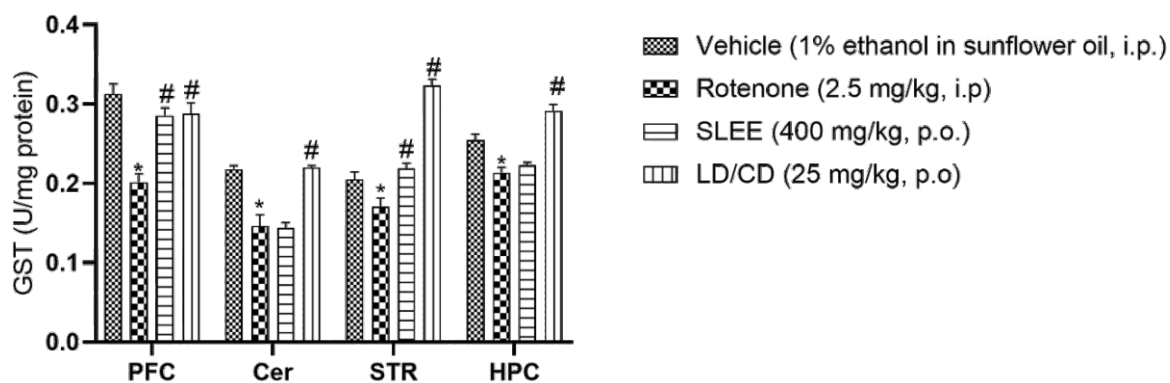


Figure 12: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on Glutathione S-Transferase (GST) Levels in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

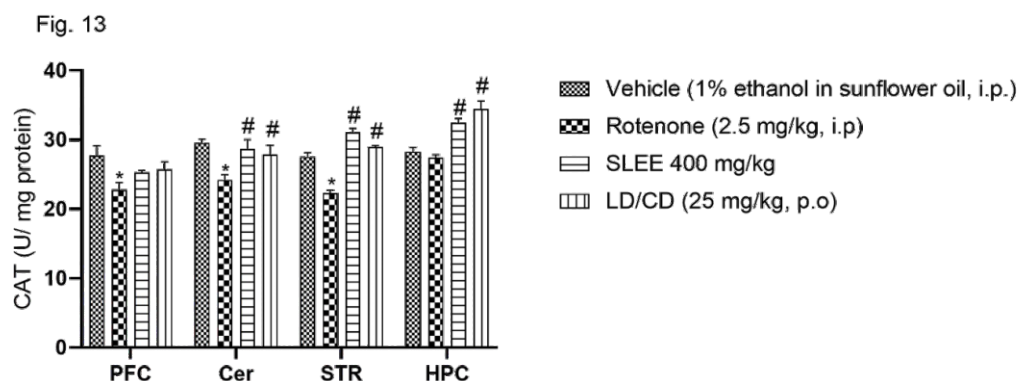


Figure 13: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on Catalase Levels in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

3.9: Effects of SLEE on Dopamine Levels in Rotenone-Induced Oxidative Stress Across Brain Regions

The concentration of dopamine in the brain regions examined was significantly altered by rotenone treatment, reflecting a clear perturbation of dopaminergic function (Fig. 14). In the prefrontal cortex (PFC), rotenone administration resulted in a significant reduction in dopamine levels (128.142 ± 5.538) compared to the control group (142.640 ± 2.542) ($p=0.0279$). Treatment with SLEE significantly reversed this decline, with dopamine levels in the PFC (159.799 ± 4.523) which approached levels seen in the control group, suggesting a potential restorative effect on dopaminergic activity. A similar pattern was observed in the cerebellum (CER) and striatum (STR), where SLEE significantly increased dopamine levels (CER: 141.114 ± 0.609 , STR: 223.042 ± 2.462) compared to the rotenone group (CER: 122.818 ± 0.600 , STR: 178.618 ± 5.283). In the hippocampus (HPC), SLEE resulted in a substantial increase in dopamine levels (260.144 ± 3.874) compared to the rotenone-treated group (186.291 ± 1.153). Levodopa/Carbidopa treatment significantly increased dopamine levels compared with the rotenone group in the PFC (195.51 ± 4.40 , $p < 0.0001$), CER (183.69 ± 1.74 , $p < 0.0001$), STR (203.84 ± 2.44 , $p = 0.0002$), and HPC (275.85 ± 4.71 , $p < 0.0001$). Two-way ANOVA revealed significant effects of treatment ($F(3,48)=497.3$, $p < 0.0001$), brain region ($F(3,48)=175.8$, $p < 0.0001$), and treatment $\times$ brain region interaction ($F(9,48)=21.02$, $p < 0.0001$).

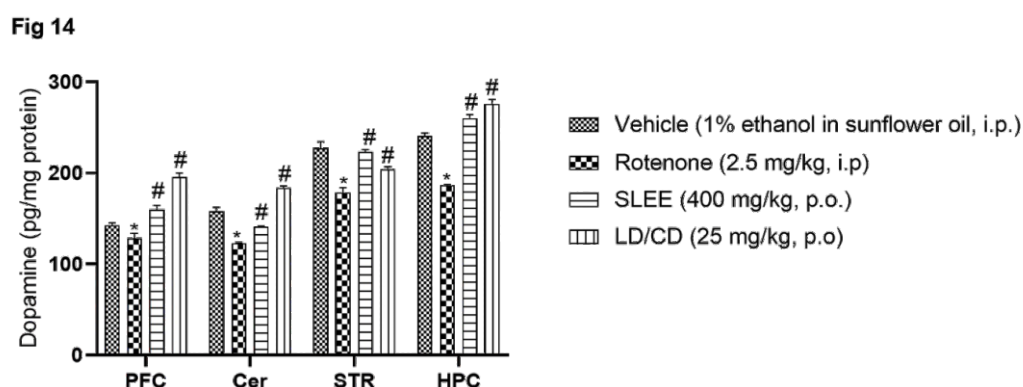


Figure 14: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on Dopamine Levels in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 4 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

3.10: Effects of SLEE on Acetylcholinesterase Activity in Rotenone-Induced Oxidative Stress

Treatment with rotenone resulted in an elevation of acetylcholinesterase (AChE) activity (Fig 15) across the prefrontal cortex (PFC), cerebellum (Cer), striatum (STR), and hippocampus (HPC), with values of 1.285 ± 0.033 , 1.234 ± 0.041 , 0.902 ± 0.022 , and 1.085 ± 0.042 , respectively, in contrast to the control group (PFC: 1.047 ± 0.045 , Cer: 0.450 ± 0.067 , STR: 0.575 ± 0.020 , HPC: 0.899 ± 0.035) respectively. Administration of SLEE (400 mg/kg) reduced AChE activity to 1.095 ± 0.080 , 1.036 ± 0.034 , 0.799 ± 0.060 , and 0.686 ± 0.040 in the PFC, Cer, STR, and HPC, respectively. SLEE significantly reduced rotenone-induced AChE activity in the PFC ($p = 0.0080$), Cer ($p = 0.0055$), and HPC ($p < 0.0001$), but the reduction in the STR was not statistically significant ($p = 0.2317$). Levodopa-carbidopa also significantly differed from the rotenone group in the PFC, Cer, STR, and HPC ($p < 0.0001$, $p < 0.0001$, $p = 0.0007$, and $p < 0.0001$, respectively). Two-way ANOVA revealed significant main effects of treatment ($F(3,64) = 43.10$, $p < 0.0001$) and brain region ($F(3,64) = 91.01$, $p < 0.0001$), as well as a significant treatment $\times$ brain region interaction ($F(9,64) = 22.89$, $p < 0.0001$).

Fig 15

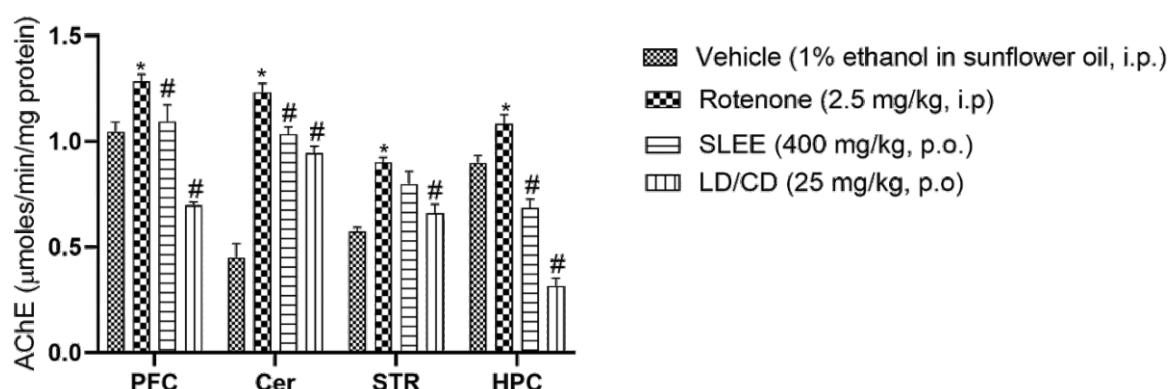


Figure 15: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on Acetylcholinesterase (AChE) Activity in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

3.10: Effects of SLEE on Proinflammatory Cytokines (TNF- α and IL-6) in Rotenone-Induced Oxidative Stress Across Brain Regions

Rotenone administration significantly increased the levels of the proinflammatory cytokine TNF- α across all examined brain regions compared with the control group (Fig. 16). In the prefrontal cortex (PFC), TNF- α levels increased from 28.306 ± 2.280 in the control group to 59.456 ± 1.639 following rotenone exposure, while SLEE treatment significantly reduced TNF- α levels to 45.300 ± 4.365 ($p = 0.0188$). Similarly, rotenone elevated TNF- α levels in the cerebellum (Cer) (59.299 ± 1.762) compared with control (25.159 ± 1.335), whereas SLEE administration significantly attenuated this increase (32.655 ± 1.353 ; $p < 0.0001$). In the striatum (STR), rotenone increased TNF- α levels from 56.284 ± 1.891 to 82.655 ± 5.034 , with SLEE significantly reducing the levels to 62.178 ± 3.822 ($p = 0.0005$). Likewise, hippocampal (HPC) TNF- α levels were elevated following rotenone treatment (110.130 ± 1.275) compared with controls (75.342 ± 2.745), while SLEE significantly restored TNF- α levels (75.004 ± 4.667 ; $p < 0.0001$). Two-way ANOVA revealed significant effects of treatment ($F(3,48) = 124.7$, $p < 0.0001$), brain region ($F(3,48) = 62.94$, $p < 0.0001$), and treatment $\times$ brain region interaction ($F(9,48) = 3.077$, $p = 0.0054$) on TNF- α levels.

A similar pattern was observed for IL-6 levels (Fig. 13), where rotenone exposure significantly increased IL-6 concentrations across all brain regions. In the PFC, IL-6 levels increased from 11.838 ± 1.542 in controls to 33.973 ± 0.664 following rotenone treatment, while SLEE reduced IL-6 levels to 19.355 ± 1.054 ($p = 0.0001$). In the Cer, rotenone increased IL-6 levels from 15.148 ± 0.221 to 36.510 ± 1.318 , whereas SLEE significantly reduced these levels to $23.590 \pm$

1.084 ($p = 0.0006$). Similarly, rotenone elevated IL-6 levels in the STR (45.965 ± 1.653) and HPC (58.922 ± 4.240) compared with controls (32.378 ± 0.717 and 33.195 ± 0.782 , respectively). SLEE administration significantly attenuated these increases in the STR (30.995 ± 1.249 ; $p < 0.0001$) and HPC (37.572 ± 5.180 ; $p < 0.0001$). Two-way ANOVA demonstrated significant effects of treatment ($F(3,48) = 50.53$, $p < 0.0001$), brain region ($F(3,48) = 69.90$, $p < 0.0001$), and treatment $\times$ brain region interaction ($F(9,48) = 4.133$, $p = 0.0006$) on IL-6 levels.

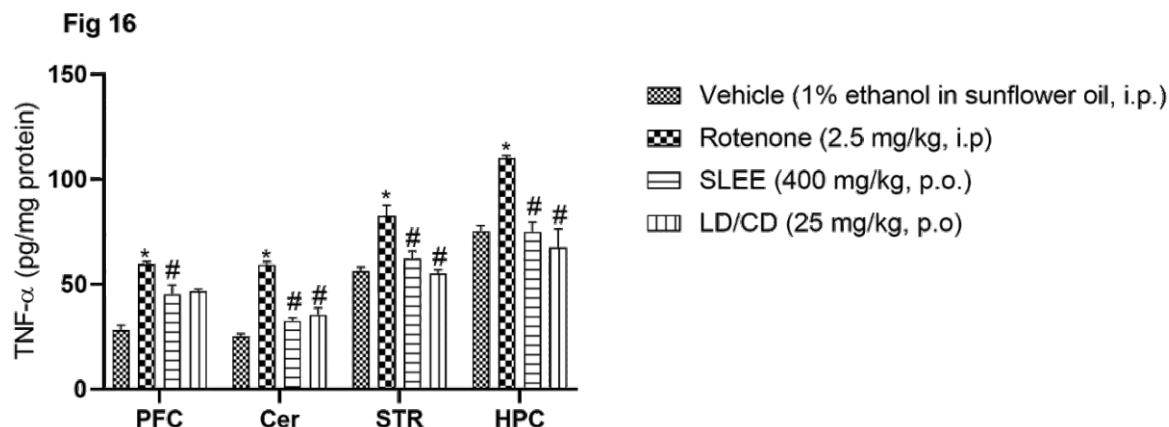


Figure 16: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on TNF- α Levels in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

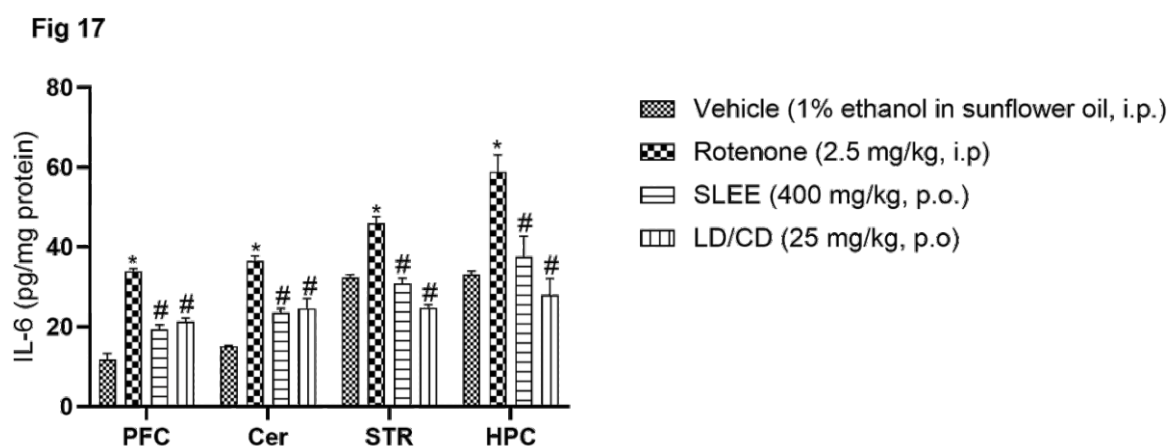


Fig 17: Effect of *Sarcocephalus latifolius* leaves Ethanol Extract on IL-6 Expression in Rotenone-Induced Parkinsonian Rats

Each vertical bar represents the mean $\pm$ SEM of 5 animals per group. Data were analyzed using two-way ANOVA with treatment and brain region as factors, followed by post-hoc Tukey's tests; * $p < 0.05$ compared with the vehicle. # $p < 0.05$ compared with the rotenone group.

SLEE – *Sarcocephalus latifolius* leaves ethanol extract; LD/CD – Levodopa/Carbidopa

4.0: DISCUSSION

The GC–MS analysis of *Sarcocephalus latifolius* leaf ethanol extract revealed a mixture of constituents, with benzoic acid, methyl ester (7.63%) as the major component. Other notable compounds included cyclopentanetridecanoic acid, methyl ester (4.80%), phenol, 2,5-bis(1,1-dimethylethyl)- (2.92%), 2,6-diisopropyl-naphthalene (2.38%), 1-dodecanol, 2-hexyl- (2.27%), 2,4,5-trichlorophenyl propenoate (2.17%), and 6,8-dichloro-2-trifluoromethyl-4-quinolinol (1.95%). The identified compounds belong to different chemical classes, including aromatic esters, substituted phenolic derivatives, and aliphatic alcohols and esters. Benzoic acid derivatives have been reported to exhibit antioxidant and antimicrobial activities^[43], indicating that the abundant methyl benzoate identified in the extract may contribute to the biological activities of the extract. Substituted phenolic compounds are known for their antioxidant properties through hydrogen donation and redox modulation mechanisms^[44]. Similarly, fatty acid ester derivatives have been associated with biological activities, including membrane interactions and modulation of inflammatory processes^[45]. The presence of these constituents, particularly the abundant benzoate and phenolic derivatives, may therefore contribute to the antioxidant and neuroprotective effects observed in the present study. However, further isolation and characterization of individual compounds are required to confirm their specific contributions. Comparative reports on other parts of *S. latifolius* indicate variation in phytochemical composition among plant tissues. Root bark extracts have been characterized by triterpenoid glycosides, monoterpene indole alkaloids, and sterols^[18, 28, 46], whereas fruit extracts have been reported to contain saponins, flavonoids, tannins, and phenolic constituents^[47]. These differences suggest that the chemical composition and biological properties of *S. latifolius* may vary depending on the plant part investigated.

The administration of rotenone in this study produced decline in locomotor activity, induced cognitive deficits, and resulted in depressive and anxiety-like behaviors. Additionally, increased pro-inflammatory cytokines, impaired cholinergic neurotransmission, and oxidative imbalance were observed in the examined brain regions of rats treated with rotenone. These findings are consistent with previous studies, which also reported similar behavioral impairments and biochemical disturbances, including oxidative stress, dopaminergic and cholinergic dysfunctions, and inflammation, following rotenone exposure^[48, 49, 50, 51].

The rats treated with rotenone exhibited a significantly

prolonged catalepsy latency and impaired performance in the negative geotaxis test, reflecting the characteristic motor dysfunction seen in PD[52]. Previous studies have demonstrated that these deficits are dopamine-dependent, with their reversibility being observable following treatment with standard PD medications such as L-DOPA or apomorphine^[53, 54]. *Sarcocephalus latifolius* ethanol extract (SLEE) at a dose of 400 mg/kg produced substantial reversal of these motor deficits. The negative geotaxis test, which evaluates motor coordination, muscle strength, and balance showed that the rotenone-treated rats took significantly longer time to reorient compared to the vehicle group. This delay indicates compromised motor coordination, balance, and bradykinesia. However, administration of SLEE resulted in a significant reduction in the latency period, suggesting an improvement in motor performance. Notably, post hoc analysis revealed no significant difference between the SLEE-treated group and the Levodopa/Carbidopa-treated group, suggesting that SLEE may have an effect comparable to the standard drug in improving motor performance. The catalepsy test, which specifically assesses immobility in an imposed posture, often related to akinesia, showed that rotenone induction led to severe catalepsy in the rats, consistent with findings in other studies^[55, 9]. Administration of SLEE produced a reduction in catalepsy latency compared with the rotenone-treated group, suggesting a trend toward attenuation of rotenone-induced motor rigidity; however, this effect did not reach statistical significance. In contrast, Levodopa/Carbidopa significantly reduced catalepsy latency, demonstrating a greater improvement in motor impairment.

Beyond the motor symptoms of Parkinson's disease (PD), non-motor symptoms, which significantly impact the quality of life of affected individuals, also play a critical role in PD progression. These non-motor symptoms include cognitive impairments, as well as depression and anxiety, all of which were assessed in the current study using the Y-Maze, Sucrose Splash, and Elevated Plus Maze tests, respectively. The Y-Maze test, used to evaluate spatial working memory, is particularly relevant as cognitive dysfunction in PD often manifests as impairments in this domain, leading to what is known as PD Mild Cognitive Impairment (PD-MCI). In our study, rotenone administration led to a significant reduction in the percentage alternation compared to the vehicle control group, indicating a marked impairment in spatial working memory. The pathophysiology of cognitive dysfunction in PD is widely attributed not only to dopaminergic degeneration but also to a reduction in cholinergic activity, particularly within the prefrontal cortex and hippocampus,

regions crucial for memory and learning [56]. Treatment with SLEE at 400 mg/kg led to a spontaneous increase in the alternation percentage, suggesting a restoration of short-term spatial memory and possibly alleviation of muscle rigidity.

The behavioural manifestations associated with Parkinson's disease, including alterations in motivation and affective responses, were examined in the present study using the Sucrose Splash test, with grooming duration employed as the principal index of response. Rotenone administration resulted in a pronounced reduction in grooming behaviour compared with vehicle-treated animals. This observation is consistent with previous reports describing the involvement of dopaminergic and monoaminergic dysfunction in the neuropsychiatric disturbances accompanying Parkinsonian pathology [57, 58]. Rotenone-induced impairment of mesolimbic dopaminergic transmission and serotonergic signalling has been implicated as an important contributor to such behavioural alterations [59]. However, grooming behaviour represents a complex motor sequence requiring intact movement initiation, muscular dexterity, and postural coordination. In rotenone-induced Parkinsonism, disruption of these motor components occurs alongside neurochemical alterations underlying the disease phenotype, such that changes in grooming behaviour represent the combined consequences of affective disturbance and motor dysfunction. Administration of *Sarcocephalus latifolius* ethanol extract (SLEE) however, produced a non significant increase in grooming duration relative to rotenone-treated animals.

In addition, the Elevated Plus Maze (EPM) test revealed a marked reduction in open-arm entries and the duration spent in the open arms following rotenone administration. Treatment with *Sarcocephalus latifolius* ethanol extract (SLEE) produced a non-significant increase in open-arm exploration relative to rotenone-treated animals. Alterations in open-arm exploration have been widely associated with anxiety-related behaviour and are reported in experimental models of Parkinson's disease, where disturbances in dopaminergic, serotonergic, and noradrenergic neurotransmission contribute to abnormalities in emotional regulation [60]. However, the behavioural manifestations of rotenone-induced Parkinsonism also encompass motor disturbances, and reduced exploratory activity within the EPM may arise from the combined influence of anxiety-related responses, bradykinesia, rigidity, and impaired locomotor function.

In the present study, rotenone administration resulted in a significant reduction in key antioxidant defenses, including glutathione (GSH) levels and the activities of catalase (CAT), superoxide dismutase (SOD), and glutathione S-transferase (GST), across most of the brain regions examined, with a concomitant increase in malondialdehyde (MDA) and nitrite levels, indicative of enhanced oxidative damage. These findings are consistent with the established contribution of oxidative stress to PD pathogenesis, where rotenone-induced inhibition of mitochondrial complex I promotes excessive reactive oxygen species generation, depletion of endogenous antioxidant reserves, and oxidative injury [61]. Similar alterations, including glutathione depletion, impaired antioxidant capacity, and increased lipid peroxidation, have been reported in human PD tissues and experimental rotenone models [62]. The observed improvements in antioxidant parameters following SLEE treatment suggests attenuation of rotenone-induced oxidative imbalance. Natural compounds such as quercetin, curcumin, piceid, and ferulic acid have been reported to restore antioxidant defenses in rotenone-induced PD models through increases in GSH levels and antioxidant enzyme activities, accompanied by reductions in lipid peroxidation and oxidative damage [63]. Analysis of the Treatment $\times$ Region interaction revealed region-specific responses across the four antioxidant markers assessed. SLEE significantly restored antioxidant parameters in the prefrontal cortex, striatum, and hippocampus, with significant recovery observed in three of the four markers assessed in each region, whereas the cerebellum showed significant recovery only in catalase activity. This distribution of response differs from previous reports describing the striatum as a region highly vulnerable to rotenone-induced oxidative injury. Rotenone has been shown to produce marked depletion of glutathione in the striatum relative to other brain regions [64, 65], and mitochondrial oxidative alterations induced by rotenone in the frontal cortex have been reported to be milder than those observed in the striatum [66]. These findings suggest that the antioxidant effects of SLEE were region-dependent. While significant improvements were observed across several brain regions, the response in the cerebellum was comparatively limited, indicating differential regional sensitivity to SLEE-mediated antioxidant effects.

Rotenone-induced oxidative stress and mitochondrial dysfunction are well-established triggers of neuroinflammation and play pivotal roles in the progression of neurodegeneration [67]. In this study, our result showed significant increase in the levels of pro-inflammatory cytokines TNF- α and IL-6, a finding consistent with prior

research^[68]. Treatment with SLEE reduced these inflammatory cytokines alongside improvements in oxidative stress markers, suggesting an anti-inflammatory effect in the rotenone model. These findings indicate that the observed reduction in inflammatory and oxidative alterations may contribute to the neurobehavioral and biochemical changes observed following SLEE treatment.

The present findings demonstrate that administration of SLEE significantly attenuated rotenone-induced dopamine depletion across several brain regions, including the prefrontal cortex, cerebellum, striatum, and hippocampus. This improvement in dopamine levels suggests an enhancement of dopaminergic function following rotenone exposure. The reduction in dopamine observed in the brain regions after rotenone administration is consistent with previous reports describing dopaminergic alterations and associated behavioural deficits in rotenone models^[69,70]. The ability of SLEE to improve dopamine levels in these regions is comparable to findings reported for other bioactive compounds, including fucoidan, baicalein, epigallocatechin gallate, fisetin, and kaempferol, which have demonstrated protective effects against rotenone-induced dopaminergic alterations through antioxidant and anti-inflammatory mechanisms^[71].

Rotenone administration produced a marked increase in AChE activity in the PFC, cerebellum, striatum, and hippocampus. This finding indicates an alteration of cholinergic enzyme activity following rotenone exposure. Rotenone-induced inhibition of mitochondrial complex I results in increased reactive oxygen species formation, and oxidative stress has been shown to influence AChE regulation through ROS-mediated signalling, calcium-dependent mechanisms, and changes in AChE expression and release^[72]. Experimental studies using rotenone-induced Parkinsonian models have demonstrated that rotenone exposure increases oxidative stress markers together with alterations in acetylcholinesterase activity, while antioxidant treatment attenuates these biochemical changes^[73]. The elevation of AChE activity observed in the present study may therefore be associated with the oxidative disturbance produced by rotenone. Administration of SLEE significantly reduced the rotenone-induced elevation of AChE activity in the PFC, cerebellum, and hippocampus. The reduction observed in the striatum did not reach statistical significance, although the value was lower than that of the rotenone-treated group. These findings indicate that SLEE exerted a region-dependent effect on rotenone-induced alterations in cholinergic enzyme activity. In Parkinsonian states, the

interaction between dopaminergic and cholinergic systems within the striatum plays an important role in the regulation of motor function^[74]. The striatum contains abundant cholinergic interneurons that interact closely with nigrostriatal dopaminergic signaling. Loss of dopaminergic input alters striatal cholinergic regulation, resulting in disturbances in dopamine–acetylcholine balance that contribute to motor abnormalities such as bradykinesia and rigidity^[75]. Experimental studies have further demonstrated that excessive activity of striatal cholinergic interneurons contributes to akinesia and bradykinesia by altering basal ganglia output pathways^[76,77]. Although this mechanism has been established mainly in dopamine-depletion models rather than rotenone models specifically, the increased AChE activity observed following rotenone administration in the present study may represent altered cholinergic regulation associated with rotenone-induced neurochemical changes. The reduction in AChE activity following SLEE treatment, together with the improvements in dopamine levels observed in this study, suggests that SLEE may influence dopaminergic and cholinergic alterations associated with rotenone exposure; however, the impact of altered AChE activity on acetylcholine availability and cholinergic signaling requires confirmation through direct measurement of acetylcholine levels.

5.0: CONCLUSION

In conclusion, the results of this study indicate that SLEE, administered at a dose of 400 mg/kg, attenuated rotenone-induced behavioural deficits and biochemical alterations. Treatment with SLEE improved both motor outcomes, increased dopamine levels, and modulated oxidative stress and inflammatory markers. These effects were observed across the brain regions examined, with more pronounced effects in the striatum and hippocampus. Therefore, SLEE warrants further investigation as a potential therapeutic candidate in Parkinson's disease models.

5.1: STUDY LIMITATION

The present study was limited by the evaluation of a single dose of *S. latifolius* ethanol extract; hence, the dose-response relationship and therapeutic range remain to be established. Although the extract demonstrated biological activity, the contribution of individual phytoconstituents and possible synergistic interactions among them require further investigation. Dopamine quantification was performed using ELISA; however, chromatographic methods such as HPLC may provide greater analytical specificity. Furthermore, the absence of histological and immunohistochemical assessments limits definitive confirmation of neuronal preservation. Finally, the use of

the sucrose splash test represents a limitation, as grooming behaviour is influenced by motor function and may not exclusively reflect motivational or affective changes in rotenone-treated animals. Future investigations incorporating dose-ranging studies, phytochemical isolation, histological evaluation, and motor-independent behavioural assessments would further clarify the therapeutic potential of SLEE.

CRediT authorship contribution statement

Akinyinka Oyedolapo Alabi: Writing – review & editing, Visualization, Software, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. Stella Afolakemi Adeyemo: Writing – original draft, Writing – review & editing. Paul Ademola Adeleke: Methodology, Investigation. Joseph Chimezie: Methodology, Investigation. Ibironke Olufunmilayo Oyenuga: Writing – original draft, Writing – review & editing. Luqman Opeoluwa Ogunjimi: Methodology, Investigation, Data curation. Abdullah Akanji Muritala: Methodology, Investigation. Elijah Oladapo Oyinloye: Methodology, Investigation. Abayomi Mayowa Ajayi: Writing – original draft, Writing – review & editing, Methodology, Investigation, Formal analysis, Data curation, Supervision, Conceptualization.

Declaration of competing interest

The authors declare that they have no financial interests or personal relationships that could be perceived as influencing the research presented in this paper.

Data availability

Data will be provided upon request

Abbreviations

PD- Parkinson's Disease
SLEE - *Sarcocephalus latifolius* ethanol extract
LDCD – Levodopa Carbidopa
p.o. – Oral
TBA- Thiobarbiturate acid
ELISA- Enzyme-Linked Immunosorbent Assay
ROS- Reactive oxygen species
i.p. - intraperitoneal
AChE- Acetylcholinesterase
GC-MS- Gas Chromatography-Mass Spectrometry
TCA- Trichloroacetic acid
GSH- Reduced glutathione
TNF- α - Tumor necrosis factor-alpha
MDA- Malondialdehyde
IL-6- Interleukin-6
SOD- Superoxidase dismutase

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