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Unlocking the neuroprotective potential of *Citrus japonica* peel oil: Gas chromatography-mass spectrometry analysis and anti-Parkinsonian activity in a paraquat rat model

Norah K. Algarzae[1], Uzma Saleem[2]*, Ifat Alsharif[3], Fahad T. Alsulami[4], Nada M. Mostafa[5], Heba A.S. El-Nashar[5], Omayma A. Eldahshan[5,6]*, Zunera Chauhdary[7], Maryam Farrukh[7], Andleeb E. Taiba[7], Mohammed B. Hawsawi[8], Fatima A. Jaber[9], Mody Albalawi[10], Reem Hasaballah Alhasani[11], Muhammad Ajmal Shah[12]*, Ana Sanches Silva[13–15]*

[1]Department of Physiology, College of Medicine, King Saud University, Riyadh, Saudi Arabia; [2]Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan; [3]Department of Biology, Jamoum University College, Umm Al-Qura University, Makkah, 21955, Saudi Arabia; [4]Clinical Pharmacy Department, College of Pharmacy, Taif university, Taif, Saudi Arabia; [5]Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo, Egypt; [6]Center for Drug Discovery Research and Development, Faculty of Pharmacy, Ain Shams University, Abbassia, Cairo, Egypt; [7]Department of Pharmacology, Faculty of Pharmaceutical Sciences, Government College University, Faisalabad, Pakistan; [8]Department of Chemistry, Faculty of Science, Umm Al-Qura University, Makkah 21955, Saudi Arabia; [9]Department of Biological Sciences, College of Science, University of Jeddah, Jeddah, Saudi Arabia; [10]Department of Biochemistry, Faculty of Science, University of Tabuk, Saudi Arabi, Arabia; [11]Department of Biology, Faculty of Science, Umm Al-Qura University, Makkah, Saudi Arabia; [12]Department of Pharmacy, Hazara University, Mansehra, Pakistan; [13]University of Coimbra, Faculty of Pharmacy, Polo III, Azinhaga de St. Comba, Coimbra, Portugal; [14]Center for Study in Animal Science (CECA), ICETA, University of Porto, Porto, Portugal; [15]Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), Lisbon, Portugal

***Corresponding Authors:** Uzma Saleem, Punjab University College of Pharmacy, University of the Punjab, Lahore, Pakistan, Email: uzma95@gmail.com. Omayma A. Eldahshan, Department of Pharmacognosy, Faculty of Pharmacy, Ain Shams University, Abbassia, 11566 Cairo, Egypt; Center for Drug Discovery Research and Development, Faculty of Pharmacy, Ain Shams University, Abbassia, 11566 Cairo, Egypt, Email: oeldahshan@pharma.asu.edu.eg; Muhammad Ajmal Shah, Department of Pharmacy, Hazara University, Mansehra, Pakistan, Email: ajmalshah@hu.edu.pk; Ana Sanches Silva, University of Coimbra, Faculty of Pharmacy, Polo III, Azinhaga de St. Comba, 3000-548 Coimbra, Portugal; Center for Study in Animal Science (CECA), ICETA, University of Porto, 4501-401 Porto, Portugal; Associate Laboratory for Animal and Veterinary Sciences (AL4AnimalS), 1300-477 Lisbon, Portugal Email: asanchessilva@ff.uc.pt

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PAPER

Abstract

Citrus japonica, commonly known as Kumquat, is an economically and pharmacologically important citrus fruit, indigenous to Asia-Pacific and South Asia. The current work seeks to investigate the neuroprotective potential of the essential oil extracted from *C. japonica* fruit's peel in a paraquat (PQT)-induced dopaminergic neurodegeneration rat model, as well as the chemical profiling by using gas chromatography-mass spectrometry (GC/MS) analysis. The Parkinson's disease (PD) rat model was prepared by administering PQT at 5-day intervals of 10 mg/kg i.p. for 3 weeks. Animals were divided into healthy (received normal saline), PD control, standard treated (L-dopa 100 mg/kg + carbidopa 25 mg/kg), and CJ 50 and 100 (received 50 µL and 100 µL *C. japonica* peel oil). Behavioral studies were performed before biochemical, neurochemical, histopathological, and gene expression analysis. The GC/MS analysis identified seven volatile chemical compounds, predominated by D-limonene (98.17%), followed by *β*-pinene (0.68%), *α*-terpineol (0.40%), germacrene D (0.36%), *α*-pinene (0.17%), terpinen-4-ol (0.15%) and

γ-terpinene (0.07%). The behavioral study indicated that *C. japonica* peel oil treatment significantly improved the motor and cognitive impairments as well as the neuromuscular coordination. Biochemical study indicated a decrease in the oxidative burden; improved hematological, liver, and renal profiles; an increase in the acetylcholinesterase level; and upregulation of the mRNA expression of IL-1α, IL-1β, TNF-α, α–synuclein, and amyloid beta precursor protein. Current findings indicate that this oil mitigates motor and nonmotor PD-aasociated symptoms, reduces oxidatiev stress, and regulates the mRNA expression of pathological genes. Consequently, it is proposed that *C. japonica* oil may be used as a novel disease-modifying therapeutic remedy in the treatment of PD.

Keywords: Limonene, *Citrus japonica,* antioxidant, neuroprotective, Parkinson disease, oxidative stress, neuroinflammation, animal behavior

Introduction

Parkinson's disease (PD) is a pervasive, age-related neurodegenerative disease. James Parkinson initially characterized bradykinesia, stiffness, and tremor—the main motor indications of the condition—in his "Essay on the Shaking Palsy." Currently, it is generally accepted that these indications point to PD (Saleem *et al.*, 2022). In nigral tissues, Lewy bodies formation with α-synuclein aggregations are distinctive characteristics of PD (Saleem *et al.*, 2019). Degeneration of dopaminergic neurons reduces the facilitation of voluntary movements. The accumulation of α-synuclein in the brain tissues increased with the advancement of disease stages (Iqbal *et al.*, 2022; Lücking, 2000; Shahnawaz *et al.*, 2020). However, most of the research in the last two decades focused on nonmotor symptoms of PD (Magistrelli *et al.*, 2021). However, even with current imaging or laboratory tests to aid the diagnostic challenges, the motor manifestations of the disease which are the most crucial parameters for a diagnosis of PD remain elusive (Giagkou *et al.*, 2020). Clinical manifestation involves rigidity, resting tremors, postural instability, and slowness of movement. In substantia nigra compacta (SNc), the selective and massive loss of dopamine-synthesizing neurons is cardinal neurodegeneration hallmarks. Till now, the etiopathogenesis of PD neuronal degeneration remains mysterious (Hunot and Hirsch, 2003).

Although multiple treatment strategies are in practice to halt the progression of disease, these do not satisfactorily alleviate PD symptoms but could further worsen the disease stage. The available drugs to treat PD are associated with numerous side effects when used for a long duration because of their shorter duration of action and fewer fluctuations in receptor permeability (Maiti *et al.*, 2017). PD susceptibility factors are connected to numerous hereditary and environmental variables. Protein aggregation build-up, mitochondrial malfunction, brain oxidative injury, inflammation, and genetic flaw are the primary pathogenic factors involved in the development of PD. Although the current treatment approaches temporarily alleviate the symptoms, their prolonged usage is linked to serious side effects. Consequently, the creation of disease-modifying, low-cost, exotic drugs with natural origins is constantly needed. (Abdallah *et al.*, 2022). Most of the valuable bioactive compounds are isolated from plants and serve as revolutionary medicaments (Jachak and Saklani, 2007). They exhibit robust antioxidant activity and hold neuroprotective potential at the cellular and molecular levels (Abdallah *et al.*, 2022; Elhawary *et al.*, 2021; Potterat and Hamburger, 2008).

There are a number of medicinal plants with exciting antioxidant and anti-inflammatory properties. The *Curcuma longa* contains curcuminoids that fight against oxidative stress. Likewise, green tea (*Camellia sinensis*) shows catechins particularly EGCG which contain free radical scavenging property and prevents oxidative cell harm. Similarly, ginger (*Zingiber officinale*) exhibits antioxidants and anti-inflammatory potential because of gingerols and shogaols. It has been discovered that *Ginkgo Biloba* has flavonoids and terpenoids which work against oxidative injury. Other useful plants include ashwagandha (*Withania somnifera*), withanolides which reveal oxidation and stress release properties. Rosemary (*Rosmarinus officinalis*) contains carnosic acid and containing rosmarinic, both have antioxidant potentials. Sage (*Salvia officinalis*) holds sageone and other polyphenols that protect from oxidative stress; St. John's Wort (*Hypericum perforatum*) is an antioxidant plant because of hyperforin and flavonoids. These plants have long been used for their therapeutic properties, and new studies are continually learning more about them (Hamedi *et al.*, 2020).

Citrus japonica, commonly known as Kumquat, is a small-size fruit with an elliptical shape, having sweet rind and acidic pulp (Lou *et al.*, 2015). Indigenous to the Indian subcontinent and Southeast Asia, kumquat is grown as an ornamental in gardens and parks in southern Turkey (Lin *et al.*, 2021). It can be consumed as fresh fruits or as pickles, candies, marmalade, or

jelly (Ozcan-Sinir *et al.*, 2018). Raw kumquat dietary content was previously reported by the United States Department of Agriculture's Agricultural Research Service to include water, carbohydrates, total sugars, total dietary fiber, protein, total lipid, minerals (potassium, calcium, magnesium, phosphate, and sodium), and vitamin C (Ozcan-Sinir *et al.*, 2018). This fruit was found to be enriched with monoterpenes, especially limonene (Baky *et al.*, 2024; El-Nashar *et al.*, 2021). Medicinally bioactive compounds, terpenoids and flavonoids, are present in favorable amounts in the peel of this fruit (Nouri and Shafaghatlonbar, 2016b). It is well known because of its multiple therapeutic activities and expectorant, deodorant, carminative, antivirus, and antiphlogistic properties (Nouri and Shafaghatlonbar, 2016b). The major compound fruit peel oil is d-limonene that possesses several pharmacological characteristics, including anti-carcinogenic, anti-inflammatory, and antioxidant activity (Farasati Far *et al.*, 2024). These bioactive compounds also have neuroprotective and antioxidant potential (Abdelghffar *et al.*, 2021; Eddin *et al.*, 2021).

Paraquat (PQT, a herbicide, also known as 1,1-dimethyl-4, 4-bipyridine, has the potential to induce PD. This compound derived from redox-cycling substances is neurotoxic via progressive degradation of dopamine-producing neurons, which is the main hallmark of PD (Tangamornsuksan *et al.*, 2019). It can perpetuate oxidative insult to brain tissues by deposition of α-synuclein aggregates, dopaminergic neurodegeneration, nigrostriatal pathway injury, and Lewy body formation (Ahmad *et al.*, 2021; Brooks *et al.*, 1999). PQT infiltrates the brain through neutral amino acid transporters, involved in redox cycling, resulting in oxidative stress, and depletion of ATP and production of reactive oxygen species (ROS), specifically targeting nigrostriatal pathways (Kumar *et al.*, 2016). The current study is designed to characterize the volatile oil isolated from *C. japonica* peel *via* GC/MS analysis and estimation of neuroprotective potential in PQT-induced PD rat model.

Materials and Methods

Chemicals

Sulfuric acid, atropine, sodium bicarbonate, gallic acid, acetylthiocholine iodide, 5,5′,dithiobis-(2-nitro benzoic acid), tricholoroacetic acid, pyrogallol, bovine serum albumin, HCl, heptanes, sodium acetate, acetic acid, formaldehyde solution, haematoxylin-eosin stain, 5,5′-dithio-bis (2-nitrobenzoic acid) (DTNB), hydrogen peroxide (H_2O_2), potassium dihydrogen phosphate, and sodium tartarate were purchased from Sigma-Aldrich (St. Louis, MO, USA). Pentylenetetrazole, sodium valproate were purchased from Reko Pharmacia (Lahore, Pakistan). All the chemicals used were of analytical grade.

Collection of C. japonica fruits and essential oil extraction

Fresh *C. japonica* fruits were acquired in May 2023 at Seoudi Hypermarket in Dokki, Giza, Egypt. Classification expert Mrs. Tereize Labib of El-Orman Botanical Garden in Giza, Egypt, identified the fruits according to their classification. A voucher specimen (PHG-P-CJ-480) has been deposited in the botanical museum collection at the Faculty of Pharmacy, Ain Shams University, Cairo, Egypt, for future reference and verification. The Clevenger apparatus was used to hydrodistill about 750 g of peels during a 4 h period. The volatile oil was isolated via steam distillation and preserved at –2°C in a tightly sealed glass vial for subsequent GC/MS examination and biological evaluation.

GC/MS analysis of peel oil

The volatile oil extracted from the peel was subjected to examination by GC/MS at the Department of Pharmacy, Ain Shams University, Cairo, Egypt. The analysis utilized a Shimadzu GCMS-QP 2010 instrument, coupled with a TRACE GC ultra gas chromatograph and a thermo mass detector. The examination employed a TG-5MS capillary column, with helium as the carrier gas, at a split ratio of 1:15 and a constant flow rate of 1.0 mL/min. The temperature of the oven was programmed to rise by 5.0°C/min, with an initial isothermal period of 2 min at 80°C. The constituents of the extracted oil were provisionally characterized by matching their GC/MS spectra, fragmentation profiles, molecular weights, and Kovats retention indices with those in the Wily and NIST databases and published literature. The retention indices were calculated relative to a homologous series of n-alkanes (C8-C28) analyzed under identical conditions. The relative abundance of each compound was determined as a percentage of the total peak area in the flame ionization detection (FID) chromatogram.

Evaluation of neuroprotective activity against PD

Experimental animals

Rats of either sex were used in this investigation. Before the trial, to acclimate the animals, they were housed in the GCUF animal house for a period of 7 days. The typical conditions, 12 h, day/night cycle, a room temperature between 25 and 30°C, and a relative humidity between 30 and 60% were met. A balanced diet and individual attention were also given.

Ethical approval

Prior to initiating animal experiments, the Ethics Committee of Government College University Faisalabad (Reference no. GCUF/ERC/54) granted approval for the use of animals in research.

Induction of PD

PQT (10 mg/kg) was injected intraperitoneally at 5-day intervals for a total of 3 weeks, inducing symptoms reminiscent of PD (Ahmad *et al.*, 2021).

Study design

Five groups (n = 6) of healthy animals were formed, with six rats in each group. Group I was given normal saline and declared to be in good health. Group II received PD control treatment with PQT 10 mg/kg administered intravenously every 5 days for 3 weeks. Group III received standard treatment with levodopa 100 mg/kg and carbidopa 25 mg/kg (p.o.) in addition to PQT 10 mg/kg (Saeed *et al.*, 2017), Group IV (CJ100) received treatment with PQT 10 mg/kg, intraperitoneal injection every 5 days for 3 weeks, and 100 µL oral C. *japonica* peel essential oil. Group V (CJ50) received treatment with PQT 10 mg/kg, an intraperitoneal injection every 5 days for 3 weeks, coupled with 50 µL oral C. *japonica* essential oil.

Every animal was given the recommended treatment for a period of 21 days. Behavioral studies were carried out after a 3-week trial period to evaluate locomotor activity and cataleptic activity. Animals were euthanized under inhalant anesthesia of isoflurane by decapitation, and blood samples were obtained for hematological and biochemical analysis. Brian tissues were divided and kept so that expression analyses could be performed.

Assessment of behavioral tests

Catalepsy test

Using a standard bar test, the cataleptic reaction of animals induced with PQT was assessed every 30 min for a maximum of 120 min. The cataleptic score was determined by placing the front limbs in an enforced position on a wooden bar that measured 3 cm in height and 1 cm in width. The animal moving its head, removing both front paws off the bar, or climbing the bar can all be considered the endpoint. Using a standard bar test, the cataleptic reaction of animals induced with PQT was assessed every 30 min for a maximum of 120 min. The front limbs were forced into an awkward posture on a wooden bar that measured 3 cm in height and 1 cm in width. If an animal moves normally, then the resulting catalepsy score was 0.5. If the animal was unable to alter its posture, forcefully imposed on the wooden box for 10 sec, the catalepsy score was 02 (Chitra *et al.*, 2017).

Exploratory behavior test

This task was performed to evaluate the trait of exploration in animals. A wooden square box of 45 cm height, 100 cm in length, and 100 cm width, with the floor divided into equal 25 squares was used. Animals were carefully introduced at the center of the box and monitored there for 2 min. The number of squares that were sniffed and looked around in, including both the center and the edges (horizontal exploration), and the number of squares that were reared (vertical investigation) were noted (Saleem *et al.*, 2021a).

Narrow beam walk test

This task was performed to investigate gait impairment and motor coordination. The apparatus was devised of two wooden platforms at both edges of a 100 cm narrow beam. Animals were gently placed at one end of the beam and movement to the other end was observed. The time to traverse the apparatus from one end to the other was noted, and foot slips were observed (Saleem *et al.*, 2021a).

Hole-board test

This task was designed to investigate the nonmotor symptoms like anxiety, cognitive decline, and depression. The apparatus consisted of a 50×50×50 cm Plexiglas box with multiple 3 cm holes evenly distributed across the floor. After completion of the experimental treatment, animals in each group were individually placed in this apparatus and observed for 5-10 minutes for head drippings. Head dipping was scored when both eyes were under the level of the hole (Saleem *et al.*, 2021b).

Swim test

It is a specific test for identifying animal depression. This test was conducted in a water-filled container that was 40 cm deep and 25 cm wide; it was maintained at an ambient temperature. Rats were gently placed in the swimming apparatus and allowed to swim for 5 min and scored as follows: 0 = when the animal was unable to swim and immersed its head, 1 = animal swam occasionally by hind paw, 2 = animals float alternately, and swam occasionally, 3 = animal swims constantly (Saleem *et al.*, 2021b).

Fear conditioned maze paradigm

Using rodents' innate tendency to avoid open, elevated spaces, this task is the gold standard for examining anxiety-like behavior in mice. The equipment used for this exercise was made up of two 50 cm by 10 cm by 40 cm open and closed arms. The arms were closed and opened, facing opposing directions, with an open canopy and a center platform. The time an animal takes to transition from an open to a closed arm is known as transfer latency (TL). TL, a gauge of memory retention, was seen following animal training in this maze (Saleem *et al.*, 2021a).

Y-maze test

In behavioral neurosciences, to explore memory, spatial memory, and cognition, Y-maze paradigm is popular in rodents (Chauhdary *et al.*, 2019). The Y-maze apparatus comprised an equilateral triangular middle region and three arms (35 cm long, 25 cm high, and 10 cm broad). Rats were introduced at the end of an arm and were

observed to explore the maze for 8 min. Spontaneous alteration and percentage of alteration were calculated according to the given formula (Aydin *et al.*, 2016).

$$\text{Alternation percentage} = \frac{\text{Actual alteration}}{(\text{Maximum spontaneous alteration})} \times 100 \quad (1)$$

$$\text{Spontaneous alterations} = \text{Total number of arms entered} - 2 \quad (2)$$

Foot printing test

This is a reliable and cost-effective method to investigate the gait abnormalities in PD rodent models. The fore and hind limbs of animals were dipped in black ink, and they were trained on a white paper sheet to walk in a straight path on the 100 cm long printing apparatus. Foot patterns on the white paper sheet were evaluated to determine the stride length.

Morphological analysis of brain tissues

Animals were sacrificed after anesthesia, and whole brains were isolated. After washing with phosphate buffer, the weight of brains were estimated. Through handheld lens, any sign of atrophy, depigmentation, or ventricular enlargement were noted. After cutting into coronal slices, size and shape of substantia nigra were noted.

Estimation of antioxidant enzymes in brain homogenate

Tissue homogenate preparation

Brain homogenate was prepared according to our previously published procedure (Saleem *et al.*, 2021c). Brain tissue homogenate (10%) was prepared in phosphate buffer by using electric tissue homogenizer and centrifuged for 10 min at 600×g, and the clear top layer was collected for biochemical analysis.

Determination of glutathione level

Trichloroacetic acid (1 mL, 10%) was added to tissue homogenate (1 mL). It was centrifuged (30 minutes at 3000 rpm), and 2 mL top layer was mixed with 0.5 mL Ellman's reagent and phosphate buffer (4 mL). Absorbance was taken at 412 nm, and the following formula was used to determine glutathione level (Saleem *et al.*, 2021c):

$$\text{Glutathione level} = \frac{\text{fold dilution}}{\text{Volume of homogenate}} \times \frac{0.00314}{0.34} \times \text{aliquot volume} \quad (3)$$

Determination of malondialdehyde (MDA) level

Tissue homogenate 1 mL was mixed with 2.5 mL of 15% trichloacetic acid, 0.25M HCl, and 0.38% (w/w) thiobarbituric acid. It was heated for 10 min at 90°C and then cooled. After centrifugation at 4000 rpm for 10 min, mixture absorbance was taken at 532 nm. The amount of MDA as nmoles/mg of tissue protein was calculated.

$$\text{MDA level} = \frac{\text{Absorbance} \times 100 \times \text{mixture volume}}{\text{E} \times \text{weight of tissue sample} \times \text{aliquot volume}} \quad (4)$$

$$E = 1.56 \times 10^5 \quad (5)$$

Superoxide dismutase (SOD) estimation

The reaction mixture was prepared by adding potassium phosphate buffer (2.8 mL) with 100 µL of homogenate and pyrogallol solution (0.1 mL). At 325 nm, absorbance was noted, and SOD calibration graph was used to express as (unit/mL).

Determination of catalase (CAT) activity

To estimate the level of catalases, tissue homogenate (50 µL) was mixed with 50 mM phosphate buffer (2 mL). This mixture was treated with 1 mL hydrogen peroxide, 30 mM. At 240 nm, optical density was calculated, and the below formula was used (Saleem *et al.*, 2021c).

$$\text{CAT} = \frac{\Delta\text{Abs}}{\varepsilon \times \text{V} \times \text{P}} \quad (6)$$

Where:

ΔAbs = Absorbance shift
ε = Extinction coefficient
V = Sample volume
P = Concentration of protein

Estimation of neurotransmitters' levels

Preparation for aqueous phase

To prepare the aqueous phase, HCl-butanol 5 mL was mixed with brain homogenate and centrifuged. The isolated top layer was added to 0.3 mL HCl and heptane (2.5 mL). After vigorous shaking, the upper layer was separated by 10 minutes of centrifugation at 2000 rpm.

Determination of noradrenaline and dopamine levels

In the isolated aqueous phase (0.2 mL), HCl (0.1 mL, 0.4 M) and EDTA solution were mixed. By addition of 100 µL iodine solution, redox reaction was started. Sodium sulfite and acetic acid (100 µL each) were added to stop the reaction. It was heated for 6 min at 100°C. At 352 nm and 452 nm, absorbance was measured for dopamine and noradrenaline, respectively (Parambi *et al.*, 2020).

Determination of serotonin levels

The serotonin level was estimated by mixing 200 µL aqueous phases with 250 µL O-phthaldialdehyde. This

mixture was maintained at high temperature for 10 min at 100°C. At 440 nm, absorbance was recorded with blank solution of HCl (Parambi *et al.*, 2020).

Synaptic activity modulation/AChE catalysis

Elman's reagent (0.1 mL) and 20 µL acetylthiocholine iodide were mixed with aqueous layer (2.6 mL) and added to phosphate buffer (0.1M; pH 8). Enzyme activity was expressed as µM per minute per milligram of tissue after taking absorbance at 412 nm (Parambi *et al.*, 2020).

Histopathological studies

Isolated brain tissues were preserved in 10% formalin solution. Under the light microscope, 10× slides were observed after staining.

Transcriptional gene analysis

Gene expression analysis was performed by using the primer sequence, as shown in Figure 1. Briefly, RNA pellets were isolated by the traizol method and transcribed to Cdna by using the cDNA kit. GADPH was used as a housekeeping gene. PCR plates were placed in a real-time PCR machine (Biorad) for 40 cycles of denaturation (95°C), annealing (60°C), and extension (72°C) (Saleem *et al.*, 2021c).

Statistical analysis

Results were statistically analyzed through graph pad prism version 5. One-way or two-ways ANOVA followed by Tukey's post-test were used to express the results after taking mean of triplicate values.

Results

GC/MS analysis of the essential oil isolated from *C. japonica* fruits

The GC/MS analysis was employed to characterize the volatile components of essential oil isolated from *C. japonica* fruits. The results of the GC/MS analysis of the essential oil are provided in Figure 2 and Table 1. The GC/MS investigation of the fruit essential oil revealed identification of seven compounds. The identified compounds accounted for 100% of the essential oil, predominated by monoterpene hydrocarbons (99.09%), and followed by traces of oxygenated monoterpenes (0.55%) and sesquiterpene hydrocarbons (0.36%), as represented in Figure 3. In addition, D-limonene showed the highest dominance with abundance percentage of 98.17%, followed by β-pinene (0.68%), α-terpineol (0.40%), germacrene D (0.36%), α-pinene (0.17%), and terpinen-4-ol (0.15%). The chemical structures of the volatile components detected in the essential oil of *C. japonica* fruits are cumulatively shown in Figure 4.

Assessment of behavioral tests

Catalepsy test

Animals in each group were observed for measurement of cataleptic response recorded at intervals of 30, 60, 90, and 120 minutes. In the PD control group, PQT (10 mg/kg i.p.) significantly (P<0.001) produced catalepsy within 30 min, with the maximum score being recorded

Bio markers	Sequence	Product size in base pair	Accession number
Acetylcholinesterase	AGGACGAGGGCTCCTACTTT	200	NM_1720091
	CATGGCATCTCTCAGGTGGG		
TNFa	GGAGGGAGAACAGCAACTCC	168	NM_012675.3
	TCTGCCAGTTCCACATCTCG		
IL-lα	CCTCGTCCTAAGTCACTCGC	102	NM_017019.1
	GGCTGGTTCCACTAGGCTTT		
IL-1β	GACTTCACCATGGAACCCGT	104	NM_031512.2
	GGAGACTGCCCATTCTCGAC		
β Secretase	CCAACCTTCGTTTGCCCAAG	197	NM_019204.2
	GCGGAAGGACTGATTGGTGA		
Alpha-synuclein	TCGAAGCCTGTGCATCCATC	156	XM_017592500.1
	CTCCCTCCTTGGCCTTTGAA		
Amyloid β precursor protein	GAGGTAGTCCGAGTTCCCAC	127	XM_006248012.3
	GCTTGGCTTCCAACCTCTCT		
GADPH	GGAGTCCCCATCCCAACTCA	173	XM_017592435.1
	GCCCATAACCCCCACAACAC		

Figure 1. List of primers with sequence and accession number.

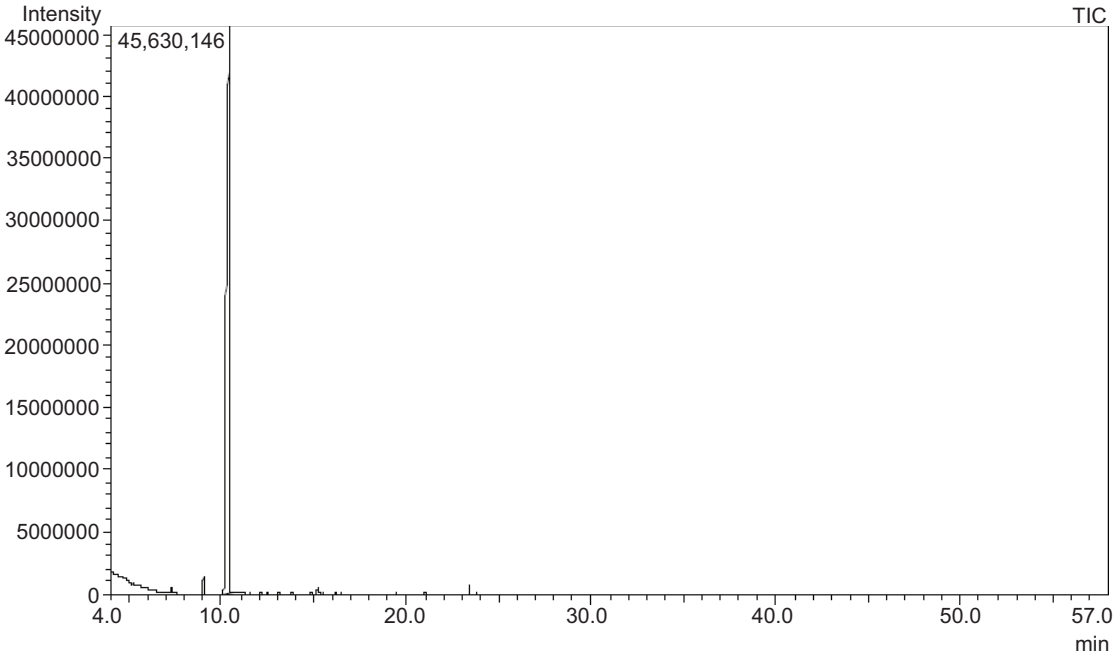

Figure 2. GC-MS chromatogram of the essential oil isolated from *Citrus japonica* fruits.

Table 1. GC-MS chemical composition (%) of the essential oil isolated from *Citrus japonica* fruits.

Compound	Retention time (t_R)	Molecular formula	Kovats index (KI)		Peak area (%)
			$KI_{Exp.}$	$KI_{Rep.}$	
α-Pinene	7.28	$C_{10}H_{16}$	914	917	0.17
β-Pinene	9.04	$C_{10}H_{16}$	978	978	0.68
D-Limonene	10.43	$C_{10}H_{16}$	1025	1025	98.17
γ-Terpinene	11.15	$C_{10}H_{16}$	1048	1050	0.07
Terpinen-4-ol	14.81	$C_{10}H_{18}O$	1166	1171	0.15
α-Terpineol	15.22	$C_{10}H_{18}O$	1179	1182	0.40
Germacrene D	23.40	$C_{15}H_{24}$	1471	1473	0.36

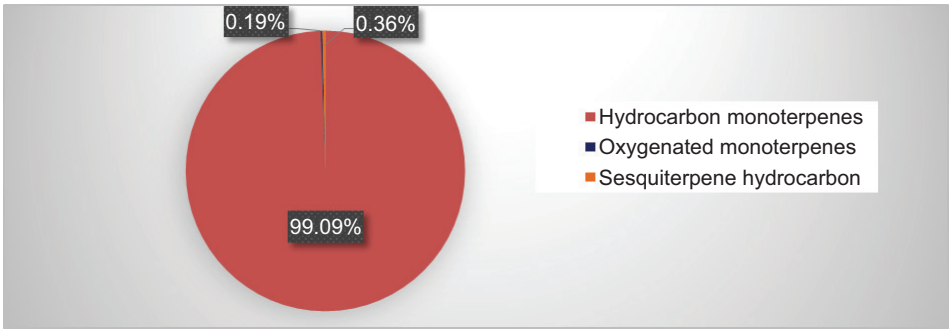

Figure 3. Pie charts display the distribution of different classes of components (%) identified in the GCMS analysis of essential oil isolated from *Citrus japonica* berries.

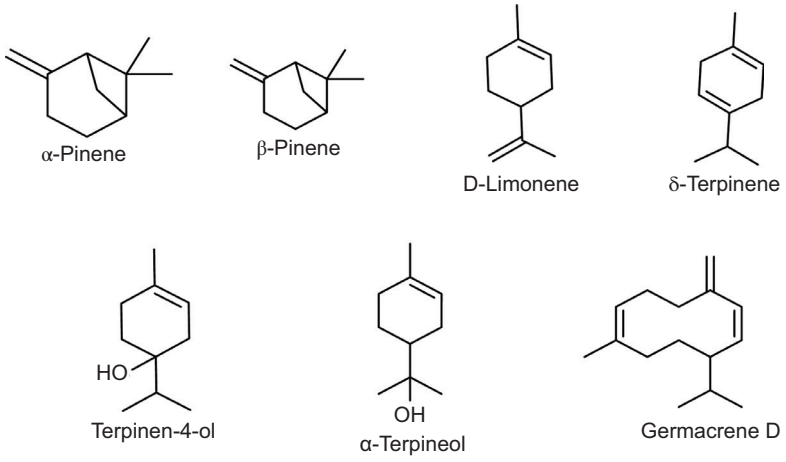

Figure 4. The chemical structures of the volatile components detected in the essential oil of *Citrus japonica* fruits.

Table 2. Effect of *Citrus japonica* essential oil on catalepsy in the paraquat-induced Parkinson's disease model.

Groups	Dose	Cataleptic scores at different time intervals			
		30 minutes	60 minutes	90 minutes	120 minutes
Healthy	-	0.0±0.0	0.0±0.0	0.0±0.0	0.0±0.0
PD control	10 mg/kg ip	3.55±0.28$^{£££}$	3.88±0.17$^{£££}$	3.98±0.22$^{£££}$	4.75±0.35$^{£££}$
Standard Treated	L-Dopa+C.dopa	1.38±0.32***	1.38±0.25***	1.36±0.28***	1.36±0.30***
C. japonica oil	CJ 50	2.16±0.30***	2.50±0.22***	1.83±0.30***	1.66±0.33***

Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

at 120 min. However, animals treated with *C. japonica* essential oil 50 µL (CJ50) and 100 µL (CJ100) showed a significant reduction (P<0.001) in cataleptic soring compared to PD control group and similar to the healthy group (Table 2).

Open field test

The protective impact of *C. japonica* essential oil on exploration, movement, and anxiety was examined using an open-field test. The total number of the lines crossed was markedly decreased (P<0.001) in the PD control group compared to the healthy group. However, the depression and anxiety-like symptoms were markedly higher in the PD control group compared to the healthy control and other treatment groups. Animals in CJ 50 and CJ 100 groups showed a significant (P<0.001) improvement in motor functions as reflected by a greater number of crossed lines and squares similar to the healthy group and standard drug-treated animals (Figures 5 and 6).

Narrow beam walk test

The findings of this task were consistent with open-field results. The latency time or time taken by animals to reach from one platform to another platform or cover the distance of 100 cm was significantly higher (P<0.001) in the PD control group compared to *C. japonica* oil-treated and healthy animals. The motor coordination and stability were markedly improved in CJ 50 and CJ 100 groups as contemplated from shorter time latency, as shown in Figure 7, akin to healthy and standard treated animals.

Hole board test

In the PD control group, PQT administration decreased the exploratory and locomotor functions of animals as manifested by significant (P<0.001) reduction in head dipping, edge sniffing, and walking (Figures 8 and 9). However, treatment with C. *japonica* oil dose dependently recovered the dopaminergic depletion and improved the exploration and motor functions of animal in treated groups as in healthy and standard treated animals.

Elevated plus maze test

Time latency, or the length of time it takes an animal to go from an open arm to a closed arm, was utilized in this assignment to account for rodents' innate tendency

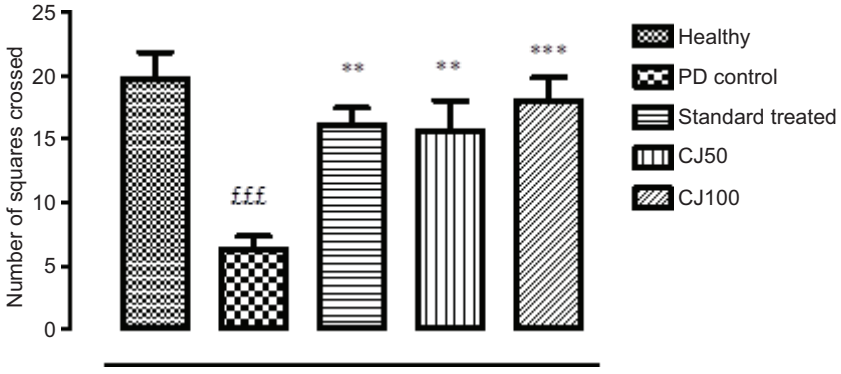

Figure 5. Number of crossed squares in the open field test. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of * less than 5%, * less than 1%, and ** less than 0.1%, respectively.

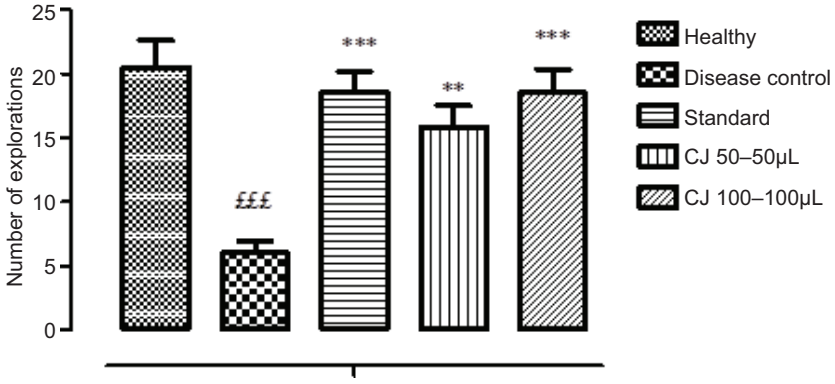

Figure 6. Central exploration counts in the test conducted in an open field. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

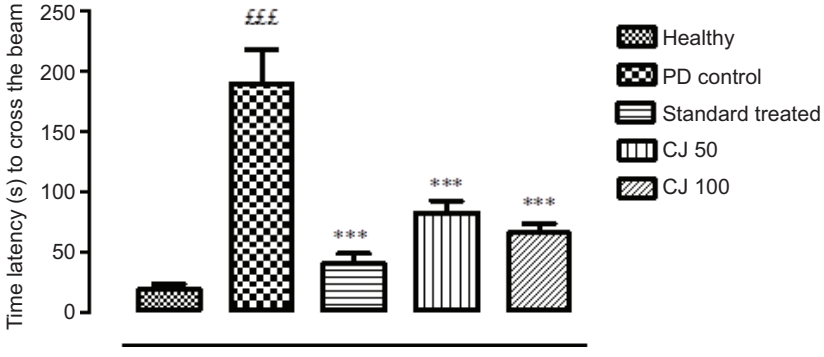

Figure 7. Effect of *Citrus japonica* essential oil on time latency (seconds) in narrow beam walk test. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

to hide out of and their fear of high and open areas. To gauge the impairment behavior, time latency was markedly ($P< 0.001$) raised in the PD control group compared to healthy, standard treated, and *C. japonica* oil treated groups dose-dependently (Figure 10).

Y-maze test

To investigate the short-term and spatial memory impairment, this task was used. The number of entries in three arms, number of triads, or spontaneous alterations were markedly decreased ($P<0.001$) in the PD control

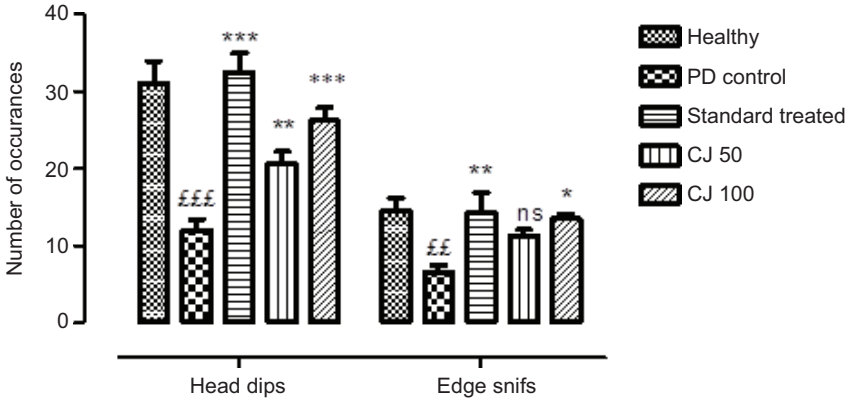

Figure 8. Effect of *Citrus japonica* essential oil on head dips and edge sniffing. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of * less than 5%, * less than 1%, and ** less than 0.1%, respectively.

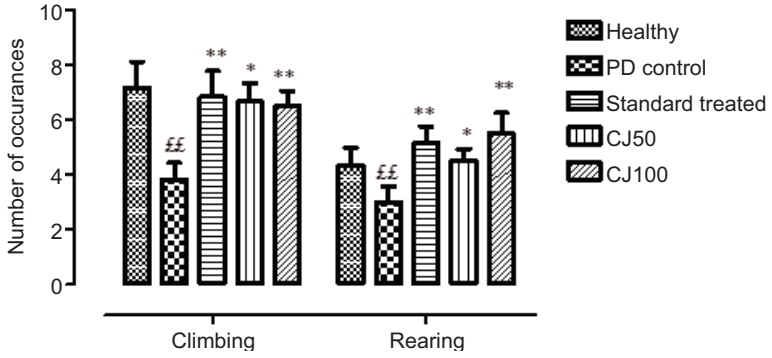

Figure 9. Impact of essential oil of *Citrus japonica* on climbing and raising. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of * less than 5%, * less than 1%, and ** less than 0.1%, respectively.

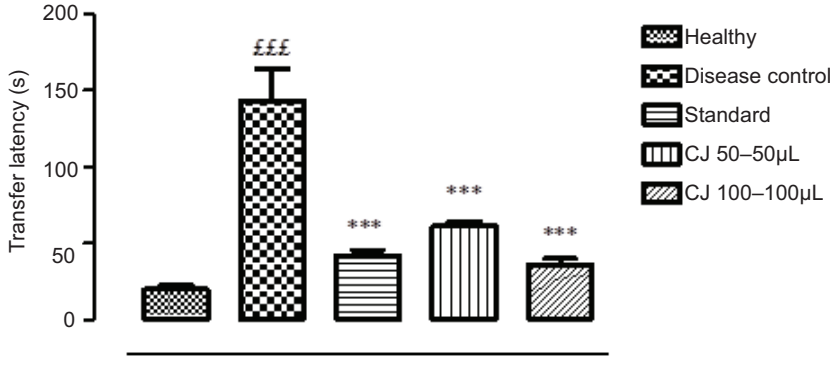

Figure 10. Test of the elevated plus maze. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of * less than 5%, * less than 1%, and ** less than 0.1%, respectively.

group compared to other treatment groups. However, CJ 50 and CJ 100 groups showed significant recovery dose-dependent like healthy and standard treated groups (Figures 11, 12, and 13).

Swim test

It is manifested from findings of the forced swimming test that the duration of immobility as an index of depression was significantly increased (P<0.01) in the PD

control group compared to healthy animals. However, the swimming duration was significantly improved (P<0.001) in CJ50 and CJ100 groups similar to healthy and standard-treated animals (Table 3).

Test for footprints

In the foot printing test, the PD control group's stride length was considerably (P<0.001) shorter than that of healthy and conventionally treated animals. However, treatment with *C. japonica* oil markedly (P<0.001) recovers the stride length and gait abnormalities induced by PQT in treated animals (Figure 14).

Effect of *C. japonica* oil on morphological parameters of brain

Morphological analysis revealed the marked alterations with obvious pathological signs of depigmentation, atrophy, and thickness in the disease control group. It was noted that the size and weight of brain tissues were significantly decreased with ventricular enlargement in diseases group compared to other treatment groups. However, *C. japonica* oil treatment decreased these pathological hallmarks in treated groups comapred to the disesae control group. The size and shape of substantia

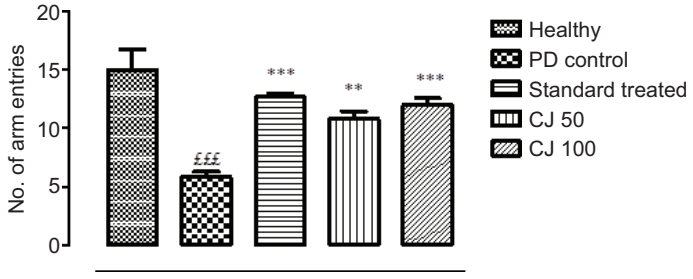

Figure 11. The Y-maze test's arm entries. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of * less than 5%, * less than 1%, and ** less than 0.1%, respectively.

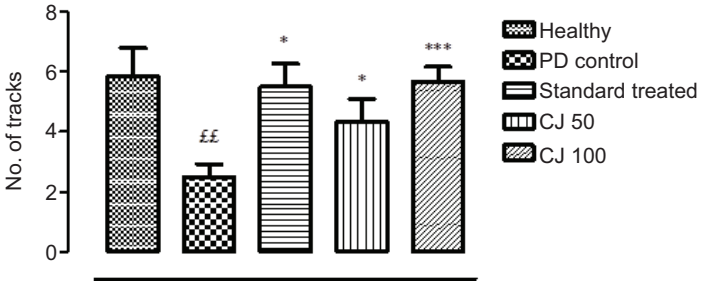

Figure 12. The Y-maze test's arm triad count. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of * less than 5%, ** less than 1%, and ** less than 0.1%, respectively.

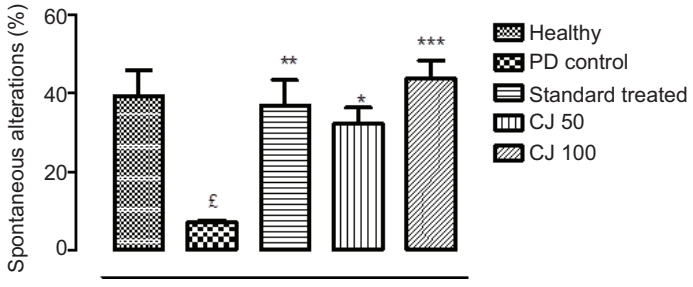

Figure 13. Percentage of spontaneous changes in the Y-maze test. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of * less than 5%, * less than 1%, and ** less than 0.1%, respectively.

Table 3. The effects of *Citrus japonica* essential oil on forced swim test in PQT-induced PD model.

Groups	Dose (mg/kg)	Swimming (s)	Climbing (s)	Immobility (s)
Healthy	-	150±3.71	29.31±3.17	67.80±5.45
PD Control	10 mg/kg ip	95.65±6.19$^{£££}$	17.65±2.40$^{£}$	131±6.89$^{£££}$
Standard treated	L-Dopa+C-Dopa	149.51±5.31***	25.69±2.57*	65.55±5.39***
C. japonica	50µL	161±2.78**	18.9±4.21**	73.36±10.59**
	100µL	167.45±2.56***	24.6±4.64***	56.89±5.78***

Research Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of * less than 5%, * less than 1%, and ** less than 0.1%, respectively.

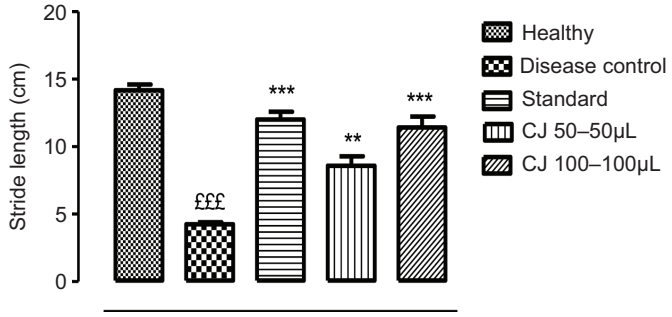

Figure 14. The effects of *Citrus japonica* peel essential oil on foot printing test in paraquat-induced Pakinson's disease model. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of * less than 5%, * less than 1%, and ** less than 0.1%, respectively.

nigra also decreased in the disesae group, but *C. japonica* treated groups showed the normal shape and size of substantia nigra and other tissues.

Determination of antioxidant enzymes

The level of first-line antioxidant enzymes was noticeably decreased in the PD control group because of oxidative stress induced by the administration of PQT. Figure 15 indicated that PQT-treated group significantly decreased the level of SOD, CAT, GSH, and protein level as compared to the normal control group and significantly increased the level of MDA. Treatment with standard drugs and *C. japonica* essential oil had significantly dose- dependently increased the level of SOD, CAT, GSH, and protein levels, and significantly decreased the level of MDA.

Estimation of neurotransmitter levels in brain

Because of PQT intoxication, the levels of noradrenaline, dopamine, serotonin, and acetyl cholinesterase were markedly (P<0.001) decreased in the PD control group. However, their level was recovered notably in *C. japonica* treatment groups dose-dependently similar to healthy and standard treated groups (Figure 16).

Estimation of acetylcholinesterase (AChE) level

Oxidative stress induced by PQT markedly (P<0.001) decreased the enzymatic activity of AChE in PD control animals. Because of PQT intoxication, the levels of noradrenaline, dopamine, serotonin, and AChE was recovered dose dependently similar to standard drug treatment (Figure 17).

Histological studies of brain tissues

As shown in Figure 18, the intact neuronal architecture and function were observed in a healthy group. Analysis of the PD control group showed a substantial loss of chromatic richness and cellular outlines. Neurodegenerative signs with extensive lipid peroxidation were obvious because of PQT-induced neuronal toxicity. However, the healthy and active neurons with

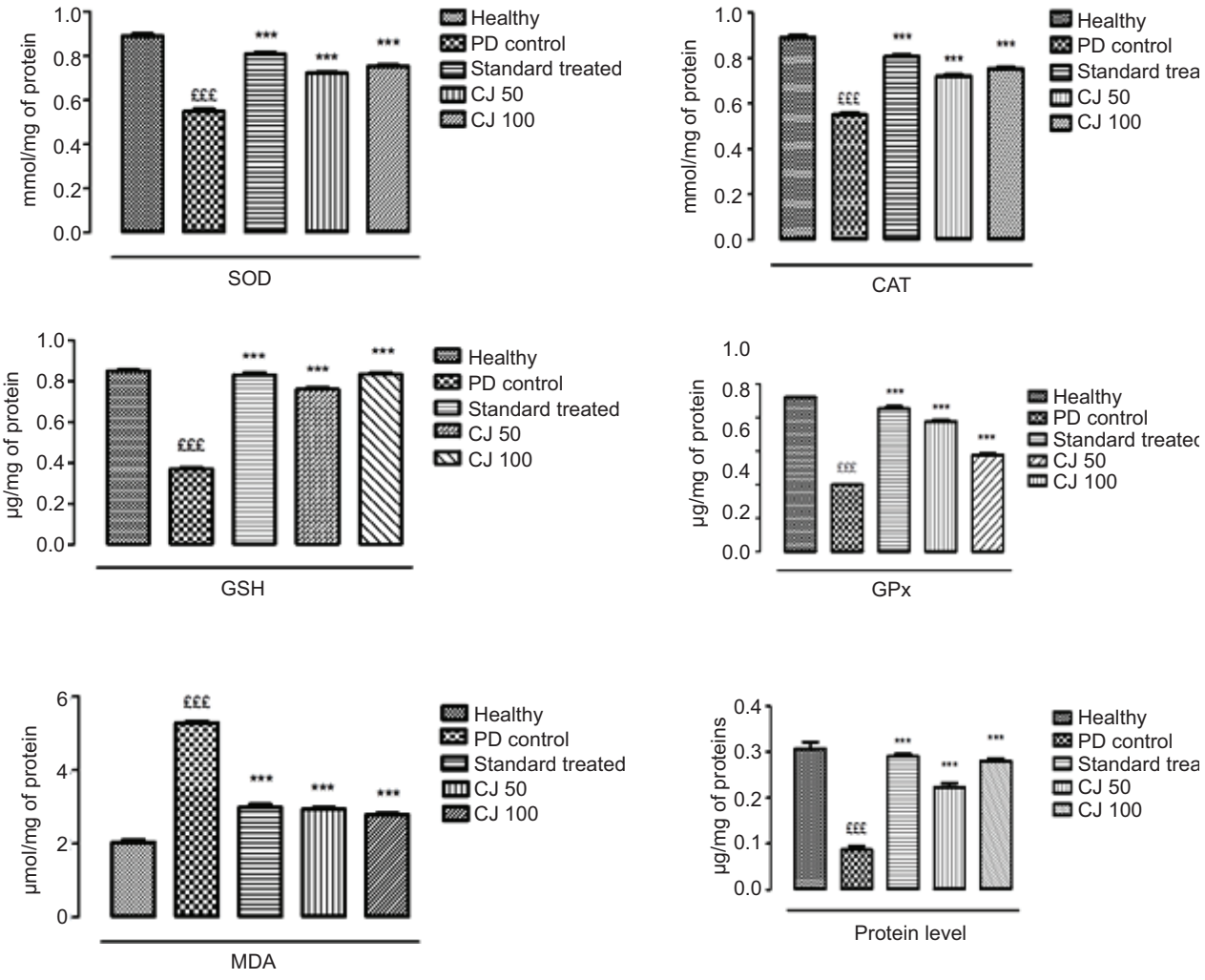

Figure 15. The effects of *Citrus japonica* essential oil on first-line antioxidant enzymes and level of proteins. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

typical morphological contours and chromatin contents were observed in healthy and standard-treated groups. In CJ 50 and CJ 100 treatment groups, reversal of neuronal and architectural loss was observed with a heavier burden of pyknotic neurons, partial vacuolation.

Effect of *C. japonica* treatment on hematological and biochemical analysis

Hematological and biochemical analyses revealed a nonsignificant difference between healthy animals and standard-treated animals. The PQT-treated group showed a marked rise in liver enzymes (ALT and AST). However, treatment with *C. japonica* recovered the raised level of liver enzymes. Hematological and biochemical analyses revealed a nonsignificant difference among healthy animals, *C. japonica,* and standard drug-treated animals. All results are shown in Tables 4–7.

Analysis of mRNA expression with qPCR

The administration of PQT induced neuroinflammation, resulting in significant P-value ($P<0.05$); upregulation in mRNA expressions of IL-1α, IL-1β, TNF-α, α–synuclein (α-synuclein); and amyloid beta precursor protein ABPP in the PD control group compared to healthy animals, standard treated, and *C. japonica* treated groups dose-dependently.

However, the AChE mRNA expression was markedly downregulated in the PD control group compared to healthy animals and other treatment groups. *C. japonica* treatment recovered the downregulated mRNA

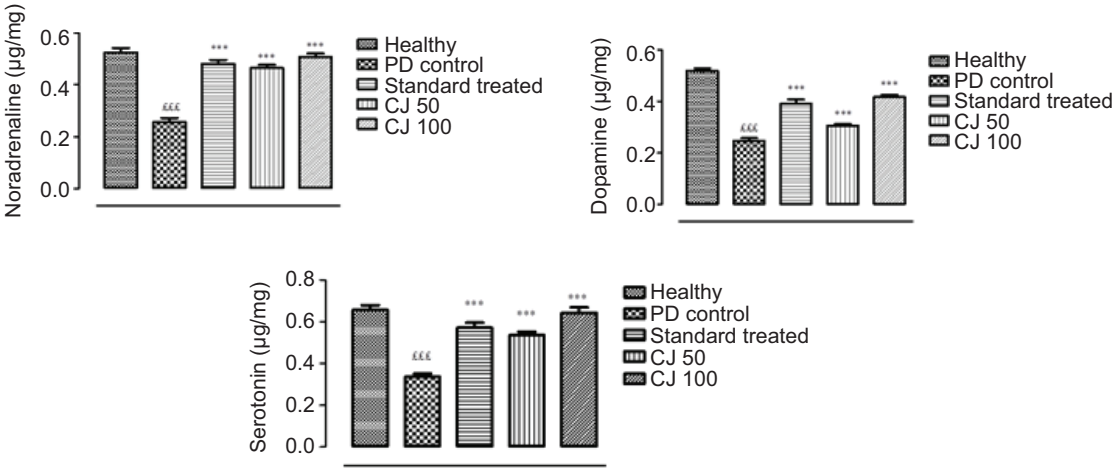

Figure 16. Effect of *Citrus japonica* essential oil on brain neurotransmitters. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

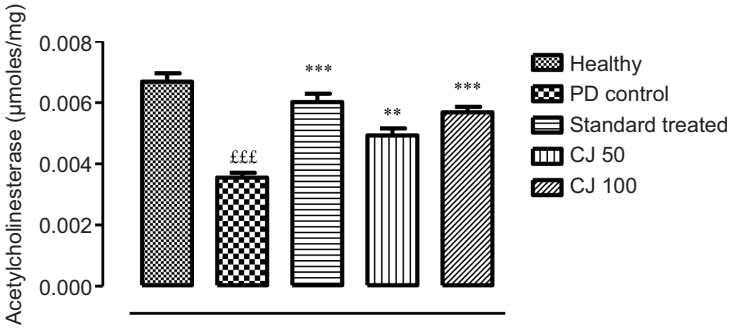

Figure 17. The effects of *Citrus japonica* essential oil on acetylcholinesterase levels in brain homogenate. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

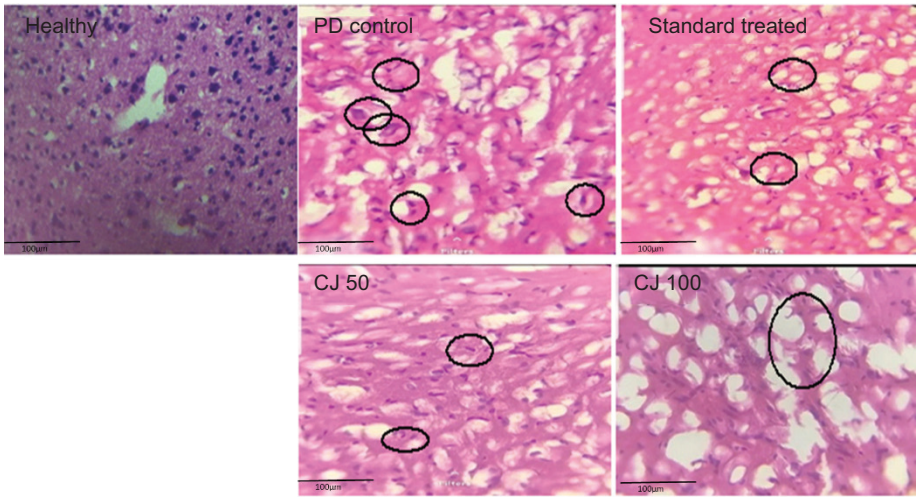

Figure 18. The histopathological analysis of brain tissue (at 10×) treated with *C. japonica* oil; NT: Neurofibrillary tangles; P: Plaques.

Table 4. The effects of *Citrus japonica* essential oil on the liver function tests profile in PQT-induced Parkinson model.

Parameters	Healthy	PD control	Standard treated	CJ 50	CJ 100
Serum Bilirubin (mg/dl)	0.47±0.11	0.51±0.01£££	0.40±0.05***	0.48±0.07*	0.46±0.08*
ALT(U/L)	45.5±3.01	54.1±2.22£££	39.7±5.51***	46.1±3.57***	37.2±3.01***
AST(U/L)	50.1±3.61	65.2±3.01£££	49.6±3.10***	45.7±4.22***	47.3±4.25***
ALP (U/L)	191.2±7.01	225±14.2£££	182±8.21***	141±8.91**	153±8.01**

Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and ** less than 0.1%, respectively.

Table 5. The effects of *Citrus japonica* essential oil on the renal function tests profile in PQT-induced Parkinson model.

Parameters	Healthy	PD control	Standard treated	CJ 50	CJ 100
Blood urea (mg/dl)	22.6±2.05	37.9±7.10£££	30±6.06***	27.8±3.71***	27.4±3.24***
Serum Creatinine (mg/dl)	0.46±0.04	0.49±0.07£££	0.44±0.02***	0.39±0.04***	0.37±0.02***
Serum uric acid (mg/dl)	3.56±0.18	4.66±0.21£££	3.33±0.24***	4.03±0.58***	3.96±0.41***

Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

Table 6. The effects of *Citrus japonica* essential oil on the lipid profile in PQT-induced Parkinson model.

Parameters	Healthy	PD control	Standard treated	CJ 50	CJ 100
Cholesterol (mg/dl)	90.1±5.81	96.7±5.43£££	96.9±6.52***	119±7.85***	120±8.52***
Triglycerides (mg/dl)	92.6±3.71	119.5±4.11£££	105.6±16.1***	125.3±3.71***	114.3±9.61***
HDL (mg/dl)	39.9±7.42	37.2±5.65£££	41.2±5.69***	47.8±3.01***	39±3.39***
LDL (mg/kg)	97.5±6.61	90.1±12.30£££	99.1±14.11***	107±8.31***	109±6.21***
VLDL (mg/dl)	27.2±3.01	30.1±3.23£££	25.6±4.01***	29.9±4.23***	27.0±4.28***

Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

Table 7. The effects of *Citrus japonica* essential oil on the complete blood count (CBC) in PQT-induced Parkinson model.

Parameters	Healthy	PD control	Standard treated	CJ 50	CJ 100
Total RBCs (10^{12}/L)	8.99±0.16	8.76±0.18£££	9.10±0.14***	8.81±0.26***	8.92±0.29***
M.C.V (fl)	52.3±4.11	56.8±7.23£££	60±4.31***	62±5.93***	58±2.15***
M.C.H (Pg)	19.5±0.21	11.10±0.31£££	13.40±0.61***	15.66±0.72***	16.21±0.86***
Hemoglobin (g/dl)	13.1±0.59	11.6±0.61£££	12.6±0.63***	12.8±0.63***	12.7±0.63***
Hematocrit (%)	38.5±3.39	42±3.62£££	39±4.52***	42±3.31***	41.6±4.01***
WBCs (K/uL)	6.91±0.91	4.95±0.31£££	5.96±0.16***	6.11±0.27***	6.78±0.31***
Platelets (K/UI)	852±8.51	742±8.92£££	894±14.1***	741±29.9***	775±15.6**

Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

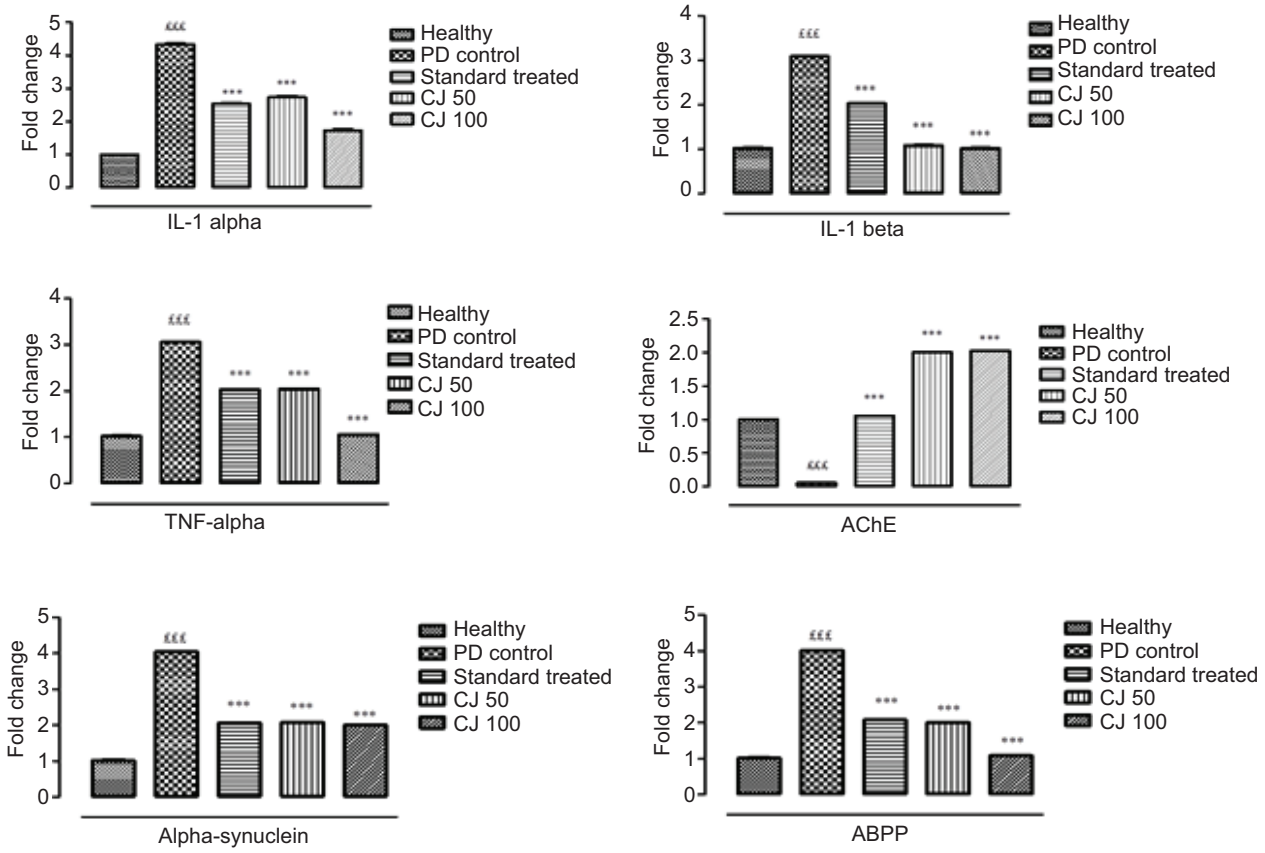

Figure 19. Impact of essential oil of Citrus *japonica* on pro-inflammatory cytokine mRNA expression and pathological indicators of Parkinson's disease. Statistical assessment showed significant variations compared to the PD control group, with P-values indicating probabilities of *less than 5%, *less than 1%, and **less than 0.1%, respectively.

expression of AChE and upregulated the expression of pro-inflammatory cytokines.

Discussion

The second-most prevalent condition is PD, a complicated and long-term neurodegenerative disorder rendering the patients to live years of mental and physical disability from diagnosis to death (Armstrong and Okun, 2020, Sveinbjornsdottir, 2016). The symptomatic characterization of PD is based on episodes of tremors and bradykinesia with postural instability, rigidity, and abnormalities such as akinesia and festinating gait (Braak H. and Braak E., 2000, Khoo *et al.*, 2013). Clinically, PD is characterized by abnormalities in motor and nonmotor functions; motor function comprises of tremors, bradykinesia, postural instability, and rigidity while nonmotor complications include sleep disorders, cardiovascular disorders, and constipation (Balestrino and Schapira, 2020). In the substantia nigra, Lewy bodies containing α-synuclein are a neuropathological sign of PD (Connolly and Lang, 2014). Dopaminergic neurons in the par's compacta of the substantia nigra disappear, which lessens the facilitation of voluntary movements. As PD progresses, the aggregation of alpha-synuclein spreads all through the brain. Chances of having PD are increased with age, as there is a 46% chance of its onset at age of 80 years compared to 40 years. Pakistan's population is more than 182 million and approximately 450,000 people are suffering from PD (Politis *et al.*, 2010). The prevalence is reported to be higher in males (63%) than in females (37%) (Ravina *et al.*, 2007).

The neuropathological mechanisms of PD are multifactorial and involve genetic as well as environmental factors. Protein accumulation, mitochondrial damage, neuroinflammation, excitotoxicity, impaired protein clearance pathways, oxidative stress, and genetic mutations are the main underlying pathological pathways (Dickson, 2018).

Several treatment strategies are currently available to temporarily mitigate the severe symptoms of PD or to moderately delay the progression of the disease (Connolly and Lang, 2014). Their drawbacks lie in the short duration of action, more side-effects on prolonged use, and

less fluctuations in receptor permeability (Zahoor *et al.*, 2018). Levodopa is the drug that works best for treating PD symptoms, particularly those caused by bradykinesia. As levodopa therapy is linked to motor side effects such as fluctuations and dyskinesias, there is ongoing dialogue about the ideal time to start levodopa therapy during PD (Münchau and Bhatia, 2000). After 5 years of levodopa therapy, the majority of individuals have motor fluctuations, dyskinesias, or other problems. One drug that has been demonstrated to decrease levodopa-induced dyskinesias without reducing levodopa dosage is amantadine. Levodopa-induced motor problems may be treated with the addition of a dopamine agonist, COMT inhibitor, or MAO-I inhibitor (Poewe *et al.*, 2017).

C. japonica, also known as Kumquat or *Fortunella margarita* peel, is rich in volatile oil (Lin *et al.*, 2021). Interestingly, it was reported to be generally recognized as safe to be used in food products (Kabara, 1991). Upon reviewing the literature, a certain study was carried out on fruits in Taiwan, which detected about 37 volatile compounds predominated by limonene, *α*-pinene, and caryophyllene in the essential oil (Peng *et al.*, 2013). Similarly in our results, limonene was reported to be the main component with an abundance value of 93.73% (Choi, 2005). In another study in Algeria, the fruit oil showed predominance of limonene (86.31%), D-germacrene (4.67%), β-myrcene (3.21%), and α-pinene (0.75%) (Lakache *et al.*, 2022). In Greece, the fruit oil was found to be rich in limonene (93.80%) and myrcene (2.70%) (Mitropoulou *et al.*, 2022). Unlike that, it was found in Brazilian fruits, there was a predominance of *β*-pinene, limonene, *β*-felandrene, *α*-guaiene, and D-germacrene (De Menezes Filho and Castro, 2019). It is assumed that these differences in the chemical composition of the above-mentioned essential oils can be ascribed to differences in the cultivation environment, geographical resources, climate, genotype, and collection time (El-Nashar *et al.*, 2022; Kamli *et al.*, 2024; Rabie *et al.*, 2023).

In the current research, the therapeutic potential of *C. japonica* peel essential oil has been evaluated for its anti-PD potential effect in a rat model. *C. japonica* essential oil maximum dose in this experiment (100 µL) showed significant neuroprotective effects. The behavioral test battery is designed to estimate motor and nonmotor function impairments. Catalepsy scoring narrow beam walk, open field, and hole board tasks were performed to estimate most of the behavioral paradigms (Meredith and Kang, 2006). *C. japonica* peel essential oil treatment recovered both the motor functions and nonmotor functions.

Oxidative stress is a critical player in the induction of neurodegeneration; therefore, the level of antioxidant enzymes markedly decreased compared to normal physiological conditions in neurotoxicity. Because of oxidative stress, the permeability of brain mitochondria is altered as a result of interruption in electron transport and autooxidation of dopamine participants in the neurotoxicity of dopaminergic neurons, resulting in the altered structure of disease-related protein structures like α-synuclein and amyloid beta precursor proteins (Uéda *et al.*, 1989). Therefore, compounds exhibiting antioxidant potential are considered a favorable therapeutic choice to treat PD. In the current work, because of the robust antioxidant power of *C. japonica* essential oil, the decreased level of first-line antioxidant enzymes SOD, CAT, and GSH was markedly recovered (Henchcliffe and Beal, 2008). However, this oil treatment mitigated the raised level of lipid peroxidation and the level of nitrite. Oxidative stress induces the structural changes of PD-related proteins, resulting in memory decline, depression, anxiety, and impairment in neuromuscular coordination (Blesa *et al.*, 2015; Henchcliffe and Beal, 2008). In PD, these symptoms are also strongly linked to depleted levels of brain neurotransmitters like dopamine, acetylcholine, adrenaline, serotonin, and noradrenaline. These neurotransmitters linked to PD pathology are thoroughly distributed in the cerebral cortex, limbic system, and basal ganglia, and take part in motor and nonmotor functions (Luchtman *et al.*, 2009). It is evident from published literature that because of the exposure of PQT, the level of dopamine and other neurotransmitters linked to motor functions are depleted. In the current work, similar to previous findings because of PQT administration, the level of these neurotransmitters decreased compared to healthy animals (Brichta *et al.*, 2013, Petzinger *et al.*, 2015). However, treatment with *C. japonica* resulted in decreased oxidative stress and consequently recovered levels of neurotransmitter dopamine, serotonin, adrenaline, and acetylcholine. As the level of brain neurotransmitters recovered, the motor and nonmotor dysfunctions were also markedly recovered (Abdel-Salam *et al.*, 2012; Selamoglu-Talas *et al.*, 2013; Sureda *et al.*, 2023). Similar to previous histopathological findings, *C. japonica* treatment also mitigated the pigmentation, neuroinflammation, and Lewy bodies formation (Bhangale and Acharya, 2016).

Oxidative stress induced structural changes and raised the level of misfolded α-synuclein protein. PQT administration resulting in oxidative stress induced higher levels of α-synuclein in the PD control group (Nemani *et al.*, 2010). PD pathology is also strongly linked to elevated levels of pro-inflammatory cytokines. As reported in previous literature, kumquat peel enriched with minerals, phenolic acids, and major component limonene hold robust antioxidant potential in 70% ethanol and also have remarkable antimicrobial properties against *Staphylococcus aureus* (Al-Saman *et al.*, 2019). *C. japonica* essential oil phytochemical screening or metabolic

fingerprinting revealed significant amounts of limonene and germacrene D; both compounds are associated with robust antioxidant potential and can be implemented to treat neurodegeneration in animal models through modulation of antioxidant signalling cascade leading to neuroprotective role (Daglia *et al.*, 2014; Nouri and Shafaghatlonbar, 2016a; Talas *et al.*, 2008). Essential oils extracted from *C. japonica* are also associated with multiple pharmacological attributes like antidiabetic, anticancer, and antiobesity. However, the current work is supported by findings of previous reported studies in which the use of *C. japonica* oil showed an antianxiety effect with significant improvement in locomotion. This study revelaed the anxiolytic potential of limonene (Satou *et al.*, 2012).

The primary function of AChE is to hydrolyze acetylcholine in order to inhibit cholinergic neurotransmission at synapses. Increased levels of synaptic isoform of AChE mRNA have been shown in previous studies to promote apoptosis in a range of cell types. AChE levels and mRNA expression were lowered in the PD model group as a result of PQT's neurotoxic effects. In line with prior research, *C. japonica* recovered AChE mRNA expression. Neuro-inflammatory cytokines like IL-1α, IL-1β, and TNF-α are also linked to debilitating symptoms of PD. Their level abnormally rose in pathological conditions as reported in a previous work. PD because of neurotoxicity induced by any neurotoxin results in elevated levels of these inflammatory cytokines (Leal *et al.*, 2013). In this work, the level of pro-inflammatory cytokines was raised in the PD control group compared to healthy and treated groups similar to a previous work (Koprich *et al.*, 2008). There is controversy over the expression of AChE in PD. But, in the current work, because of the neurotoxic effect of PQT, the protein structure of AChE was altered, resulting in lower expression in the PD control group compared to other healthy and treatment groups (Jiang and Zhang, 2008). However, the raised level of these enzymes in the PD model group was reported contrary to this work (Ben-Shaul *et al.*, 2006).

The neuroprotective effects of *C. japonica* essential oil may be attributed to the major component, limonene, as evident in many experimental models (Beserra-Filho *et al.*, 2023; Eddin *et al.*, 2021; Eddin *et al.*, 2023). Limonene reduced oxidative stress, altered NF-κB/MAPK signaling and motor impairment, and downregulated the levels of proinflammatory cytokines and inflammatory mediators in the brain in the rotenone-induced Parkinson model (Eddin *et al.*, 2021). Moreover, it might change mTOR signaling and inhibit Hippo signaling and the intrinsic apoptotic pathway (Eddin *et al.*, 2023). In reserpine-induced parkinsonism mice, limonene remarkably delayed the onset of catalepsy behavior and protected against memory deficit (Beserra-Filho *et al.*, 2023). Cholinesterase and butrylcholinestersae were both inhibited by limonene (Szwajgier and Baranowska-Wójcik, 2019). This action was linked to limonene's hydrocarbon skeleton, which is thought to function as a hydrophobic ligand that interacts with the AchE hydrophobic active site (Conforti *et al.*, 2007). Therefore, by considering the current work's findings, it is suggested that *C. japonica* peel essential oil will be helpful in the mitigation of PD hallmarks like gait abnormalities, depression, anxiety, impairment of motor functions, and loss of psychomotor skills. It should be considered as a neuroprotective agent to treat neurodegenerative disorders through its multiple mechanistic pathways to inhibit neuroinflammation and oxidative stress. The study offers important insights into the neuroprotective effects of *C. japonica*, but it also has some limitations that require future research. Firstly, since the study was conducted on animal models, it provides a basic understanding, but further human clinical trials are essential to validate its efficacy.

Conclusions

In view of the above findings, it is deduced that *C. japonica* peel oil is rich in limonene and could mitigate neurotoxicity induced by environmental and other risk factors. This essential oil modulated the behavioral, biochemical, and neurochemical parameters in PQT-induced PD rats. Also, the oil could lessen PD motor defects and protect the brain tissues from oxidative stress. The gene expression analysis revealed that *C. japonica* peel oil treatment downregulated the mRNA expression of pathological neuro-inflammatory biomarkers. Therefore, *C. japonica* peel oil should be considered as adjuvant therapy for the management of neurodegenerative disorders such as PD. Molecular mechanisms and clinical trials are recommended for the full characterization of the tested oil as an herbal drug.

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Author Contributions

US, OEA, MBH, MAS, and ASS conceived and supervised the study. NKA, IF, NMM, HASEN, ZC, MF, AET, and FAJM performed the experiments. IF, FTA, HASEN, OAE, MBH, MA, RHA and MAS analyzed and interpreted the results. All authors have equally contributed to writing, editing, and revising the final draft.

Conflicts of Interest

All authors declared no conflict of interest.

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