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Research Article

Phytochemical-Enriched Walnut Energy Bar Attenuates Chlorpromazine-Induced Neurotoxicity via Modulation of Oxidative Stress and TNF-α-Mediated Inflammation

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Abstract

Objective: This study aimed to investigate the neuroprotective activity of a walnut-based energy bar in drug-induced Parkinsonism in rats. **Methodology:** Wistar rats (n = 24) were divided into four groups: control (standard diet), disease (standard diet + chlorpromazine 3 mg/kg, intraperitoneal), standard treatment (standard diet +carbidopa+ levodopa (100 + 25 mg/kg, oral)), and walnut energy bar-treated (standard diet + walnut energy bar 1 g/kg/day for one month). Motor functions were assessed using standard behavioral tests. The antioxidant potential of the energy bar was analyzed using the 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assay. The walnut energy bar was analyzed by gas chromatography-mass spectrometry (GC-MS), confirming the presence of bioactive compounds, and high-performance liquid chromatography (HPLC) was used to quantify ferulic acid and cinnamic acid. Tumor necrosis factor-alpha (TNF-α), glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) levels were measured, and histopathological examination was performed on rat brain tissue. **Results:** Parkinsonism was successfully induced in rats, as evidenced by motor dysfunction, higher TNF-α and MDA levels, and lower GSH, along with a decline in antioxidant defense mechanisms (CAT, SOD). These biochemical alterations were improved in the walnut energy bar-treated group, suggesting neuroprotective and anti-inflammatory effects. Bioactive compounds identified by GC–MS, along with ferulic and cinnamic acids quantified by HPLC, were associated with the neuroprotective potential of the bar. Enzyme-linked immunosorbent assay (ELISA) and oxidative stress markers further supported these findings. These results were further validated by histopathological findings that showed neurodegeneration in the disease group and preserved neuronal architecture in the group treated with the walnut energy bar. **Conclusion:** The walnut energy bar-treated group exhibited significant neuroprotective, antioxidant, and anti-inflammatory activity. These findings suggest that a functional walnut energy bar may serve as a dietary intervention for managing drug-induced Parkinsonism symptoms.

Keywords: Parkinson's disease; antioxidants; neuroinflammation; ELISA

1. Introduction

Parkinson's disease (PD) is a progressive neurodegenerative disorder characterized by dopamine depletion in the striatum due to selective death of dopaminergic neurons in the substantia nigra. This reduction in dopamine levels leads to a neurochemical imbalance, manifested by motor symptoms such as postural instability, bradykinesia, stiffness, and tremor, along with non-motor symptoms, including cognitive decline and neuronal damage driven by oxidative stress [1]. Approximately 1% to 2% of people over the age of 60 are affected by PD. Carbidopa and levodopa are standard treatments; however, the long-term use of these agents is limited by motor dysfunction [2]. These limitations highlight the necessity of safe and multi-targeted supplementary therapy.

Neuroinflammation and oxidative stress are key factors in the development of PD. Dopaminergic neuron death, protein misfolding, and mitochondrial dysfunction are all facilitated by excessive reactive oxygen species (ROS) generation and compromised antioxidant defenses [3]. Thus, functional foods fortified with bioactive substances have gained attention for their potential to reduce inflammation and oxidative stress [4]. Plant-derived bioactive compounds, including unsaturated fatty acids, polyphenols, flavonoids, and tocopherols, exhibit neuroprotective properties through scavenging ROS, modifying apoptotic pathways, and maintaining mitochondrial activity [5].

A significant body of research has examined the influence of dietary bioactive compounds and functional foods on neurodegenerative processes in PD. Experimental studies using toxin-induced models of Parkinsonism,

including those induced by 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), 6-hydroxydopamine (6-OHDA), and antipsychotic agents, have demonstrated that polyphenol-rich diets can significantly reduce dopaminergic neuronal loss and enhance motor performance [6]. Specifically, flavonoids have been documented to modulate essential signaling pathways pertinent to neuronal survival, including inhibition of neuro-inflammatory mediators such as nuclear factor kappa B (NF-κB) and attenuation of proinflammatory cytokines [7]. Meanwhile, omega-3 fatty acids, especially α-linolenic acid, help make cell membranes more flexible, reduce oxidative stress, and support the ability of the brain to form and adapt connections, thereby protecting nerve cells [8].

Moreover, various studies emphasize the key role of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), and glutathione (GSH), in mitigating oxidative stress-induced neuronal injury. Plant-derived antioxidant-rich diets have proven effective in boosting these natural defenses, thereby lowering lipid peroxidation and safeguarding mitochondrial activity [9]. Specific foods, including walnuts, oats, pumpkin seeds, and figs, have undergone separate evaluations, yielding evidence of enhanced antioxidant profiles, decreased neuroinflammation, and modest recovery of dopamine concentrations in animal models.

For example, ferulic acid found in oats reduce oxidative stress, support dopaminergic neurons, and increase dopamine levels [10]. The ellagitannins, melatonin, and α-linolenic acid found in walnuts modulate neuroinflammation and mitochondrial function [11]. Carotenoids, B vitamins, and minerals found in pumpkin seeds promote neuroprotection, strengthen antioxidant defenses, and shield dopaminergic neurons [12]. Phenolic compounds, which are prevalent in figs, have anti-inflammatory and antioxidant properties [13]. Medium-chain triglycerides from coconuts have shown neuroprotective activity by reducing oxidative stress, apoptosis, and mitochondrial dysfunction [14]. Previous research has also shown that energy bars made from nuts or cereals have cardiometabolic and antioxidant benefits [15]; however, it remains unknown whether these bars exert neuroprotective effects against dopaminergic neurodegeneration.

Currently, to our knowledge, no functional nut-based formulation has been evaluated *in vivo* for its potential to treat chlorpromazine-induced Parkinsonism. To address this gap in the literature, this study evaluated a walnut-based energy bar for its neuroprotective effect. Specifically, this study aimed to:

(1) Examine how diet supplementation with a walnut bar affects motor impairments induced by chlorpromazine using standardized behavioral assessments.

(2) Assess how the walnut bar supplementation performs compared with the usual levodopa/carbidopa treatment.

(3) Examine the contribution of the main enzymatic antioxidant defense system to the observed effects.

2. Methodology

2.1 Drugs, Chemicals, and Reagents

2, 2-Diphenyl-1-picrylhydrazyl (DPPH), methanol, L-ascorbic acid, (±)-6-hydroxy-2, 5, 7, 8-tetramethylchromane-2-carboxylic acid, ferulic acid, and cinnamic acid were purchased from Sigma-Aldrich (Steinheim, Germany). For high-performance liquid chromatography (HPLC), acetonitrile (isocratic grade), ethanol, potassium dihydrogen phosphate, orthophosphoric acid, and propyl 4-hydroxybenzoate (propylparaben; internal standard) were obtained from Merck (Darmstadt, Germany). The reagents for Thiobarbituric acid reactive substances (TBARS), including malondialdehyde (MDA), trichloroacetic acid, and 2-thiobarbituric acid, were procured from Sigma-Aldrich (USA). Enzyme-linked immunosorbent assay (ELISA) kits for tumor necrosis factor-alpha (TNF-α) (Version 13.0 SEA133Ra, Cloud-Clone Corp., Wuhan, Hubei, China) and glutathione (GSH) (Version 13.0 CEA294Ge, USCN Life Science Inc., Wuhan, Hubei, China) were used. Micro-method assay kits for superoxide dismutase (SOD) (D799594-0100, Sangon Biotec, Shanghai, China) and catalase CAT (D7799598-0100, Sangon Biotec, Shanghai, China) were used for the assessment of antioxidant enzyme activities. Chlorpromazine (Largactil) was from Sanofi-Aventis Pakistan Ltd (Karachi, Sindh, Pakistan), while carbidopa/levodopa (Sinemet) was obtained from Searle Pakistan Ltd (Karachi, Sindh, Pakistan). Only analytical grade chemicals were used in this study.

2.2 Bar Composition and Preparation

The energy bar was formulated using the following functional ingredients: oats (*Avena sativa*), pumpkin seeds (*Cucurbita moschata*), figs (*Ficus carica*), coconut (*Cocos nucifera*), and walnuts (*Juglans regia)*. The energy bar was designed in accordance with internationally recognized dietary guidelines, ensuring that the daily intake of nuts, seeds, and oats in the formulation fell within recommended consumption limits [16], thereby providing a safe and nutritionally relevant intervention for experimental use. All components were obtained from the local market and authenticated, and Ziauddin University allotted herbarium numbers: oats (031-25-ASS), fig (036-25-FCF), pumpkin (0335-25-CPS), walnut (035-25 JRS), and coconut (032-25-CNH). Ingredients were ground, mixed with coconut water (used as a binding agent and to enhance palatability for rats), molded (10 × 3 × 2 cm), and baked at 130 °C for 10 min [17]. The bar was cooled and stored in airtight containers. The composition and caloric contribution of each ingredient per 70 g walnut energy bar (268 kcal) are presented in Table 1:

Table 1. Composition of energy bar.

Ingredient	Amount in bar	Recommended daily intake (g/day)	Calories per walnut bar (kcal)
Oats	25 g	40–80	97.25
Figs	10 g	40–80	24.90
Walnut	10 g	30	65.20
Pumpkin seeds	10 g	20–30	44.60
Coconut	10 g	10–15	35.40
Coconut water	5 mL	-	0.95

Table 1: Values represent the amount of each ingredient (g) used to prepare the energy bar.

Overall, the walnut energy bar (70 g) provides 268 kcal, consistent with daily dietary recommendations for nuts, seeds, and grains [16].

2.3 Experimental Animals

Healthy Wistar albino rats (n = 24) of both sexes (12 weeks old, 150–200 g) were used. Rats were housed 6 per cage under a 12:12 h light/dark cycle, with controlled temperature and humidity, and were provided a standard pellet diet and water *ad libitum*. Animals were habituated for one week before the experiment [18].

2.4 Experimental Design

Chlorpromazine (3 mg/kg, intraperitoneal (i.p.)) was administered daily for 30 days to induce the Parkinsonism model [19]. Animals were randomly assigned to four groups: Group I (control), standard diet + water. Group II (disease control), standard diet + chlorpromazine 3 mg/kg, i.p. Group III (standard treatment), standard diet + chlorpromazine 3 mg/kg, i.p., plus carbidopa/levodopa + (100 + 25 mg/kg, oral). Group IV (test group; walnut bar), standard diet + chlorpromazine 3 mg/kg, i.p., followed by walnut energy bar at 1 g/kg body weight/day orally for 30 days.

Rationale for choosing 1 g/kg/day: This dose falls within the reported safe oral dose range for rats (1–3 g/kg/day) and is scaled to achieve therapeutic relevance based on the total bar content (70 g) [20]. The results of the preliminary acute and sub-chronic toxicity studies, including gross behavioral, hematological, renal, and hepatic parameters, are provided in the **Supplementary Material (Tables 1–4)**. Behavioral assessments were conducted on day 30. After completion of these tests, animals were anesthetized through intraperitoneal injection of ketamine (100 mg/kg) combined with xylazine (10 mg/kg). Animals were closely monitored until the complete loss of reflexes. Exsanguination was then performed under deep anesthesia for euthanasia. The absence of heartbeat, corneal reflex, pedal withdrawal reflex, and cessation of vital signs confirmed death. The brains were then carefully removed, cleaned, and processed for biochemical analysis [21].

2.5 Antioxidant Activity: DPPH Radical Scavenging Assay

The DPPH assay was used to determine the free radical-scavenging activity of the energy bar extract. In brief, 1 mL of the sample solution (2 g/50 mL in methanol) was mixed with 3 mL of 0.1 mM DPPH in methanol and incubated in the dark for 30 min. The absorbance was recorded at 517 nm against a blank. Ascorbic acid was used as a standard. The radical scavenging activity (%) was expressed as ascorbic acid equivalents (AAEs, mM/100 g) as presented in Table 2 [22].

2.6 Gas Chromatography–Mass Spectrometry (GC-MS) Analysis

The chemical composition of energy bar extracts was analyzed using an Agilent Technologies 7000 GC-MS Triple Quad (TQQQ) system. Helium was used as carrier gas, and separation was achieved on an OPTIMA-5 column (30 m × 250 µm × 0.25 µm). A sample (2.5 µL) was injected, and electron ionization was performed at 70 eV. Internal standards and calibration curves were included for semi-quantitative analysis. Compounds were identified by comparing mass spectra with the NIST database, and retention indices were calculated using Kovats' formula [22].

2.7 High-Performance Liquid Chromatography (HPLC)

2.7.1 Sample and Standard Preparation

For sample preparation, 5 g of the energy bar was placed in a conical flask, soaked in 50 mL of methanol, and left for 24 h. The extracts were then filtered with Whatman No. 1 filter paper. Cinnamic acid and ferulic acid standards were prepared in HPLC-grade methanol. Following 15 minutes of sonication, all standard and sample solutions were filtered through a 0.45 µm membrane filter before analysis by HPLC [23].

2.7.2 Chromatographic Conditions for HPLC

HPLC analysis was performed on a Shimadzu LC-20 AT system with an ultraviolet (UV)-visible (Vis) detector (SPD-10A), and Class LC-20 software version 1.62, Kyoto, Japan. Separation was conducted at room temperature in isocratic mode on a C18 reversed-phase column (150 mm × 4.6 mm, 5 µm particle size). The flow rate was 1 mL/min, and the injection volume was 20 µL [24].

2.7.3 Selection of Wavelength and Mobile Phase

The sample and standard solutions were scanned across a broad UV range, and detection was performed at

Table 2. DPPH radical scavenging activity of the walnut energy bar extract.

Extract	Concentration (mg/mL)	Percentage inhibition (%)	IC_{50}	AAE (mM/100 g)
Walnut energy bar	20	53.1	19.2	0.15
	18	48.07		0.13
	16	30.83		0.12
	14	22.02		0.12
	12	16.35		0.13

DPPH, 2, 2-Diphenyl-1-picrylhydrazyl; AAE, ascorbic acid equivalent.

270 nm. The mobile phase consisted of methanol and water (80:20, v/v) containing 0.1% orthophosphoric acid. The internal standard and other sample components were effectively resolved from the cinnamic acid and ferulic acid peaks. Calibration curves generated using standard solutions exhibited good linearity over the investigated range (R^2 ≥0.99) [25]. Ferulic and cinnamic acids were quantified by HPLC using standard curves (y = 2484.9x + 177674, R^2 = 0.9656) and (y = 2736.4x + 279574, R^2 = 0.9336), respectively.

2.8 Behavioral Assessment

2.8.1 Bar Test

Rats were placed with their forepaws on a 5 mm horizontal metal bar, and the time for which each rat maintained the pose was recorded. The endpoint was recorded when the rat moved. The cut-off time was 180 s. Readings were recorded on days 1, 15, and 30 [26].

2.8.2 Rota-Rod Test

Motor coordination was evaluated using a rod with a 9 cm diameter that rotated at 15 rpm. The time for which the rats maintained balance was recorded on days 1, 15, and 30 [27].

2.9 Biochemical Assays

2.9.1 Tumor Necrosis Factor-Alpha (TNF-α)

Brain tissues were isolated and stored at –40 °C. Samples and standard dilutions were added to microplate wells pre-coated with TNF-α antibodies, followed by biotin-conjugated antibody, avidin-horseradish peroxidase (HRP), and tetramethylbenzidine (TMB) substrate. Color change was measured at 450 nm after the reaction was terminated with sulfuric acid [28].

2.9.2 Glutathione (GSH)

To assess GSH levels, the brain tissue homogenate was quantified. A total of 50 µL of standards, blank, and samples were added to designated wells, followed immediately by 50 µL of Detection Reagent A. Plates were sealed and incubated at 37 °C for 1 h. After incubation, wells were washed three times with 1× wash buffer. Then, 100 µL of detection reagent B was added and incubated at 37 °C for about 30 min, followed by 5 wash cycles. The reaction mixture received 90 µL of substrate solution and was incubated at 37 °C in the dark for 20 min to develop the color.

The reaction was terminated by adding 50 µL of stop solution, resulting in a color change from blue to yellow. Absorbance was measured immediately at 450 nm using a microplate reader, and the results are expressed as µmol/mg protein [29].

2.10 Analysis of Antioxidant Enzymes and Oxidative Stress

CAT activity was measured using a micro-method that monitored hydrogen peroxide breakdown via absorbance at 240 nm. The absorbance values were recorded with a Shimadzu UV-1800 spectrophotometer. CAT activity was calculated and expressed as (U/mg protein) using the formula = ΔA × 459 / protein concentration.

SOD activity was measured using a reaction mixture containing xanthine and xanthine oxidase to produce superoxide radicals. Enzyme activity was evaluated by measuring absorbance at 560 nm, and SOD activity is expressed as percentage inhibition, calculated using the formula: SOD (U/mg protein) = ΔA × 459 / protein concentration.

Thiobarbituric acid reactive substances were used to assess lipid peroxidation. To extract the supernatant, rat brain tissues were homogenized in ice-cold 0.1 M phosphate buffer (pH 7.4) and then centrifuged at 4 °C for 10 min. Subsequently, 1 mL of 0.6% thiobarbituric acid (TBA) and 1 mL of 10–20% trichloroacetic acid (TCA) were mixed with a 0.2 mL aliquot of the sample. The sample was vortexed for 30 s and then heated in an acidic boiling water bath (95–100 °C) for 15–20 min. After heating, samples were allowed to cool at room temperature before being centrifuged for 10 min. The absorbance of the pink chromogen formed at 532 nm, relative to a reagent blank, was measured with a UV-Vis spectrophotometer. A standard curve prepared with 1,1,3,3-tetramethoxypropane was used to calculate the MDA concentration, which was then expressed as nmol MDA/mL or nmol MDA/mg protein. A molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} was used for the calculations. Readings were recorded using a UV-Vis spectrophotometer (Shimadzu UV-1800, Shimadzu Corporation, Kyoto, Japan) [30].

2.11 Histopathology

At the end of the study, rats from all groups were sacrificed, and their brain tissues were isolated for histopathological analysis. Brain tissue from each group was removed, cleaned with normal saline, and then stored in

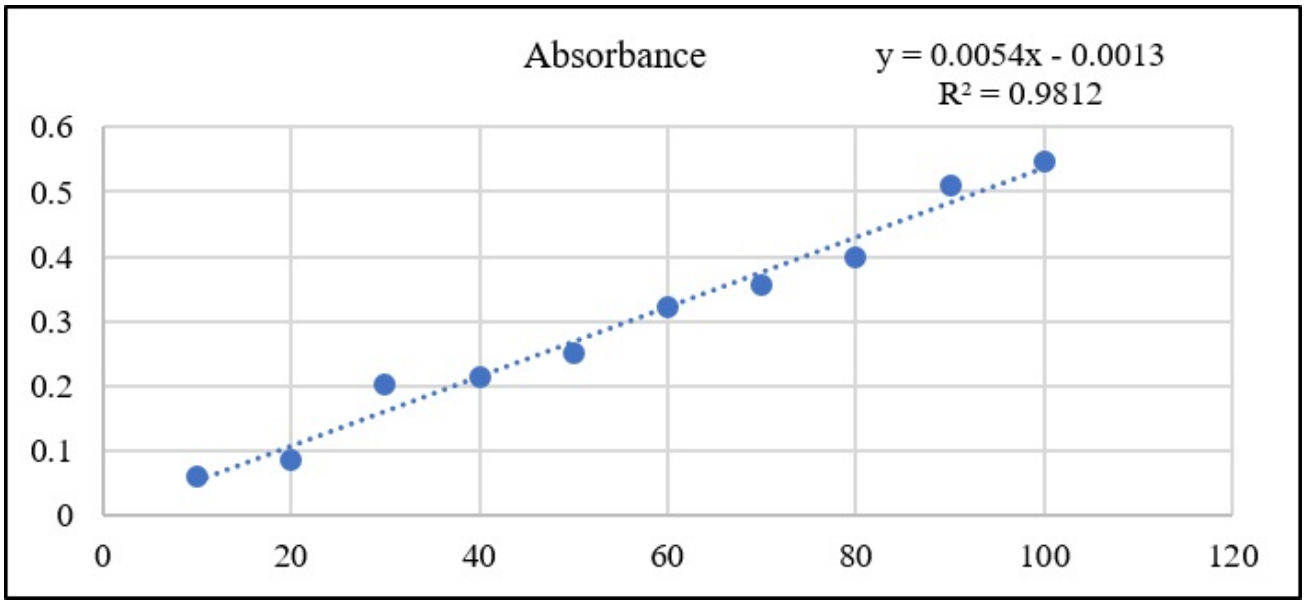


Fig. 1. Calibration curve of ascorbic acid standard for DPPH assay.

10% formalin. Tissue slices were prepared and stained with hematoxylin and eosin (H&E) for microscopic analysis [31].

2.12 Statistical Analysis

Data were analyzed using two-way analysis of variance (ANOVA) followed by Tukey's post hoc test to determine differences between groups and across time points. A p-value < 0.05 was considered statistically significant. SPSS version 22.0 (IBM Corp., Armonk, NY, USA) was used for analysis.

3. Results

3.1 2, 2-Diphenyl-1-picrylhydrazyl (DPPH)

Table 2 and Fig. 1 show the percent inhibition and IC_{50} (half-maximal inhibitory concentration) values of walnut energy bar extract. The % inhibition ranged from 53.1% (20 mg/mL) to 16.35% (12 mg/mL), with an antioxidant content of 0.12-0.15 mM AAE/100 g. The IC_{50} was 19.2 mg/mL, showing a clear dose-related effect. Fig. 1 represents the ascorbic acid standard curve.

3.2 GC-MS Analysis

GC-MS analysis of the energy bar revealed the presence of several bioactive compounds associated with antiparkinsonian and neuroprotective activity. A total of four major compounds were identified. Compound 1, octanoic acid, showed the highest percentage (84%) with a retention time of 13.8 min and a retention index of 1173. Compound 2, n-decanoic acid, was detected at 82.5%, with a retention time of 17.6 min and a retention index of 1372. Compound 3, lauric anhydride, was present with 68% area, retention time of 25.4 min, and retention index of 2710. Compound 4, an eicosanoid acid, showed 62.4% and had a retention time of 76.6 min and a retention index of 5926. All identified compounds are associated with antiparkinsonian and neuroprotective potential (Table 3, Ref. [32,33,34,35]). Peaks of all compounds are displayed in the spectra in Fig. 2.

3.3 High-Performance Liquid Chromatography (HPLC)

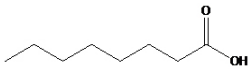
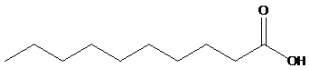
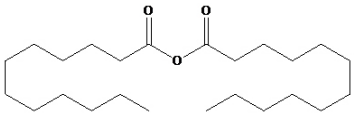
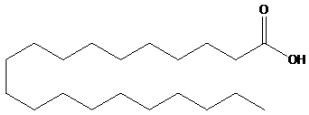
Fig. 3 shows the sample chromatogram with well-differentiated peaks that match the typical retention times of cinnamic and ferulic acids, indicating their presence at approximately 2.61 and 3.12 min, respectively, confirming the presence of hydrocinnamic acid derivatives in the walnut energy bar. Figs. 4,5 show the standard calibration curves for ferulic and cinnamic acids. Table 4 shows the amounts of ferulic acid and cinnamic acid, which were 11.74 ± 0.31 (0.023%) and 18.3 ± 0.51 (0.036%), respectively, in the walnut energy bar. The HPLC system suitability parameters are presented in Table 5.

3.4 Behavioral Test

3.4.1 Bar Test

The results presented in Fig. 6 revealed that at 15 and 30 days, the disease group showed a highly significant increase in the time taken to turn on the bar, confirming the induction of catatonia compared to the control group. In the standard treatment group, immobility time was significantly reduced on both days 15 and 30 compared to the disease group. The group treated with the walnut energy bar also showed a significant reduction in immobility time on days 15 and 30 compared with the disease group, indicating improved motor function; however, latency remained slightly higher than in the treatment group. Within-group comparisons showed a significant difference between day 30 and day 15 across all groups.

Table 3. Compounds identified in the walnut energy bar by GC-MS.

S. no.	Compound	% match	Area (%)	Retention time	Retention index (IU)	Antiparkinsonian
1	Octanoic acid 	84%	0.70	13.8	1173	[32]
2	n-decanoic Acid 	82.5%	0.11	17.6	1372	[33]
3	Lauric anhydride 	68%	0.06	25.4	2710	[34]
4	Eicosanoid acid 	62.4%	0.17	76.6	5926	[35]

RI, retention index (optima-5 column); area %, percentage calculated by their relative percentages of total chromatogram area; GC-MS, gas chromatography-mass spectrometry. Sample (walnut energy bar).

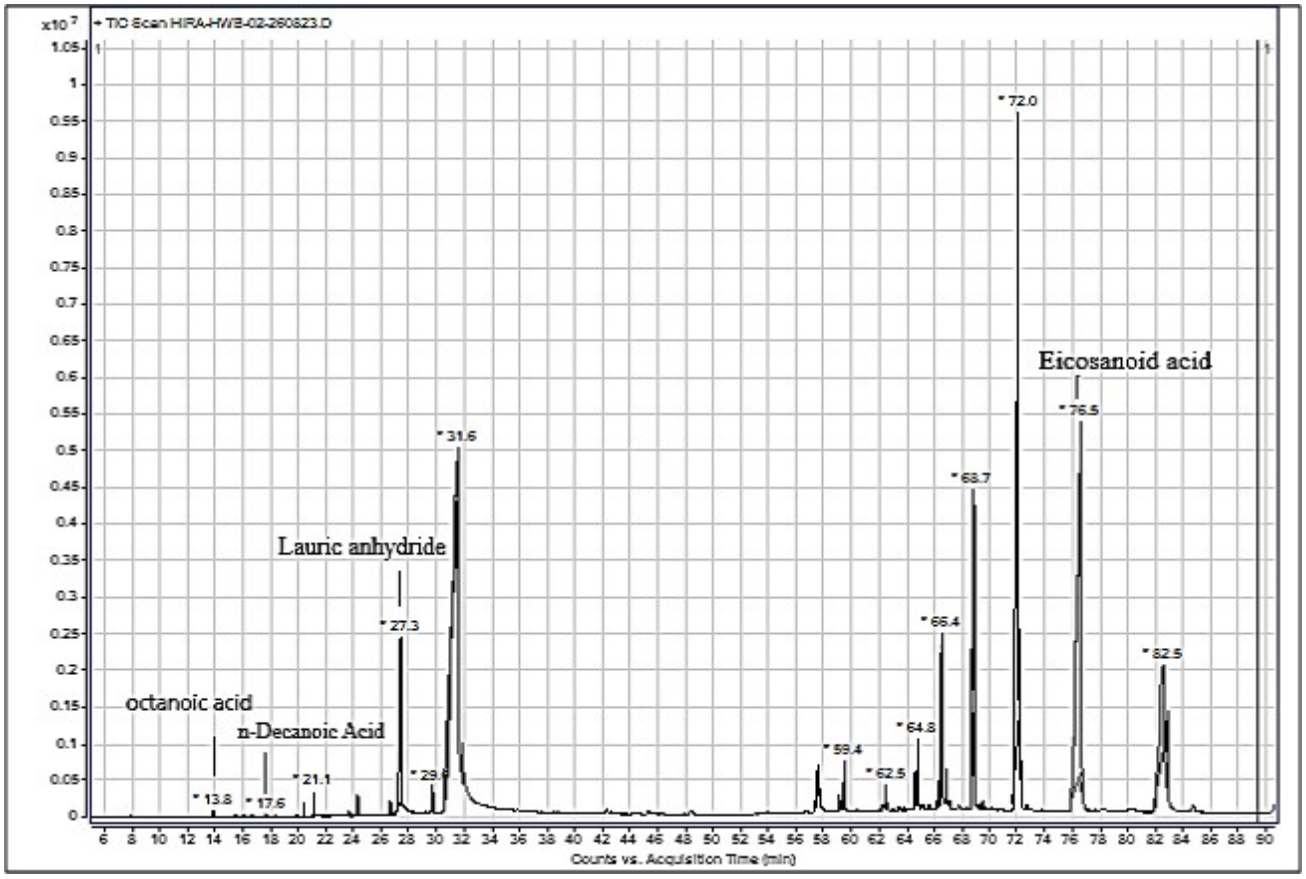


Fig. 2. GC-MS chromatogram of the analyzed compounds.

3.4.2 Rota-Rod Test

As shown in Fig. 6, among the disease group, falling time was significantly reduced ($p < 0.05$) on days 15 (a) and 30 (ab) compared with days 1 and 15, respectively. In the standard-treated group, falling time was significantly increased ($p < 0.05$) on days 15 (a) and 30 (ab) compared with the disease group. The group fed the walnut energy bar showed a significant increase in falling time ($p < 0.05$) on day 15 (a) compared with day 1, with a further significant improvement on day 30 (ab) compared with day 15.

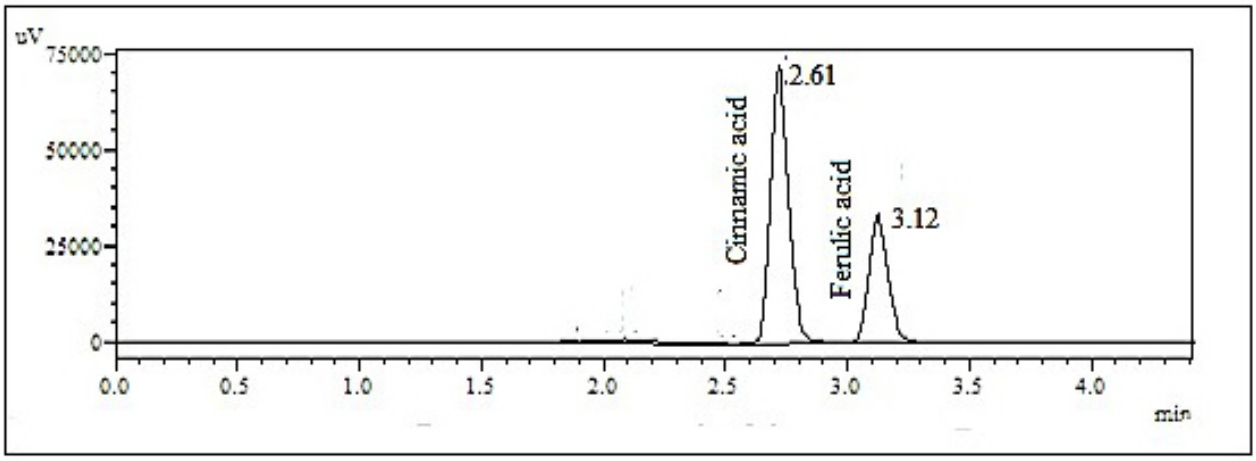


Fig. 3. Chromatogram peaks for the cinnamic and ferulic acids identified in the walnut energy bar.

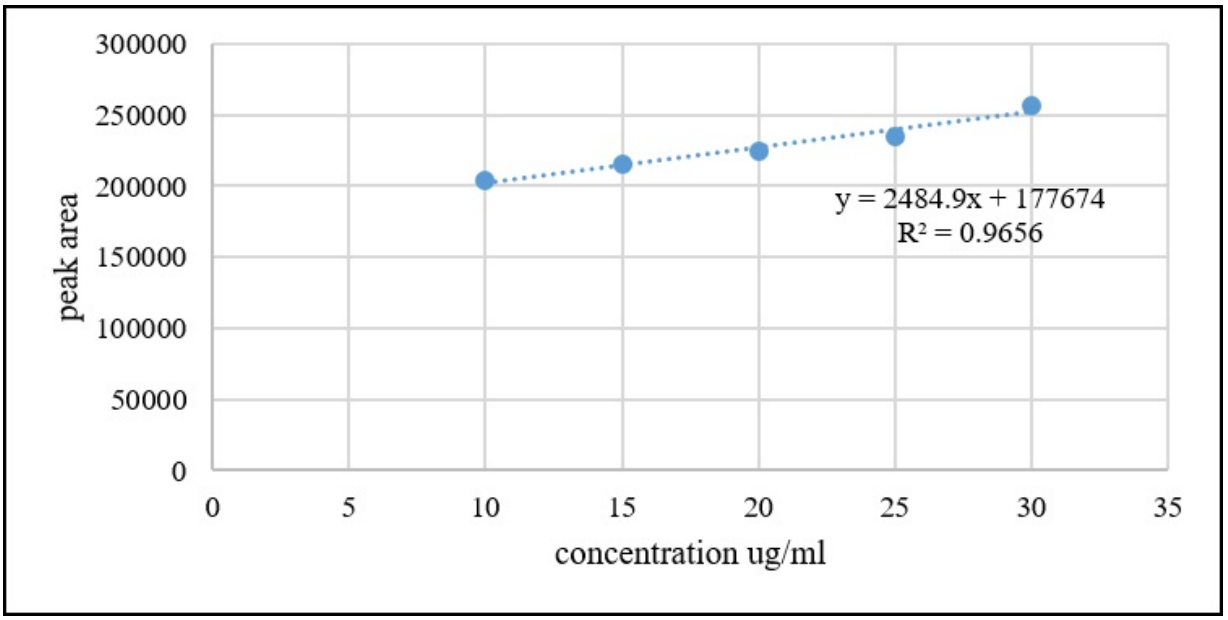


Fig. 4. Standard calibration curve for ferulic acid.

Table 4. Quantification of ferulic and cinnamic acids in the walnut energy bar (n = 3).

Compound	Amount (µg/mL)	Content (%)
Ferulic acid	11.74 ± 0.31	0.023
Cinnamic acid	18.30 ± 0.51	0.036

Table 4: Values are represented as µg/mL ± S.D.

Table 5. System suitability parameters of the HPLC method for the identification of phenolic acids in the walnut energy bar.

Parameters	Walnut energy bar	
	Cinnamic acid	Ferulic acid
Retention time, R (min)	2.61	3.12
Capacity factor, k’	0.293	1.020
Theoretical plates, n	3197	3101
Tailing factor, T	1.173	1.01
Resolution, R	0.985	0.483
Coefficient of determination	0.9336	0.9656

HPLC, high-performance liquid chromatography.

3.5 Biochemical Assays

3.5.1 Tumor Necrosis Factor-Alpha (TNF-α)

TNF-α, a proinflammatory cytokine, was significantly elevated in the chlorpromazine-treated (disease) group compared with the control group, indicating an increased inflammatory response. Treatment with the standard drug group resulted in normalized TNF-α levels compared with the disease group. The walnut energy bar group showed a noticeable reduction in TNF-α levels compared to the dis-

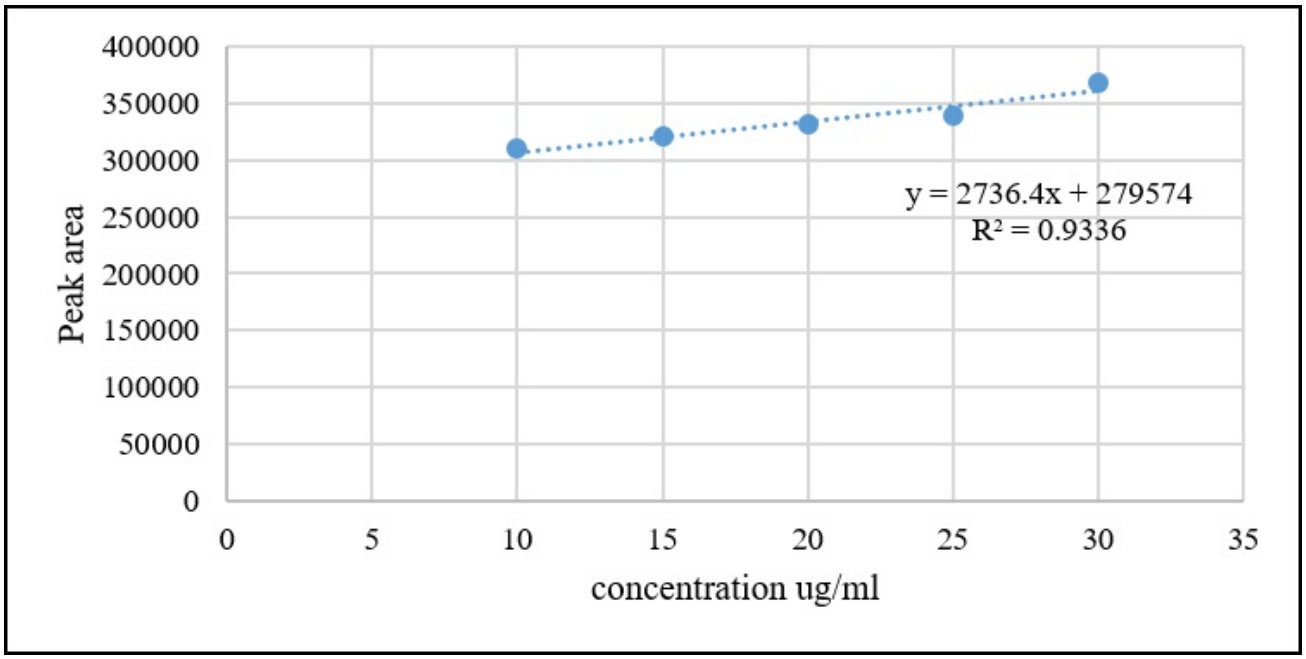


Fig. 5. Standard calibration curve for cinnamic acid.

ease group, rendering their levels comparable to those observed with standard treatment as shown in Fig. 7.

3.5.2 Glutathione (GSH)

GSH, a key endogenous antioxidant, was significantly decreased in the disease group compared with the control group, indicating enhanced oxidative stress induced by chlorpromazine. In contrast, standard drug treatment restored GSH levels to normal, whereas the group treated with the walnut energy bar showed a comparable protective effect to the standard treatment, suggusting its potencial role in attenuating oxidative stress Fig. 7.

3.6 Analysis of Antioxidant Enzymes and Oxidative Stress

The antioxidant enzymes CAT and SOD were reduced in the disease group, while MDA levels were significantly elevated compared with the control group. The standard group showed restored CAT and SOD levels with reduced MDA levels compared to the disease groups. The walnut energy bar-treated group also showed improved antioxidant activity, with increased CAT and SOD levels and reduced MDA levels compared to the disease group; overall, the walnut energy bar group demonstrated a significant difference compared with the standard treatment groupas illustrated in Fig. 8.

The treatment showed a statistically significant difference compared with the standard group.

3.7 Histopathology

Fig. 9 illustrates histopathological analysis of the rat brain tissue (cerebral cortex), supporting the behavioral and biochemical findings. (a) At 40× magnification, the tissue showed no signs of neuronal damage or gliosis; the overall structure was intact and well-organized, with no evidence of vacuolization or cellular infiltration. Red arrows show glial cells, and black arrows show normal neuronal cells. (b) At 40× magnification, the tissue shows preserved architecture; no pathological changes were observed. The red arrow shows glial cells, whereas the black arrows indicate normal neurons. (c) At 40× magnification, the tissue reveals mostly preserved architecture with minimal vacuolization, normal neuronal cells marked with black arrows, and glial cells indicated by a red arrow. (d) At 40× magnification, there is evidence of disrupted architecture, vacuolization, and gliosis marked with red arrows, indicative of chronic neurodegenerative or ischemic changes. Black arrows show cellular infiltration, suggesting an inflammatory response.

4. Discussion

Chronic neuroinflammation is a key factor in dopaminergic neuronal death. When microglia and astrocytes are activated, proinflammatory cytokines such as TNF-α, IL-1β, and IL-6 are released. These cytokines exacerbate oxidative stress, disrupt mitochondrial function, and trigger apoptotic pathways in dopaminergic neurons. In parallel, activation of nitric oxide synthase and NADPH oxidase increases oxidative damage and ROS generation [36].

In this study, the dopamine D2 receptor antagonist chlorpromazine was used to induce Parkinsonian symptoms. Prolonged D2 blockage disrupts basal ganglia circuits, inhibits nigrostriatal dopaminergic transmission, and causes bradykinesia, stiffness, and postural instability. Moreover, dopamine metabolism under oxidative

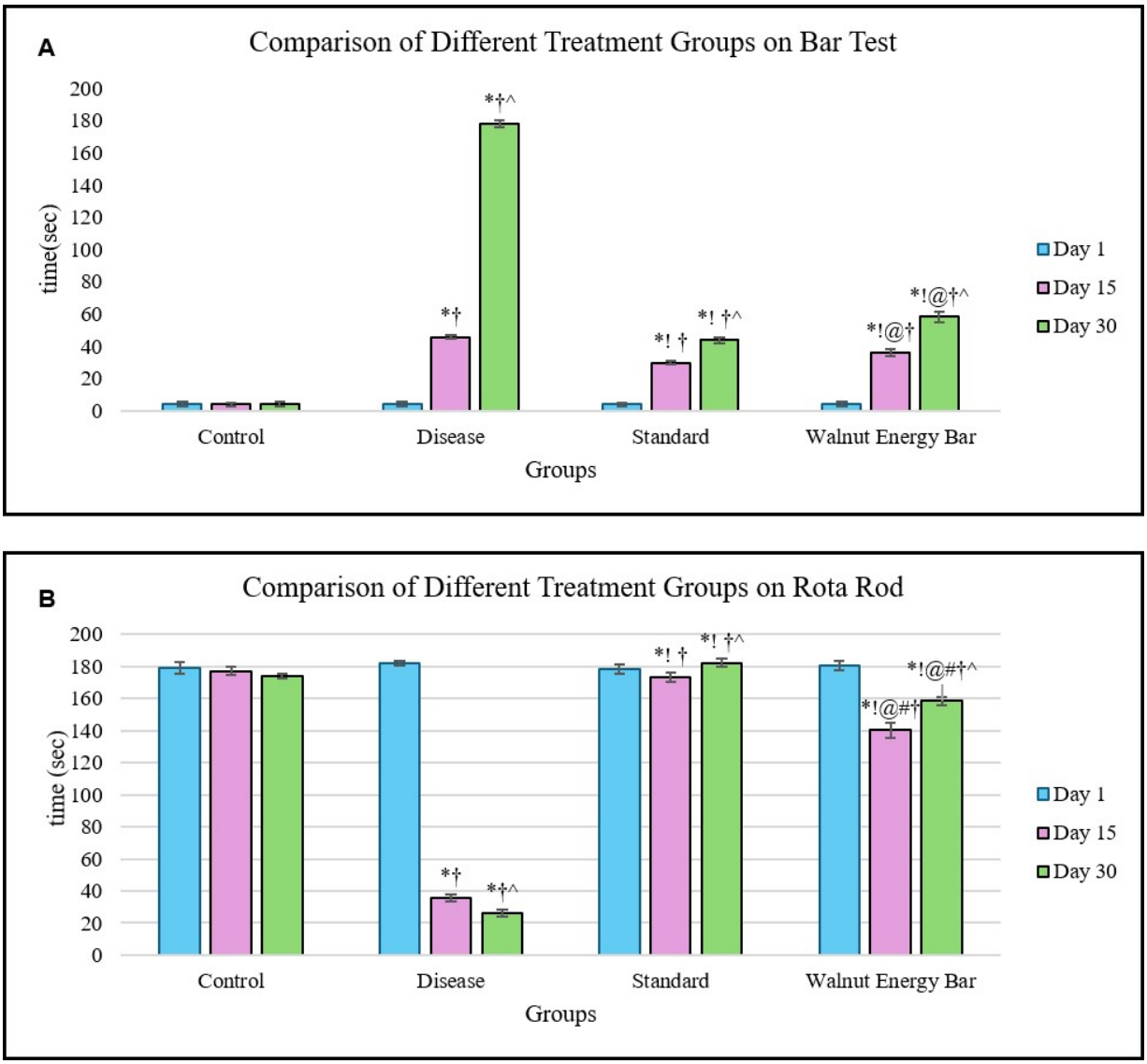


Fig. 6. Behavioral assessment. Values are presented as the mean ± standard deviation (SD) from six rats per group (two-way analysis of variance (ANOVA) followed by Tukey's honestly significant difference (HSD) test). (a) Comparison of different treatment groups on the bar test. (b) Comparison of different treatment groups on the Rota-rod. * marked difference compared with the control; ! shows a substantial difference compared with the disease; @ shows a notable difference compared with the standard; # shows a notable difference compared with Almond bar; (†) shows a significant comparison to day 1; (^) shows a significant comparison to day 15.

stress generates ROS that modify proteins and lipids [37]. Dopaminergic neuronal damage, lipid peroxidation, and functional impairments are caused by the highly neurotoxic environment generated by this dual mechanism of receptor blockade and ROS overproduction [38]. On days 15 and 30, the shorter fall times on the Rota-rod in the disease group confirm motor impairment and loss of coordination.

Dietary supplementation with an antioxidant-enriched energy bar significantly enhanced motor performance. Ferulic acid and cinnamic acid were the main phenolic compounds identified by HPLC profiling. Moreover, ferulic acid provides neuroprotection by scavenging ROS, activating nuclear factor erythroid 2- related factor 2 (Nrf2)-dependent antioxidant enzyme expression, maintaining mitochondrial membrane integrity, and inhibiting lipid peroxidation [39]. Indeed, by inhibiting cytochrome c release and caspase activation, ferulic acid further modulates apoptotic pathways and reduces dopaminergic neuronal death. Meanwhile, cinnamic acid supports the antioxidant effects of the bar by reducing neuroinflammation, inhibiting the release of proinflammatory cytokines, and activating peroxisome proliferator-activated receptor-alpha (PPAR-α) in astrocytes [40,41].

Neuroprotective fatty acids were recognized by complementary GC–MS profiling. These fatty acids preserve membrane integrity, promote mitochondrial activity, and reduce microglial activation. Previous literature has also reported the involvement of fatty acids in anti-parkinsonian

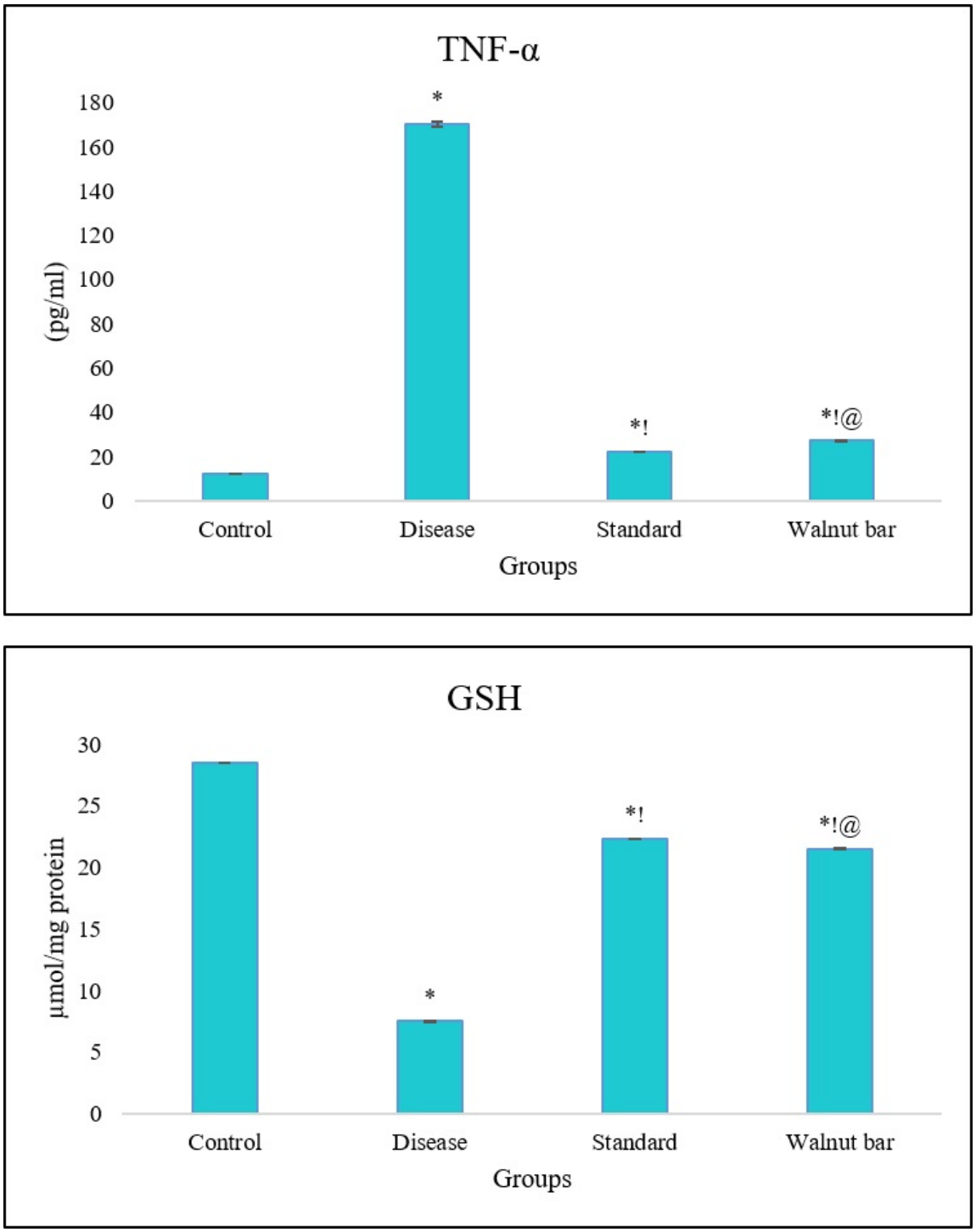


Fig. 7. Tumor necrosis factor-alpha (TNF-α) and glutathione (GSH) analysis in the brain tissue of rats. * Shows a marked difference compared with the control. ! shows a substantial difference compared with the disease group. @ shows a notable difference compared with the standard treatment.

activity (Table 3). Strong antioxidant activity was further demonstrated by 2, 2-Diphenyl-1-picrylhydrazyl (DPPH) assays, which revealed the presence of bioactive compounds with free-radical-scavenging potential.

Biochemically, the walnut energy bar-treated group showed restored redox balance, with considerably lower MDA levels, higher GSH content, and greater SOD and CAT activity compared with the disease group. Additionally, the lower TNF-α levels observed in the walnut bar-treated group compared with the disease group indicate an anti-inflammatory effect.

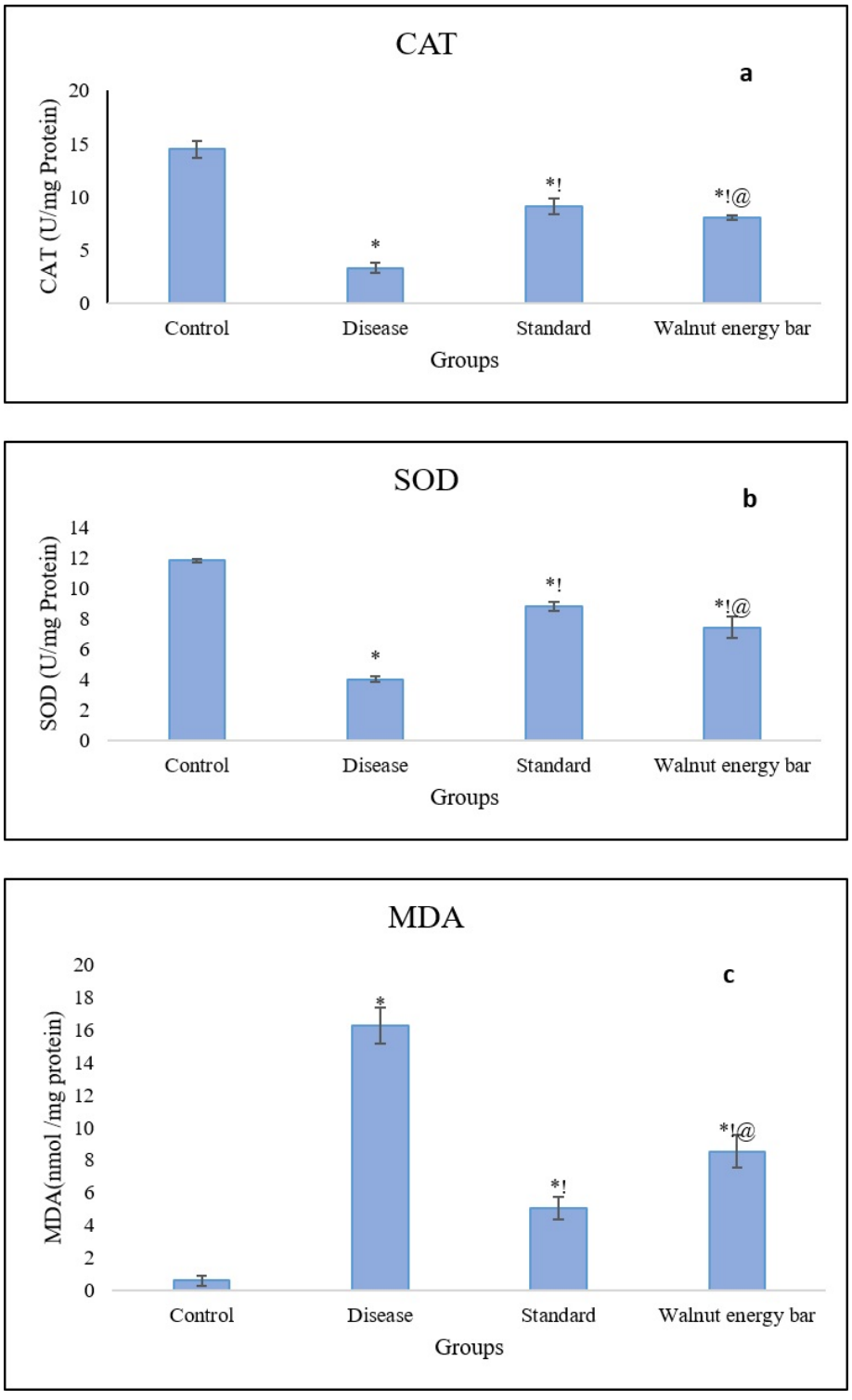


Fig. 8. Catalase (CAT), superoxide dismutase (SOD), and malondialdehyde (MDA) levels in the brain tissue of rats. (a) CAT (U/mg protein). (b) SOD (U/mg protein). (c) MDA (nmol/mg protein). * shows a marked difference compared with the control. ! shows a substantial difference compared with the disease group. @ shows a notable difference compared with the standard treatment.

Histopathological analysis also confirmed these findings. The disease group showed inflammatory infiltration, altered neuronal architecture, vacuolization, and widespread gliosis. Conversely, walnut bar-treated rats showed largely preserved neuronal organization with negligible vacuolization. Therefore, the walnut bars appear to provide comprehensive neuroprotection, as indicated by consistent results from motor, biochemical, and histological assessments.

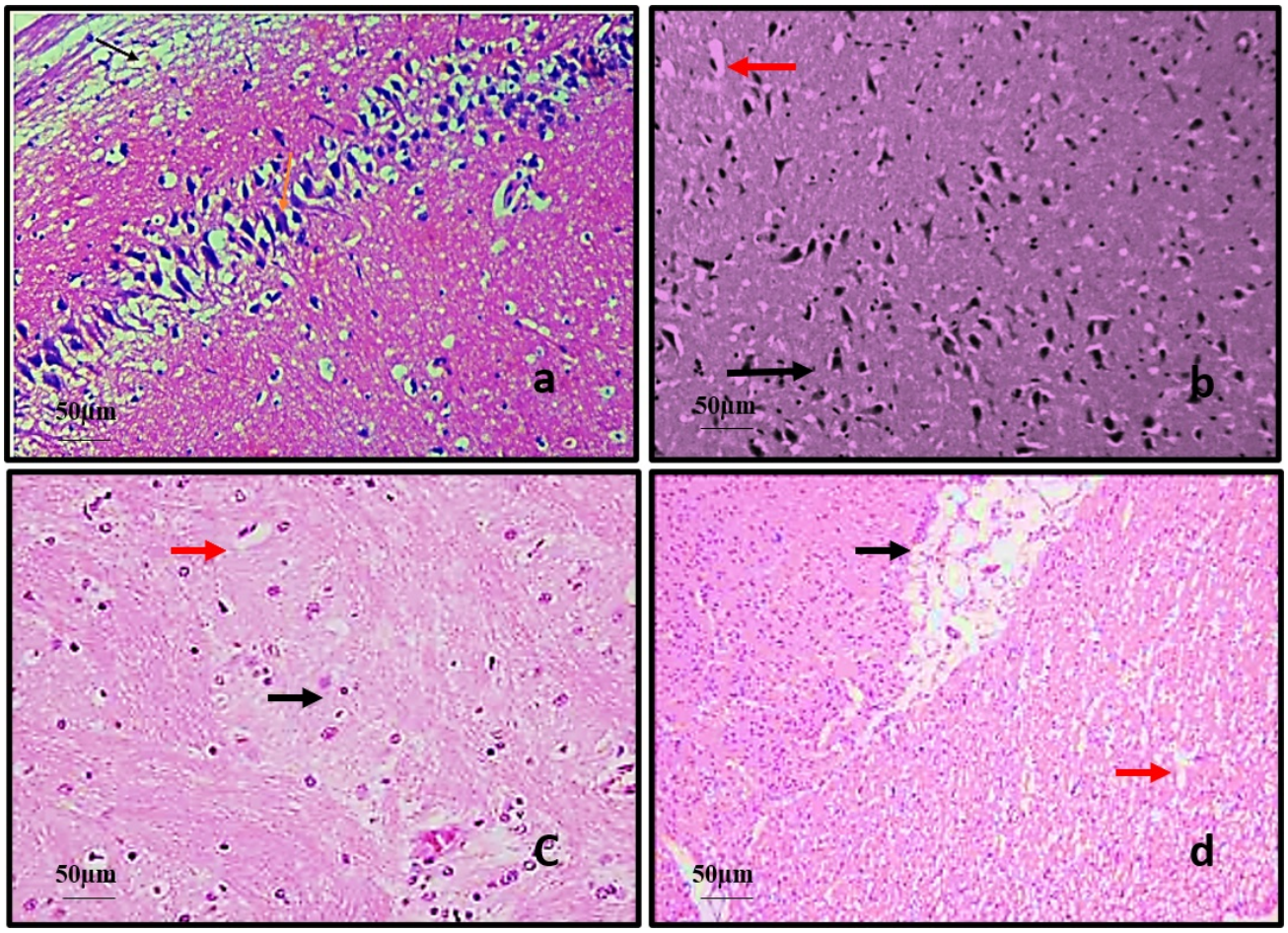


Fig. 9. Photomicrographs of the histopathological changes in the cerebral cortex of experimental groups. (a) Control group with normal cortical architecture. (b) Walnut energy bar-treated group with preserved neuronal architecture. (c) Standard-treated group with partial restoration of cortical structure. (d) Disease group with neuronal degeneration and cellular disorganization (hematoxylin and eosin (H&E) staining, magnification ×40 and scale bar = 50 µm). Red arrows indicate normal glial cells and black arrows indicate normal neuronal cells (a–c). Red arrow indicates gliosis, and black arrow indicates cellular infiltration (d).

5. Mechanistic Highlights

Notably, chlorpromazine causes Parkinsonian features by blocking the dopamine D2 receptor, producing excessive ROS, disrupting basal ganglia circuits, damaging mitochondria, and inducing neuronal apoptosis. Meanwhile, energy bars enriched with ferulic and cinnamic acids counteract these processes by scavenging free radicals, strengthening SOD, CAT, and GSH defenses, stabilizing mitochondria, and reducing TNF-α-mediated inflammation. Neuroprotective fatty acids also promote mitochondrial activity and membrane integrity. Thus, owing to the associated increases in phenolic and fatty acid content, the walnut bar offers multimodal protection through these combined mechanisms, thereby restoring redox balance, minimizing neuroinflammation, and preserving neuronal architecture.

6. Translational Relevance

Dietary interventions rich in phenolic acids, such as cinnamic and ferulic acids, which have potent anti-inflammatory and antioxidant properties, may help preserve neurons, enhance motor function, and slow the progression of PD. Although more research in humans is required, these results suggest the potential of nutraceuticals as adjuncts to pharmaceutical treatments.

7. Limitations

The sample size was small and may have reduced statistical power and limited the generalizability of the findings. Moreover, evaluating a single dose over a short period of time limits conclusions about long-term efficacy. Additionally, underlying molecular mechanisms were not explored in detail beyond oxidative stress and inflammatory markers. Future preclinical and clinical studies are needed to validate these findings.

8. Conclusion

Dopamine receptor blockage is exacerbated by oxidative stress and neuroinflammation in Parkinsonism caused

by chlorpromazine. These degenerative processes are reduced by energy bars high in ferulic and cinnamic acids and neuroprotective fatty acids, which strengthen antioxidant defenses, inhibit proinflammatory signaling, and maintain mitochondrial and neural integrity. The improved neuroprotection observed with the walnut energy bar, which has higher bioactive content, highlights the therapeutic potential of dietary antioxidant therapies in PD models.

Availability of Data and Materials

Data are available from the corresponding author on reasonable request.

Author Contributions

HR and SS designed the study. HR performed the experiments. SS analyzed the results and verified the data. FQ contributed to data analysis and interpretation of the results. MMA and MR assisted with additional experimental support and data interpretation. AE and SZ contributed to manuscript preparation, revision, and search reference. All authors reviewed and approved the final version of the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript. All authors have participated sufficiently in the work and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

Ethical approval for this study was obtained from the Institutional Bioethical Committee, University of Karachi, Pakistan (IBC-KU-356/2023). The study was conducted in accordance with the relevant institutional ethical standards and animal welfare principles for the care and use of laboratory animals, following the specifications described by HUBRECHT and Kirkwood (2010). The study was also reported in accordance with the ARRIVE guidelines.

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Conflicts of Interest

The authors declare no conflicts of interest.

Supplementary Material

Supplementary material associated with this article can be found in the online version at https://doi.org/10.31083/IJP51826.

References

[1] Ramesh S, Arachchige ASPM. Depletion of dopamine in Parkinson's disease and relevant therapeutic options: A review of the literature. AIMS Neuroscience. 2023; 10: 200–231. https://doi.org/10.3934/Neuroscience.2023017

[2] Bogetofte H, Alamyar A, Blaabjerg M, Meyer M. Levodopa Therapy for Parkinson's Disease: History, Current Status and Perspectives. CNS & Neurological Disorders Drug Targets. 2020; 19: 572–583. https://doi.org/10.2174/1871527319666200722153156

[3] Chakrabarti S, Bisaglia M. Oxidative Stress and Neuroinflammation in Parkinson's Disease: The Role of Dopamine Oxidation Products. Antioxidants (Basel, Switzerland). 2023; 12: 955. https://doi.org/10.3390/antiox12040955

[4] Soumya NP, Mini S, Sivan S, Mondal S. Bioactive compounds in functional food and their role as therapeutics. Bioactive Compounds in Health and Disease. 2021; 4: 24–39. https://doi.org/10.31989/bchd.v4i3.786

[5] Shoaib S, Ansari MA, Fatease AA, Safhi AY, Hani U, Jahan R, et al. Plant-Derived Bioactive Compounds in the Management of Neurodegenerative Disorders: Challenges, Future Directions and Molecular Mechanisms Involved in Neuroprotection. Pharmaceutics. 2023; 15: 749. https://doi.org/10.3390/pharmaceutics15030749

[6] Kujawska M, Jodynis-Liebert J. Polyphenols in Parkinson's Disease: A Systematic Review of In Vivo Studies. Nutrients. 2018; 10: 642. https://doi.org/10.3390/nu10050642

[7] Chiang MC, Tsai TY, Wang CJ. The Potential Benefits of Quercetin for Brain Health: A Review of Anti-Inflammatory and Neuroprotective Mechanisms. International Journal of Molecular Sciences. 2023; 24: 6328. https://doi.org/10.3390/ijms24076328

[8] Zhang M, Wang S, Mao L, Leak RK, Shi Y, Zhang W, et al. Omega-3 fatty acids protect the brain against ischemic injury by activating Nrf2 and upregulating heme oxygenase 1. The Journal of Neuroscience: the Official Journal of the Society for Neuroscience. 2014; 34: 1903–1915. https://doi.org/10.1523/JNEUROSCI.4043-13.2014

[9] Salehi B, Azzini E, Zucca P, Maria Varoni E, V. Anil Kumar N, Dini L, et al. Plant-derived bioactives and oxidative stress-related disorders: a key trend towards healthy aging and longevity promotion. Applied Sciences. 2020; 10: 947. https://doi.org/10.3390/app10030947

[10] Nagarajan S, Chellappan DR, Chinnaswamy P, Thulasingam S. Ferulic acid pretreatment mitigates MPTP-induced motor impairment and histopathological alterations in C57BL/6 mice. Pharmaceutical Biology. 2015; 53: 1591–601. https://doi.org/10.3109/13880209.2014.993041

[11] Tan B, Wang Y, Zhang X, Sun X. Recent Studies on Protective Effects of Walnuts against Neuroinflammation. Nutrients. 2022; 14: 4360. https://doi.org/10.3390/nu14204360

[12] Akomolafe SF, Oriyomi IA, Akintoye OO, Ajayi OO, Ojo OO, Ajayi OB, et al. Dietary raw and roasted pumpkin seed supplementation exerts neuroprotective effects in healthy rats as demonstrated by biochemical and histopathological analysis. Discover Food. 2025; 5: 305. https://doi.org/10.1007/s44187-025-00612-2

[13] Park S, Han J, Im K, Whang WK, Min H. Antioxidative and anti-inflammatory activities of an ethanol extract from fig (Ficus carica) branches. Food Science and Biotechnology. 2013; 22: 1071–1075. https://doi.org/10.1007/s10068-013-0185-7

[14] N P D, Kondengadan MS, Sweilam SH, Rahman MH, Muhasina KM, Ghosh P, et al. Neuroprotective role of coconut oil for the prevention and treatment of Parkinson's disease: potential mechanisms of action. Biotechnology & Genetic Engineering Reviews. 2024; 40: 3346–3378. https://doi.org/10.1080/02648725.2022.2122296

[15] Mietus-Snyder ML, Shigenaga MK, Suh JH, Shenvi SV, Lal A, McHugh T, et al. A nutrient-dense, high-fiber, fruit-based supplement bar increases HDL cholesterol, particularly large HDL, lowers homocysteine, and raises glutathione in a 2-wk trial. FASEB Journal: Official Publication of the Federation of American Societies for Experimental Biology. 2012; 26: 3515–3527. https://doi.org/10.1096/fj.11-201558

[16] Juul F, Bere E. Ultra-processed foods - a scoping review for Nordic Nutrition Recommendations 2023. Food & Nutrition Research. 2024; 68: 10.29219/fnr.v68.10616. https://doi.org/10.29219/fnr.v68.10616

[17] Chandegara M, Chatterjee B, Sewani N. Development of novel chocolate energy bar by using nuts. International Journal of Food and Fermentation Technology. 2018; 8: 93–97. https://doi.org/10.30954/2277-9396.01.2018.12

[18] Mahdizadeh F, Fazaeli A, Rahimi S, Ojarudi M, Sobhi P, Bahrami M, et al. Combined effects of metformin and coenzyme Q10 on doxorubicin-induced cardiotoxicity in male wistar rats. Scientific Reports. 2025; 15: 20725. https://doi.org/10.1038/s41598-025-07576-4

[19] Bais S, Gill NS, Kumar N. Neuroprotective effect of Juniperus communis on chlorpromazine induced Parkinson disease in animal model. Chinese Journal of Biology. 2015; 2015: 542542. https://doi.org/10.1155/2015/542542

[20] Jacob J, Amalraj A, Divya C, Janadri S, Manjunatha PM, Gopi S. Oral toxicity study of sports nutritional powder in Wistar rats: A 90 day repeated dose study. Toxicology Reports. 2018; 5: 497–503. https://doi.org/10.1016/j.toxrep.2018.04.001

[21] Forouzanfar F, Hosseini M, Ahmadzadeh AM, Pourbagher-Shahri AM. Neuroprotective effect of cedrol in a male rat model of Parkinson's disease. Physiological Reports. 2025; 13: e70309. https://doi.org/10.14814/phy2.70309

[22] Farooqi SS, Naveed S, Qamar F, Sana A, Farooqi SH, Sabir N, et al. Phytochemical analysis, GC-MS characterization and antioxidant activity of *Hordeum vulgare* seed extracts. Heliyon. 2024; 10: e27297. https://doi.org/10.1016/j.heliyon.2024.e27297

[23] Oktaviyanti ND, Setiawan F, Kartini K, Azminah A, Avanti C, Hayun H, et al. Development of a simple and rapid HPLC-UV method for ultrasound-assisted deep eutectic solvent extraction optimization of ferulic acid and antioxidant activity from Ixora javanica flowers. South African Journal of Chemical Engineering. 2022; 40: 165–175. https://doi.org/10.1016/j.sajce.2022.03.004

[24] Shetty V, Chellampillai B, Kaul-Ghanekar R. Development and validation of a bioanalytical HPLC method for simultaneous estimation of cinnamaldehyde and cinnamic acid in rat plasma: Application for pharmacokinetic studies. New Journal of Chemistry. 2020; 44: 4346–4352. https://doi.org/10.1039/c9nj03183a

[25] Khezeli T, Daneshfar A, Sahraei R. A green ultrasonic-assisted liquid-liquid microextraction based on deep eutectic solvent for the HPLC-UV determination of ferulic, caffeic and cinnamic acid from olive, almond, sesame and cinnamon oil. Talanta. 2016; 150: 577–585. https://doi.org/10.1016/j.talanta.2015.12.077

[26] Al-Abbasi FA. Neuroprotective effect of butin against rotenone-induced Parkinson's disease mediated by antioxidant and anti-inflammatory actions through paraoxonase-1-induction. Journal of Taibah University for Science. 2022; 16: 944–953. https://doi.org/10.1080/16583655.2022.2128561

[27] von Wrangel C, Schwabe K, John N, Krauss JK, Alam M. The rotenone-induced rat model of Parkinson's disease: behavioral and electrophysiological findings. Behavioural Brain Research. 2015; 279: 52–61. https://doi.org/10.1016/j.bbr.2014.11.002

[28] Azadbakht AA, Radahmadi M, Javanmard SH, Reisi P. The effects of doxepin on stress-induced learning, memory impairments, and TNF-α level in the rat hippocampus. Research in Pharmaceutical Sciences. 2015; 10: 460–5.

[29] Kuriakose J, Lal Raisa H, A V, Eldhose B, M S L. Terminalia bellirica (Gaertn.) Roxb. fruit mitigates CCl_4 induced oxidative stress and hepatotoxicity in rats. Biomedicine & Pharmacotherapy. 2017; 93: 327–333. https://doi.org/10.1016/j.biopha.2017.06.080

[30] Aziz S, Khatoon H, Aziz A, Imran M, Athar Ishaqui A. Lauric acid mitigates doxorubicin-induced cardiotoxicity in rats: Modulation of oxidative stress and inflammation via NF-κB p65 attenuation. Pakistan Journal of Pharmaceutical Sciences. 2026; 39: 487–502. https://doi.org/10.36721/PJPS.2026.39.2.REG.14740.1

[31] Chen XY, Li J, Qi WQ, Shen SH. Experimental change on dopaminergic neurons in striatum of Parkinson disease rats. Histology and Histopathology. 2007; 22: 1085–1090. https://doi.org/10.14670/HH-22.1085

[32] Joniec-Maciejak I, Wawer A, Turzyńska D, Sobolewska A, Maciejak P, Szyndler J, et al. Octanoic acid prevents reduction of striatal dopamine in the MPTP mouse model of Parkinson's disease. Pharmacological Reports: PR. 2018; 70: 988–992. https://doi.org/10.1016/j.pharep.2018.04.008

[33] Sanguanphun T, Sornkaew N, Malaiwong N, Chalorak P, Jattujan P, Niamnont N, et al. Neuroprotective effects of a medium chain fatty acid, decanoic acid, isolated from *H. leucospilota* against Parkinsonism in *C. elegans* PD model. Frontiers in Pharmacology. 2022; 13: 1004568. https://doi.org/10.3389/fphar.2022.1004568

[34] Zaidi AA, Khan MA, Shahreyar ZA, Ahmed H. Lauric acid: Its role in behavioral modulation, neuro-inflammatory and oxidative stress markers in haloperidol induced Parkinson's disease. Pakistan Journal of Pharmaceutical Sciences. 2020; 33: 755–763.

[35] Luchtman DW, Meng Q, Song C. Ethyl-eicosapentaenoate (E-EPA) attenuates motor impairments and inflammation in the MPTP-probenecid mouse model of Parkinson's disease. Behavioural Brain Research. 2012; 226: 386–396. https://doi.org/10.1016/j.bbr.2011.09.033

[36] Peterson LJ, Flood PM. Oxidative stress and microglial cells in Parkinson's disease. Mediators of Inflammation. 2012; 2012: 401264. https://doi.org/10.1155/2012/401264

[37] Juárez Olguín H, Calderón Guzmán D, Hernández García E, Barragán Mejía G. The Role of Dopamine and Its Dysfunction as a Consequence of Oxidative Stress. Oxidative Medicine and Cellular Longevity. 2016; 2016: 9730467. https://doi.org/10.1155/2016/9730467

[38] Guo JD, Zhao X, Li Y, Li GR, Liu XL. Damage to dopaminergic neurons by oxidative stress in Parkinson's disease (Review). International Journal of Molecular Medicine. 2018; 41: 1817–1825. https://doi.org/10.3892/ijmm.2018.3406

[39] Li X, Zhang J, Rong H, Zhang X, Dong M. Ferulic Acid Ameliorates MPP^+/MPTP-Induced Oxidative Stress via ERK1/2-Dependent Nrf2 Activation: Translational Implications for Parkinson Disease Treatment. Molecular Neurobiology. 2020; 57: 2981–2995. https://doi.org/10.1007/s12035-020-01934-1

[40] Iranshahy M, Javadi B, Sahebkar A. Protective effects of functional foods against Parkinson's disease: A narrative review on pharmacology, phytochemistry, and molecular mechanisms. Phytotherapy Research: PTR. 2022; 36: 1952–1989. https://doi.org/10.1002/ptr.7425

[41] Prorok T, Jana M, Patel D, Pahan K. Cinnamic Acid Protects the Nigrostriatum in a Mouse Model of Parkinson's Disease via Peroxisome Proliferator-Activated Receptorα. Neurochemical Research. 2019; 44: 751–762. https://doi.org/10.1007/s11064-018-02705-0