

Chamuangone-enriched rice bran oil ameliorates neurodegeneration in haloperidol-induced Parkinsonian rat model via modulation of neuro-inflammatory mediators and suppression of oxidative stress markers

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Abstract

A natural bioactive compound chamuangone extracted from Thai salad *Garcinia cowa* leaves exhibited robust medicinal properties, targeting central oxidative stress pathways, and having neuroprotective potential. Chamuangone-enriched rice bran oil (CERBO), with 1.97 mg/mL chamuangone, was obtained through green extraction. The study was designed to evaluate the anti-Parkinson's activity of CERBO in the haloperidol-induced Parkinsonian rat model. Animals were categorized into six groups as control, disease control and treatment groups. Parkinson's disease (PD)-like symptoms were induced by administration of haloperidol 1 mg/kg, intraperitoneally; CERBO treatment groups received 2.5, 5, and 7.5 mg/kg orally before the administration of haloperidol for 21 days. Neurobehavioral, biochemical, neurochemical, and histopathological studies along with gene expression analysis were performed at the completion of the study. CERBO markedly recover the motor and non-motor PD-like symptoms in treatment groups dose-dependently. The levels of antioxidant enzymes, such as catalase,

superoxide dismutase, reduced glutathione, and glutathione peroxidase, increased, while malondialdehyde levels decreased dose-dependently in CERBO-treated groups. CERBO dose-dependent elevations were observed in neurotransmitters (dopamine, serotonin, and noradrenaline). PD-associated specific biomarker (α -synuclein) decreased dose-dependently with downregulation in messenger RNA expression of neuro-inflammatory mediators (interleukin α , interleukin 1β , and tumor necrosis factor- α). Histopathological studies revealed recovery in neuronal loss, formation of Lewy's bodies, and neurofibrillary tangles in the treatment groups. It was concluded from the data that CERBO possessed good anti-Parkinson's activity and could be a novel, safe, and effective remedy for the treatment of PD.

Keywords: animal behavior; anti-Parkinson; chamuangone; neuro-inflammation; nutraceutical; phytomedicine; rice bran oil

Introduction

Neurodegenerative disorders are a group of nervous conditions that progress slowly and affect the brain, spinal cord, and peripheral nerves (Uddin *et al.*, 2018). In the list of neurological disorders, following Alzheimer's disease in terms of predominance, Parkinson's disease (PD) has unidentified origins (Tysnes and Storstein, 2017). PD has 1% predominance among people aged >60 years and is the most prevalent disorder of movement worldwide. Age is a leading factor in the prognosis of PD (Uwishema *et al.*, 2022). PD decreases mortality and is directly linked with deficiency in automaticity. Clinical features of PD are motor (postural instability, tremor, rigidity, loss of voluntary movements, and akinesia) and non-motor clinical manifestations (disorganization, depression, hyposmia, sialorrhea, dysphagia, and dementia) (Akhtar *et al.*, 2021).

In the substantia nigra pars compacta (SNpc), dopaminergic neurons gradually decline in their structure and function (Chinta and Andersen, 2005; Park *et al.*, 2022). Numerous environmental and genetic factors contribute in the pathological processes of PD, which results in inflammation, apoptosis, protein aggregation, and excitotoxicity as well as mitochondrial complex-1 suppression (Saleem *et al.*, 2020). The motor features of PD in animals are linked to oxidative stress with dopaminergic neuronal demyelination. Oxidative stress is a common mechanism contributing to atonicity (Dias and Junnand, 2013). Oxidative stress and antioxidants directly or indirectly entangle in the pathogenesis of PD (Nikam *et al.*, 2009). Brain tissues are more likely to be affected by oxidative stress because of mitochondrial dysfunction in PD (Guzman *et al.*, 2010; Nottia *et al.*, 2017). Dopaminergic neuronal loss in PD is correlated with the production of Lewy's bodies, microglial activation, oxidative stress, and mitochondrial dysfunction (Jenner and Olanow, 2006). Currently, available neuroprotective agents, such as L-dopa, carbidopa, amantadine, selegiline, rasagiline, and orphenadrine, effectively increase the activation of dopaminergic

receptors in the substantia nigra (Saleem *et al.*, 2020). These agents have a long list of adverse effects, including toxicity, ulceration, depression, and hypertension (Wang *et al.*, 2022). Therefore, the essential medical necessity is an efficient complementary herbal therapeutic agent.

Haloperidol (HP) belongs to group of typical antipsychotics; because of its highly potent disposition, it is used in the management of psychosis, mania, hyperactivity, aggressiveness, and delirium. HP serves as a high affinity D2 receptor antagonist in the basal ganglia and causes the destruction of dopaminergic neurons (Andreassen *et al.*, 2001). Haloperidol causes extrapyramidal toxicity and drug-induced PD; therefore, it is mostly used in experimental models for the induction of PD.

Garcinia cowa Roxb. Ex Choisy called "Chamuang" in Thai, is an edible plant that belongs to the Clusiaceae family (Sae-Lim *et al.*, 2019b). The plant is a small-to-medium size tree, 30 m in height. Fruit is digestible with an unpleasant taste (Wahyuni *et al.*, 2015). Parts of *Garcinia cowa* are used for medicinal purposes and have pharmacological activities, such as antidepressant, antiviral, antifungal, antioxidant, antitumor, anti-leishmaniasis, and anti-obesity actions (Sae-Lim *et al.*, 2022; Sarma *et al.*, 2014; Ritthiwigrom *et al.*, 2013). Traditionally, it has been used as an expectorant as well as for the improvement of blood circulation and indigestion. Its major phytoconstituents are flavonoids, xanthones, phloroglucinols, chamuangone, cowaxanthone, and garcicowanone (Paudel *et al.*, 2022; Ritthiwigrom *et al.*, 2013). Recent studies have suggested the robust antioxidant and neuroprotective potential of polyoxygenated xanthones and garcicowanone through the mechanism of reducing Ca^{+2} influx and by prohibiting the synthesis of reactive oxygen species (ROS; Nguyen *et al.*, 2022).

Chamuangone isolated from *G. cowa* with an IC_{50} value (10.7 μ M) exhibited cytotoxic activity against *Leishmania major*, manifesting strong anticancer activity against lung adenocarcinoma and leukemia cell lines (Sakunpak *et al.*, 2017). Chamuangone exhibits potential

chemotherapeutic effect by inhibiting cell proliferation, migration, and inducing apoptosis in HeLa cell lines (Sae-Lim *et al.*, 2020). A previous study revealed the significant antimicrobial property of active compound chamuangone (Sakunpak and Panichayupakaranant 2012). Chamuangone-enriched rice bran oil (CERBO), obtained through green microwave-assisted extraction, manifested vigorous anticancer activity against human cancer cell lines and noninvasive to normal cells (Sae-Lim *et al.*, 2019a). In light of the recently reported pharmacological activities of chamuangone, this study was planned to explore the neuroprotective potential of CERBO against haloperidol-induced Parkinsonism rat model.

Material and Methods

Chamuangone rich extract in rice bran oil

Standardized CERBO enriched with chamuangone was prepared through green microwave-assisted extraction technology and standardized by high-performance liquid chromatography (HPLC; 1.97 mg/mL) at Phytomedicine and Pharmaceutical Biotechnology Excellence Centre, Faculty of Pharmaceutical Sciences, Prince of Songkla University, Hat Yai, Songkhla, Thailand (Sae-Lim *et al.*, 2019a).

Chemicals and drugs

Haloperidol was procured from Searle Pakistan Pvt. Ltd. Levodopa and carbidopa were procured from Platinum Pharmaceuticals Pvt. Ltd. Triazole (Invitrogen), cDNA kit (Thermo Scientific), cyber green (SYBER green master mix Bio-Rad), and primers (ThermoFischer Scientific) were used in different experimental assays and tests.

Evaluation of anti-Parkinson activity

Study design and participants

This study was performed on rats of either gender. The animals were obtained from Government College University Faisalabad, Pakistan. Prior to starting the experiments, rats were housed for 1 week in animal house for familiarizing them with the environment. The standard laboratory conditions (12-h light and dark cycles; 22±3°C room temperature; and 30–60% humidity) were assured. All rats were provided with proper diet and personalized care. Animal studies were approved by the Ethical Committee of Government College University, Faisalabad, Pakistan (GCUF/ERC/258).

All rats excluding the control group, were daily injected haloperidol (1 mg/kg) intraperitoneally (i.p.) for 21 days

to induce disease prior to starting the treatment. Haloperidol solution was prepared in distilled water. All 30 rats were categorized into six groups (n = 6). The control group was injected distilled water. Haloperidol (1 mg/kg, i.p) was administered to diseased control group. The standard group received levodopa+carbidopa (100+25 mg/kg, orally). CERBO treatment groups received 2.5-, 5-, and 7.5-mg/kg CERBO orally.

Neurobehavioral studies were carried out in the last week of the study duration. The rats were slaughtered under anesthesia (isoflurane 10%) by cervical dislocation. Blood samples were collected along with brain tissues and other vital organs for biochemical, neurochemical, histopathological, and real-time polymerase chain reaction (RT-PCR) analyses. For biochemical analysis, the brains were kept in an ice-cold phosphate buffer (pH 7.4). For histopathological analyses, vital organs (heart, liver, kidney, and lungs) were removed and kept in 10% formalin solution.

Neurobehavioral studies

Catalepsy

A vertical grid that was 25.5-cm wide by 44-cm high with 1 cm of space between each wire lifted each rat by its paws. Rats were monitored for the time they took to move, including moving their paws. *Scoring of the catalepsy*: all the animals were made to maintain the imposed position for not less than 30 sec. The time must be noted in seconds. Catalepsy test was done on the 7th, 14th, and 21st day of the treatment (Chitra *et al.*, 2017).

Hang test

The hang test was intended to assess the animal's neuromuscular capability. A standard linear wire hang apparatus was made comprising a large box, which was 55 cm in length, 40 cm in width, and 35 cm in depth with a 2.5-mm strand hanging in the top center. Rats were caught up by their tails and gently held with all four paws in the middle of the wire. The stopwatch was stopped when the rats dropped from the wire or when they crawled to the end of the wire. The reading was taken when the rats felt the stopwatch after 30 s (Tillerson and Miller, 2003).

Foot printing test

Fore limbs and hind limbs of all animals were painted with harmless green and red paint to obtain the footprints. The animals were then permitted to proceed down a runway that was 100-cm long and 10-cm wide. For one and all, a clean white paper was spread out on the ground. The stride length was estimated in centimeters. It was observed by analyzing the footprint patterns (Madiha *et al.*, 2017).

Grip walk test

The grip walk test was performed through a ladder inclined at an acute angle with 25 steps, 2 cm apart from each other. The rats were allowed to climb one by one onto the ladder and the time duration between different steps was determined (Cummings *et al.*, 2007).

Forced swimming test

This test was designed by placing rat inside a water tank with a temperature of $27 \pm 4^\circ\text{C}$. The apparatus had 25-cm width, 12-cm depth, 40-cm length, and 16-cm height. All rats were placed inside the water tank one by one and swim score was recorded (Selvakumar *et al.*, 2014).

Open field test

The apparatus was a wooden box with a floor divided into 16 squares, measuring 72×72 cm by 36 cm. Each rat was positioned in the middle of the box, and it had 5 min to move around. The parameters were noted for 10 min. *Rearing*: rat's capability to stand on hind paws. *Grooming*: rat undergoes stroking and licking itself in a standing position (Saleem *et al.*, 2021).

Biochemical analysis

Biochemical analysis of brain tissues

Brain homogenates were prepared for estimation of superoxide dismutase (SOD), glutathione peroxidase (GPx), lipid peroxidation, catalase (CAT) and total protein contents, and reduced glutathione (GSH).

Assay for superoxide dismutase

Sodium phosphate buffer (pH 7.4), 1.2 mL, 100 μL of tissue homogenate, 100 μL of phenazine methosulfate (186 mM), 200 μL of Triton X, and 300 μL of nitro blue tetrazolium chloride (300 mM) were combined to form reaction mixture, which was incubated at 30°C for 95 s. The chemical process was stopped by adding glacial acetic acid. After incorporating 4 mL of n-butanol, it was vigorously combined before being allowed to settle for 10 min to have a distinct layer of n-butanol centrifuged at 1,000 rpm. The chromogen color intensity was assessed at 560 nm (Hira *et al.*, 2018).

Assay for catalase

Potassium phosphate buffer 1.95 mL (pH 7.4) consisted of 50 μL of brain homogenates. Hydrogen peroxide, 1 mL (30 mM) mixture was incorporated to an assay mixture and absorbance was obtained at 240 nm to estimate CAT activity using the following formula (Sharma and Bafna, 2012):

$$\text{Catalases activity} = \frac{\text{difference in absorbance per 60 seconds}}{0.071 \text{ mmol} \times \text{sample volume} \times \text{weight of protein (mg)}}$$

Extinction co-efficient: 0.071 mmol/cm.

Assay for malondialdehyde (MDA)

Brain homogenate was centrifuged for 5 min at 1,500 revolutions/minute (rpm). Thiobarbituric acid (TBA) mixture, 3 mL, was combined with 1 mL of supernatant. TBA mixture was prepared by mixing 0.38% weight by weight (w/w) of TBA, 2.5 mL of HCl (0.25 M), and 15% trichloroacetic acid (TCA). The assay mixtures were shaken and allowed to settle down for 15 min, refrigerated, and centrifuged at 3,500 rpm for 10 min. Absorbance at 532 nm was obtained. The MDA level was measured as nmol/mg of tissue protein (Bhangale and Acharya, 2016):

$$\text{Malondialdehyde} = \frac{\text{sample absorbance at 532 nm} \times 100 \times \text{admixture volume}}{\text{coefficient} \times \text{tissue weight} \times \text{aliquot volume}}$$

The coefficient value was 1.56×10^5 .

Assay for glutathione

Trichloroacetic acid 10% (1 mL) was mixed with homogenate (1 mL). The preparation was continued by adding 4 mL of sodium phosphate buffer solution (0.1 molar) having pH 7.4 followed by 5,5-dithiobis-2-nitrobenzoic acid reagent (DTNB) (0.5 mL). DTNB solution was prepared by mixing 29.78-mg DTNB in 25-mL methanol. Absorbance was recorded at 412 nm to check reduced level of GSH (Bhangale and Acharya, 2016; Saleem *et al.*, 2021):

$$\text{Glutathione} = \frac{\text{Absorbance of sample} - 0.00314}{0.0314 \times \text{dilution factor} \times \text{tissue weight} \times \text{aliquot volume}}$$

Estimation of protein level

Tissue homogenate, 0.2 mL, was combined with 4.5 mL of reagent one (48 mL of solution A [2% sodium carbonate in 0.1-N NaOH], 1 mL of solution B [1% aqueous solution of sodium potassium tartrate], and 1 mL of solution C [0.5% aqueous solution of copper sulfate]) and the resultant mixture was incubated for 10 min. After incubation, 0.5 mL of reagent 2 (an equal amount of Folin-phenol [2N] and H_2O) was added, and the solution was subjected to 30 min of incubation. Absorbance at 660 nm was obtained. Different concentrations of bovine serum albumin were used to plot regression line (Lowry, 1951).

Quantification of neurotransmitters

Preparation of aqueous phase

Brain homogenate preparation was carried out in 5-mL hydrochloric acid butanol, centrifuged at 2,000 rpm for 10 min. The aliquot of the supernatant obtained after centrifugation was incorporated in 2.5-mL heptane and

0.31-mL hydrochloric acid, shaken vigorously and again centrifuged for 10 min at 2,000 rpm. After centrifugation, the mixture was separated into two separate layers and 0.2 mL of the lower aqueous layer was used for further analysis of neurotransmitters (Hira *et al.*, 2020).

Measurement of dopamine and noradrenaline levels

Hydrochloric acid (0.4 M) 50 μ L was mixed to 200- μ L aqueous phase. Then 100 μ L of EDTA solution was added in the reaction mixture. Oxidation was started by adding 100- μ L iodine solution. After 1.5 min, oxidation was stopped by adding 100- μ L Na₂SO₃ and 100- μ L acetic acid to the reaction mixture. The remaining solution was heated at 100°C for 6 min. Absorbance for dopamine was measured at 352 nm and for noradrenalin at 452 nm (Saleem *et al.*, 2021).

Measurement of serotonin level

For estimation of serotonin, 0.2 mL of aqueous phase was mixed with 0.25-mL O-phthalaldehyde and heated for 10 min at 100°C. Hydrochloric acid was used as a blank. The absorbance of the blank and sample solution was observed at 440 nm (Rahman and Eswaraiyah, 2008).

Measurement of acetylcholinesterase (AChE) activity

A phosphate buffer solution (0.1 M), pH 8, was combined with acetylthiocholine iodide (20 μ L) and 2,4 dithiobisnitrobenzoic acid (100 μ L) in small quantities of brain homogenate (0.4 mL). The acetylthiocholine reaction with 2,4 dithiobisnitrobenzoic acid produced yellow color and absorbance was measured at 412 nm (Lakshmi *et al.*, 2015).

Determination of histology of brains and vital organs

The rats were slaughtered by cervical dislocation. Whole brains of all animals were isolated. Incisions were made in the abdominal areas of rats, and the kidneys, livers,

spleens, lungs, and hearts were removed. Each organ was then weighed separately on a weighing balance. Isolated brain and vital organs were stored in 10% formalin solution. After staining the slides with hematoxylin and eosin (H&E \times 10), photomicrographs were taken for microscopic examination.

Determination of liver function tests (LFTs), renal function tests (RFTs), lipid profile, and complete blood count (CBC)

Blood samples were collected by cardiac puncture for evaluating serum marker enzymes of the liver, including alanine transaminase (ALT), bilirubin level, aspartate aminotransferase (AST), renal function markers, such as creatinine and blood urea. CBC and lipid profiles were investigated by using commercially available kits.

Real-time polymerase chain reaction (RT-PCR) amplification

After RNA extraction by the Triazole method, complementary DNA (cDNA) was synthesized using the cDNA synthesis kit according to manufacturer's guidelines. RT-PCR plate was prepared by using forward and reverse primers (Table 1) and cyber green master mix. The following conditions were met before heat cycling with RT-PCR: after 5 min at 95 °C, 40 cycles of 15 s of denaturation at 95 °C, 20 s of annealing at 60 °C, and 20 s of extension at 72 °C were carried out. The Livak method was used to calculate fold changes in messenger RNA (mRNA) expression (Saleem *et al.*, 2020).

Statistical analysis

Data were presented as mean and standard error of mean (SEM). GraphPad Prism version 5.0 was used to analyze the data using one-way and two-way ANOVA, followed by a post-test Bonferroni results.

Table 1. List of primers used for RT-PCR.

Biomarkers	Sequence	Accession No.
IL-1 α	CCTCGTCCAAGTCACTCGC GGCTGGTTCCACTAGGCTTT	NM_017019.1
IL-1 β	GACTTCACCATGGAACCCGT GGAGACGAGGGCTCCTACTTT	NM_031512.2
TNF- α	GGAGGGAGAACAGCAACTCC TCTGCCAGTTCACATCTCG	NM_012675.3
α -synuclein	GGAGGGAGAACAGCAACTCC TCTGCCAGTTCACATCTCG	XM_017592500.1
GAPDH	GGAGTCCCCATCCCAACTCA GCCATAACCCCCACAACAC	XM_017592435.1

GAPDH: glyceraldehyde 3-phosphate dehydrogenase; IL-1 β : interleukin 1 beta; IL-1 α : interleukin 1 alpha; TNF- α : tumor necrosis factor- α .

Results

Effect of CERBO on neurobehavioral parameters

Catalepsy

Table 2 shows that catalepsy scoring was remarkably ($p < 0.001$) elevated in the disease control group comparable to the control group from 0.5 to 3 h. CERBO-treated groups at 2.5-, 5.0-, and 7.5-mg/kg doses exhibited remarkable ($p < 0.001$) reduction in the level of catalepsy compared to the disease control group. The standard group (levodopa+carbidopa) showed remarkable ($p < 0.001$) alteration in cataleptic scoring.

Hang test

Latency time was remarkably ($p < 0.001$) decreased in the disease control group comparable to the control group. Chamuangone-rich extract treatment groups at 2.5-, 5-, and 7.5-mg/kg doses remarkably ($p < 0.001$) improved

the latency time in a dose-dependent manner regarding the disease control group. The standard group remarkably ($p < 0.001$) enhanced the latency time more than the disease control group (Figure 1).

Foot printing test

Stride length was assessed by foot printing test, and it showed a remarkable ($p < 0.001$) reduction in stride length in the disease control group compared to the control group. CERBO-treated groups showed remarkable ($p < 0.001$) efficacy in motor function and mitigated akinesia by increasing the stride length (Figure 2).

Grip walk test

The grip walk test was designed to investigate the neuroprotective efficacy of CERBO on grip walk. The disease control group remarkably ($p < 0.001$) raised the climbing time in contrast to the control group. Treatment groups at 2.5-, 5.0-, and 7.5-mg/kg doses exhibited

Table 2. Effect of chamuangone-enriched rice bran oil on catalepsy scoring in the Parkinson's disease model.

Groups	Dose (mg/kg)	Time (hour)					
		0.5	1	1.5	2	2.5	3
Control		0	0	0	0	0	0
Disease control (haloperidol)	1	0.85±0.02###	1.06±0.05###	1.15±0.03###	1.14±0.01###	1.69±0.12###	1.82±0.09###
Standard (levodopa+carbidopa)	100+25	0.37±0.11***	0.51±0.04***	0.84±0.07***	0.67±0.03***	0.73±0.31***	0.54±0.06***
Treatment groups	2.5	0.55±0.21***	0.66±0.07***	1.26±0.05*	1.13±0.04***	0.96±0.02***	0.84±0.05***
	5.0	0.52±0.15***	0.63±0.02***	1.14±0.01	1.03±0.05***	0.80±0.03***	0.62±0.07***
	7.5	0.46±0.23***	0.52±0.01***	0.87±0.09***	0.85±0.11***	0.55±0.04***	0.40±0.03***

Mean±SEM of all figures with sample size (n = 6) is presented. ###Control group difference to disease control group $p < 0.001$, and disease control group difference to other groups *** $p < 0.001$.

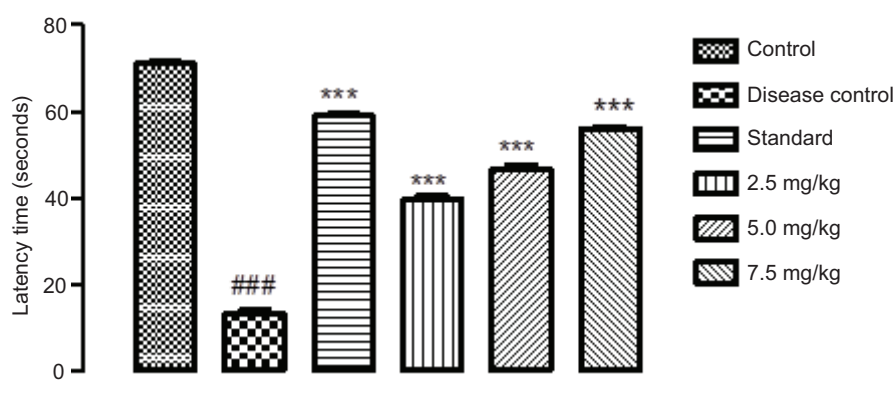


Figure 1. Effect of chamuangone-enriched rice bran oil on hang test in Parkinson's disease model. Mean±SEM of all figures with sample size (n=6) is presented. Control group difference to disease control group ### $p < 0.001$ and disease control group difference to other treatment groups *** $p < 0.001$.

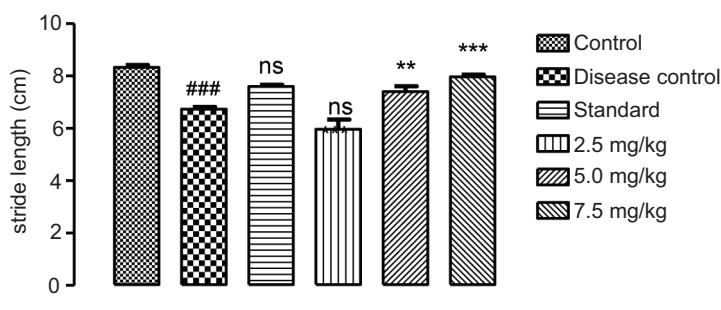


Figure 2. Effect of chamuangone-enriched rice bran oil on stride length in foot printing test in Parkinson's disease model. Mean±SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ### $p < 0.001$ and disease control group difference to other treatment groups *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$.

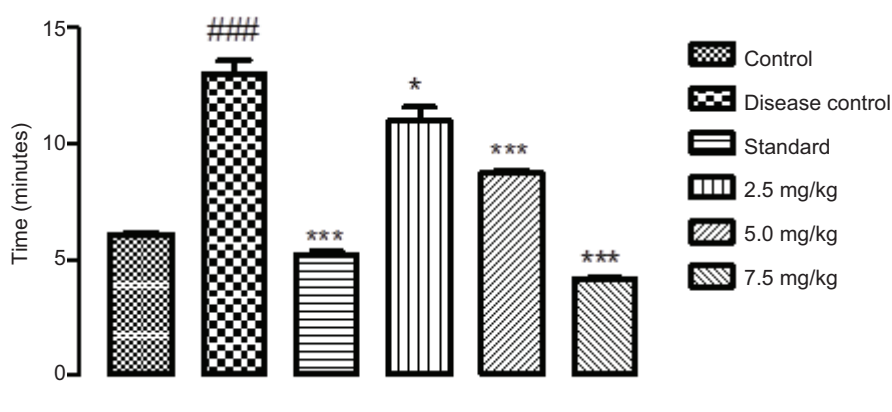


Figure 3. Effect of chamuangone-enriched rice bran oil on grip walk test in Parkinson's disease model. Mean±SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ### $p < 0.001$ and disease control group difference to other treatment groups *** $p < 0.001$, * $p < 0.05$.

dose-dependent improvement in climbing regarding the disease control group (Figure 3).

Forced swimming test

A swimming test was used to investigate depression in Parkinsonian rat model. Swim score remarkably ($p < 0.001$) decreased in the disease control group compared to the control group. The rats treated with CERBO at 2.5-, 5.0-, and 7.5-mg/kg doses remarkably ($p < 0.001$) raised the swim score comparable to the disease control group (Figure 4).

Open field test

An open field test was performed to evaluate the neuroprotective effect of CERBO on locomotion, exploration, and depression. Parameters such as the number of lines crossed; grooming, and frequency of rearing declined remarkably ($p < 0.001$) in the disease control group compared to the control group. Locomotion and motor functions recovered remarkably after treatment with chamuangone-rich extract treatment

($p < 0.001$) comparable to the disease control group (Table 3).

Biochemical analysis

Effect of CERBO on superoxide dismutase level

The SOD level of the disease group was remarkably ($p < 0.001$) decreased in contrast to the control group. Chamuangone-rich extract-treated groups had a remarkable ($p < 0.001$) dose-dependent increase in SOD level against the disease control group. CERBO at 2.5 mg/kg showed a value (2.58 ± 0.01) near to the standard value (2.95 ± 0.01). High doses of CERBO showed improvement in SOD level, compared to the standard group. CERBO at 7.5 mg/kg exhibited better results (3.36 ± 0.01), compared to 5.0-mg/kg CERBO (3.15 ± 0.01) (Table 4).

Effect of CERBO on catalase level

Table 4 shows that CAT level in brain tissue of the disease control group was remarkably ($p < 0.001$) decreased

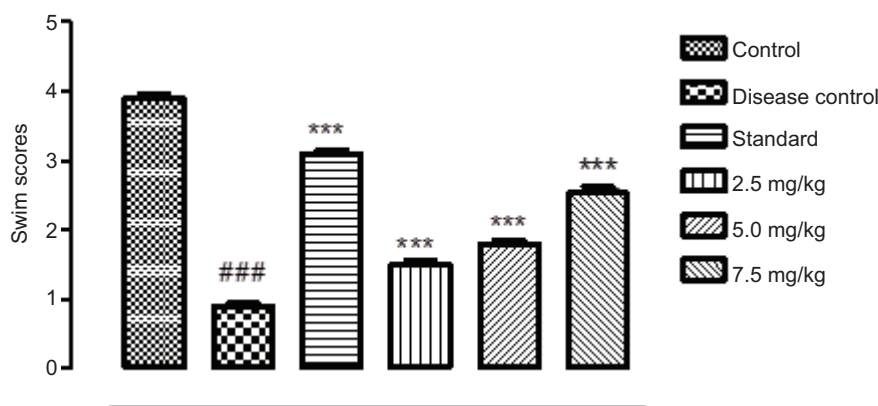


Figure 4. Effect of chamuangone-enriched rice bran oil on swimming test in Parkinson's disease model. Mean±SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ### $p < 0.001$ and disease control group difference to other treatment groups *** $p < 0.001$.

Table 3. Effect of chamuangone-enriched rice bran oil on open field test in Parkinson's disease model.

Groups	Dose (mg/kg)	Open field test		
		No. of lines crossed	No. of groomings	No. of rear rings
Control		14.5±0.28	5.57±0.02	10.49±0.07
Disease control (haloperidol)	1	7.34±0.02###	0.00±0.00###	4.15±0.17###
Standard (carbidopa+levodopa)	100+25	14.86±0.06***	3.22±0.11***	8.93±0.09***
Treatment groups	2.5	10.55±0.02***	2.97±0.01***	7.54±0.02***
	5.0	12.53±0.02***	3.54±0.02***	8.93±0.16***
	7.5	14.697±0.02***	3.77±0.01***	12.55±0.02***

Mean±SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ### $p < 0.001$ and disease control group difference to other treatment groups *** $p < 0.001$.

compared to the control group. CERBO-treated groups at 2.5-, 5.0-, and 7.5-mg/kg doses had a remarkable ($p < 0.001$) dose-dependent increase in CAT levels, comparable to the disease control group. CERBO at 5.0 mg/kg exhibited (2.14±0.01) value close to that of standard (2.15±0.01) group. At 7.5 mg/kg, CERBO showed (2.29±0.10) better results, compared to the standard group.

Effect of CERBO on malondialdehyde level

The level of MDA in brain tissue of the disease control group was remarkably ($p < 0.001$) elevated comparable to the control group. Treatment groups had a remarkable ($p < 0.001$) decline in MDA level in contrast to the disease control group. Chamuangone-rich extract at a 2.5-mg/kg dose exhibited a result (7.61±0.01) close to the standard group (7.55±0.01). Treatment groups at 5.0- and 7.5-mg/kg doses indicated the reversal of MDA level (Table 4).

Effect of CERBO on glutathione level

Table 4 shows that GSH level in the brain tissue of the disease control group was remarkably ($p < 0.001$) decreased

than the control group. CERBO-treated groups had remarkable ($p < 0.001$) efficacy at 2.5- and 5.0-mg/kg doses compared to the disease control group. CERBO at 7.5 mg/kg showed (0.79±0.001) value near to the standard (0.85 ± 0.001) value.

Effect of CERBO on glutathione peroxidase level

Glutathione peroxidase level in the brain tissue of disease control group was remarkably ($p < 0.001$) declined compared to the control group. Treatment groups at 2.5-, 5.0-, and 7.5-mg/kg doses had remarkable ($p < 0.001$) effectiveness compared to the disease control group. CERBO at 5.0-mg/kg dose exhibited 3.27±0.001 result, compared to 3.16 ± 0.001 result with 2.5-mg/kg dose. CERBO at 7.5-mg/kg dose showed better result (3.40±0.001) compared to the standard result (3.37±0.001) (Table 4).

Effect of CERBO on protein level estimation

Table 5 shows that protein levels were remarkably ($p < 0.001$) decreased in the disease control group in comparison to the control group. Chamuangone-rich extract-treated groups at 2.5-, 5.0-, and 7.5-mg/kg doses

Table 4. Effect of chamuangone-enriched rice bran oil on biochemical markers in the brain homogenate.

Groups	Dose (mg/kg)	Catalase (mmol/mg)	Superoxide dismutase ($\mu\text{g}/\text{mg}$ of proteins)	Malondialdehyde ($\mu\text{mol}/\text{mg}$ of proteins)	Reduced glutathione ($\mu\text{g}/\text{mg}$ of proteins)	Glutathione peroxidase ($\mu\text{g}/\text{mg}$ of proteins)	Proteins ($\mu\text{mol}/\text{g}$)
Control		2.30 \pm 0.01	3.56 \pm 0.01	6.53 \pm 0.11	0.66 \pm 0.01	3.43 \pm 0.21	98 \pm 0.57
Disease control (haloperidol)	1	1.67 \pm 0.04 ^{###}	2.26 \pm 0.01 ^{###}	8.25 \pm 0.01 ^{###}	0.59 \pm 0.01 ^{###}	2.36 \pm 0.02 ^{###}	40 \pm 0.57 ^{###}
Standard (levodopa+carbidopa)	100+25	2.15 \pm 0.01 ^{***}	2.95 \pm 0.02 ^{***}	7.55 \pm 0.01 ^{***}	0.85 \pm 0.21 ^{***}	3.37 \pm 0.01 ^{***}	64 \pm 0.57 ^{***}
Treatment groups	2.5	1.75 \pm 0.02 ^{***}	2.58 \pm 0.01 ^{***}	7.61 \pm 0.31 ^{***}	0.91 \pm 0.01 ^{***}	3.16 \pm 0.21 ^{***}	53 \pm 0.33 ^{***}
	5.0	2.14 \pm 0.02 ^{***}	3.15 \pm 0.02 ^{***}	7.68 \pm 0.02 ^{***}	0.97 \pm 0.41 ^{***}	3.27 \pm 0.51 ^{***}	63 \pm 0.88 ^{***}
	7.5	2.29 \pm 0.10 ^{***}	3.36 \pm 0.02 ^{***}	7.81 \pm 0.01 ^{***}	0.79 \pm 0.61 ^{***}	3.40 \pm 0.71 ^{***}	60 \pm 0.57 ^{***}

Mean \pm SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ^{###} $p < 0.001$ and disease control difference to other treatment groups ^{***} $p < 0.001$.

Table 5. Effect of chamuangone-enriched extract on dopamine level in brain homogenate.

Groups	Dose (mg/kg)	Protein ($\mu\text{mol}/\text{g}$)	Dopamine ($\mu\text{g}/\text{mg}$)	Serotonin ($\mu\text{g}/\text{mg}$)	Noradrenaline ($\mu\text{g}/\text{mg}$)
Control		98 \pm 0.57	0.65 \pm 0.02	0.86 \pm 0.05	0.71 \pm 0.03
Disease control (haloperidol)	1	40 \pm 0.57 ^{###}	0.04 \pm 0.03 ^{###}	0.33 \pm 0.02 ^{###}	0.31 \pm 0.05 ^{###}
Standard (levodopa + carbidopa)	100+25	64 \pm 0.57 ^{***}	0.70 \pm 0.11 ^{***}	0.82 \pm 0.01 ^{***}	0.68 \pm 0.01 ^{***}
Treatment groups	2.5	53 \pm 0.33 ^{***}	0.33 \pm 0.21 ^{***}	0.39 \pm 0.21 ^{***}	0.36 \pm 0.22 ^{***}
	5.0	63 \pm 0.88 ^{***}	0.47 \pm 0.012 ^{***}	0.58 \pm 0.11 ^{***}	0.44 \pm 0.25 ^{***}
	7.5	60 \pm 0.57 ^{***}	0.59 \pm 0.31 ^{***}	0.76 \pm 0.31 ^{***}	0.59 \pm 0.03 ^{***}

Mean \pm SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ^{###} $p < 0.001$ and disease control group difference to other treatment groups ^{***} $p < 0.001$.

indicated remarkable improvement in the disease control group. The treatment group at 5.0-mg/kg dose showed (63 \pm 0.33) result near the standard group (64 \pm 0.57) result.

Quantification of neurotransmitters

Effect of CERBO on dopamine level

Dopamine level in the brain tissue decreased remarkably ($p < 0.001$) in the disease control group compared to the control group. Dopamine level was remarkably ($p < 0.001$) restored in the substantia nigra of the treatment groups comparable to the disease control group. Treatment groups at 2.5-, 5.0-, and 7.5-mg/kg doses exhibited dose-dependent restoration of dopamine level than in the disease control group (Table 5).

Effect of CERBO on serotonin level

Table 5 shows that the serotonin level was remarkably ($p < 0.001$) reduced in the disease control group compared to the control group. CERBO-treated groups at 2.5-,

5.0-, and 7.5-mg/kg doses exhibited a dose-dependent improvement in serotonin level compared to the disease control group. CERBO at 5.0-mg/kg dose showed remarkable ($p < 0.001$) enhancement in serotonin level (0.58 \pm 0.01), compared to 2.5-mg/kg dose (0.39 \pm 0.01) in the disease control group.

Effect of CERBO on noradrenaline level

Noradrenaline level in the substantia nigra of the disease control group was remarkably ($p < 0.001$) reduced compared to the control group. CERBO-treated groups at 2.5-, 5.0-, and 7.5-mg/kg doses exhibited remarkable ($p < 0.001$) increase in noradrenaline compared to the disease control group. The standard group remarkably ($p < 0.001$) restored the noradrenaline level in comparison to the disease control group (Table 5).

Effect of CERBO on acetylcholinesterase activity

Acetylcholinesterase level was remarkably ($p < 0.001$) increased in the disease control group compared to the control group. CERBO at 7.5-mg/kg dose exhibited

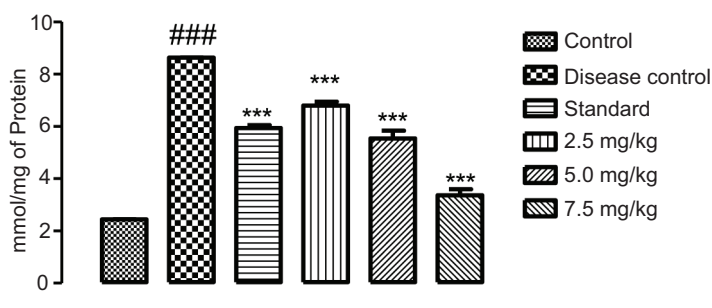


Figure 5. Effect of chamuangone-enriched bran oil on acetylcholine level in brain homogenate. Mean \pm SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ### $p < 0.001$ and disease control group difference to other treatment groups *** $p < 0.001$.

remarkably ($p < 0.001$) decreased AChE level in comparison to the standard, 2.5-mg/kg and 5.0-mg/kg dose (Figure 5).

Effect of CERBO on hematological examination

Red blood cells (4.6 ± 0.15), hemoglobin (11 ± 0.57), and hematocrit (42 ± 0.57) were remarkably ($p < 0.001$) decreased in the disease control group compared to the respective control group values (5.4 ± 0.05 , 14 ± 0.57 , and 47 ± 0.57) but within a normal range (Hb, F: 12–16, M: 12–17, and HCT 35–55%). WBC (13 ± 0.57), neutrophils (77 ± 0.57), lymphocytes (18 ± 0.57), monocytes (11 ± 0.57), and platelets (281 ± 0.88) were remarkably ($p < 0.001$) increased comparable to the control group values (WBC 6.6 ± 0.33 , neutrophils 69 ± 0.57 , lymphocytes 36 ± 0.57 , monocytes 8 ± 0.57 , and platelets 251 ± 0.88) but within normal range (WBC $4\text{--}11 \times 10^3/\mu\text{L}$, neutrophils 40–75%, lymphocytes 20–45 %, monocytes 02–07%, and platelets $150\text{--}450 \times 10^3/\mu\text{L}$). However, chamuangone-rich extract treatment at different doses did not produce any adverse effect on hematological parameters (Table 6).

Effect of CERBO on liver function test

The AST level of the disease control group showed a remarkable ($p < 0.001$) increase in liver enzymes (73 U/L) compared to the control group (47 U/L). The standard group (levodopa+carbidopa) was associated with mild increase in AST level (54 U/L). The alanine transferase (ALT) level (72 U/L) of the disease control group was remarkably ($p < 0.001$) increased compared to the control group (41 U/L). However, CERBO treatment controlled the increased level of AST in a dose-dependent manner. The bilirubin level (1.8 mg/dL) of the disease control group increased remarkably ($p < 0.001$) compared to the control group (0.57 mg/dL). CERBO treatment groups showed dose-dependent recovery in bilirubin level (Table 7).

Effect of CERBO on renal function tests

The serum creatinine level (1.4 ± 0.03) of the disease control group increased remarkably ($p < 0.001$) compared to the control group (0.7 ± 0.03). Treatment groups at 2.5-, 5.0-, and 7.5-mg/kg doses showed dose-dependent

improvement in serum creatinine level compared to the disease control group. Urea level (30 ± 0.57) in the disease control group was increased remarkably ($p < 0.001$) compared to the control group (21 ± 0.33). Chamuangone-rich extract-treated groups at 2.5-, 5.0-, and 7.5-mg/kg doses showed remarkable relapse in a dose-dependent manner ($p < 0.001$) in disease control group (Table 7).

Effect of CERBO on lipid profile

Cholesterol level (65 ± 0.57) of the disease control group was increased remarkably ($p < 0.001$) against the control group value (52 ± 0.57). Standard group showed a decrease in cholesterol level (55 ± 0.57) against the disease control group. Chamuangone-rich extract treatment remarkably decreased the cholesterol level in a dose-dependent manner in treatment groups. Triglycerides, high density lipoprotein (HDL), and low-density lipoprotein (LDL) were remarkably ($p < 0.001$) increased in the disease control group compared to the control group within normal range. Dose-dependent elevations were observed in HDL but decline in LDL in the treatment groups. Therefore, chamuangone-rich extract increased the level of good fats in a dose-dependent manner (Table 8).

RT-PCR analysis of Parkinson's disease biomarkers

mRNA expression of pathological genes and inflammatory cytokines (interleukin 1 alpha [IL-1 α], interleukin 1 beta [IL-1 β], alpha synuclein [α -synuclein], and tumor necrosis factor alpha [TNF- α]) was increased remarkably ($p < 0.001$) in the diseased model group treated with haloperidol. However, treatment with chamuangone-rich extract markedly recovered the mRNA expression of these markers in treatment groups similar to the control group (Figure 6).

Effect of CERBO on the histology of brain and vital organs

Histopathological examination of brain

Control group animals showed normal architecture of brain tissues. Decline was observed in neuronal count,

Table 6. Effect of chamuangone-enriched rice bran oil on hematology of rat.

Parameters	Units	Control	Disease control	Standard	Treatment groups		
					2.5 mg/kg	5.0 mg/kg	7.5 mg/kg
Hemoglobin	g/dL	14±0.57	11±0.57 ^{###}	16±0.57 ^{***}	13±0.57 ^{***}	14±0.01 ^{**}	17±0.57 ^{***}
Red blood cells (RBC)	×10 ⁶ /μL	5.4±0.05	4.6±0.15 ^{###}	5.8±0.05 ^{***}	4.7±0.05 [*]	5.2±0.08 ^{**}	5.5±0.12 ^{***}
Hematocrit	%	47±0.57	42±0.57 ^{###}	50±0.57 ^{***}	39±0.57 ^{***}	44±0.88	46±0.88 ^{**}
Mean corpuscular volume	%	80±0.57	85±0.33 ^{###}	90±0.57 ^{***}	77±0.57 ^{***}	82±0.57 ^{**}	85±0.33
Mean corpuscular hemoglobin	pg	29±0.57	21±0.88 ^{###}	31±0.57 ^{***}	23±0.57 ^{***}	25±0.57 [*]	27±0.57 ^{***}
Mean corpuscular hemoglobin concentration	%	31±0.57	28±0.57 [#]	32±0.57 ^{***}	25±0.57 ^{***}	28±0.57 [*]	29±0.57 ^{**}
Red cell distribution width	%	12±0.33	17±0.57 ^{###}	14±0.33 [*]	15±0.33 ^{***}	12±0.57 ^{***}	16±0.57 ^{***}
White blood cells (WBC)	10 ³ /μL	6.6±0.33	13±0.57 ^{###}	10±0.33 ^{**}	12±0.57 [*]	8±0.57 ^{***}	9±0.33 ^{***}
Neutrophils	%	69±0.57	77±0.57 ^{###}	74±0.57 ^{***}	76±0.57 ^{**}	72±0.57 ^{**}	71±0.57 ^{***}
Lymphocytes	%	36±0.57	18±0.57 ^{###}	33±0.57 ^{***}	21±0.57 [*]	26±0.57 ^{***}	29±0.57 ^{***}
Monocytes	%	8±0.57	11±0.57 ^{###}	14±0.57 ^{***}	5±0.57 ^{**}	7±0.57 [*]	9±0.57 ^{***}
Platelets	10 ³ /μL	251±0.88	28±0.88 ^{###}	271±0.57 ^{***}	241±0.57 ^{***}	246±0.57 ^{***}	248±0.57 ^{***}

Mean±SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ^{###}p < 0.001 and disease control group difference to other treatment groups ^{***}p < 0.001, ^{*}p < 0.05, ^{**}p < 0.01.

Table 7. Effect of chamuangone-enriched rice bran oil on liver function tests.

Groups	Dose (mg/kg)	Liver function test (LFT; mg/dL)			Renal function tests (RFT; mg/dL)	
		AST	ALT	Bilirubin (total)	Creatinine	Urea
Control		47±0.57	41±0.33	0.57±0.01	0.7±0.03	21±0.33
Disease control (haloperidol)	1	73±0.57 ^{###}	72±0.57 ^{###}	1.8±0.05 ^{###}	1.4±0.03 ^{###}	34±0.33 ^{###}
Standard (carbidopa + levodopa)	100+25	54±0.57 ^{***}	52±0.57 ^{***}	0.53±0.05 ^{***}	0.6±0.03 ^{***}	20±0.33 ^{***}
Treatment groups	2.5	50.09±0.57 ^{***}	55.02±0.57 ^{***}	0.7±0.01 ^{***}	1.5±0.03 ^{***}	27.04±0.33 ^{***}
	5.0	49.1±0.57 ^{***}	48.6±0.57 ^{***}	0.6±0.01 ^{***}	1.1±0.03 ^{**}	25.02±0.33 ^{***}
	7.5	48.09±0.57	42.01±0.57 ^{**}	0.54±0.05 ^{***}	0.8±0.03	21.07±0.33 ^{***}

Mean±SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ^{###}p < 0.001 and disease control group difference to other treatment groups ^{***}p < 0.001.

Table 8. Effect of chamuangone-enriched rice bran oil on lipid profile.

Groups	Dose (mg/kg)	Lipid profile (mg/dL)			
		Cholesterol	Triglycerides	HDL	LDL
Control		52±0.57	35±0.57	47.04±0.57	48±0.57
Disease control (haloperidol)	1	65±0.57 [#]	42±0.57 ^{###}	34.0±0.57 ^{###}	56±0.57 ^{###}
Standard (carbidopa+levodopa)	100+25	55±0.57 ^{***}	31±0.57 ^{***}	41±0.57 ^{***}	43±0.57 ^{***}
Treatment groups	2.5	47±0.57 ^{***}	38±0.57 ^{***}	44±0.57 ^{***}	45±0.57 ^{***}
	5.0	50±0.57 ^{***}	35±0.57 ^{***}	47±0.57 ^{***}	44±0.57 ^{***}
	7.5	46±0.57 ^{***}	32±0.57 ^{***}	51±0.57 [*]	42±0.57 ^{***}

Mean±SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ^{###}p < 0.001 and disease control group difference to other treatment groups ^{***}p < 0.001.

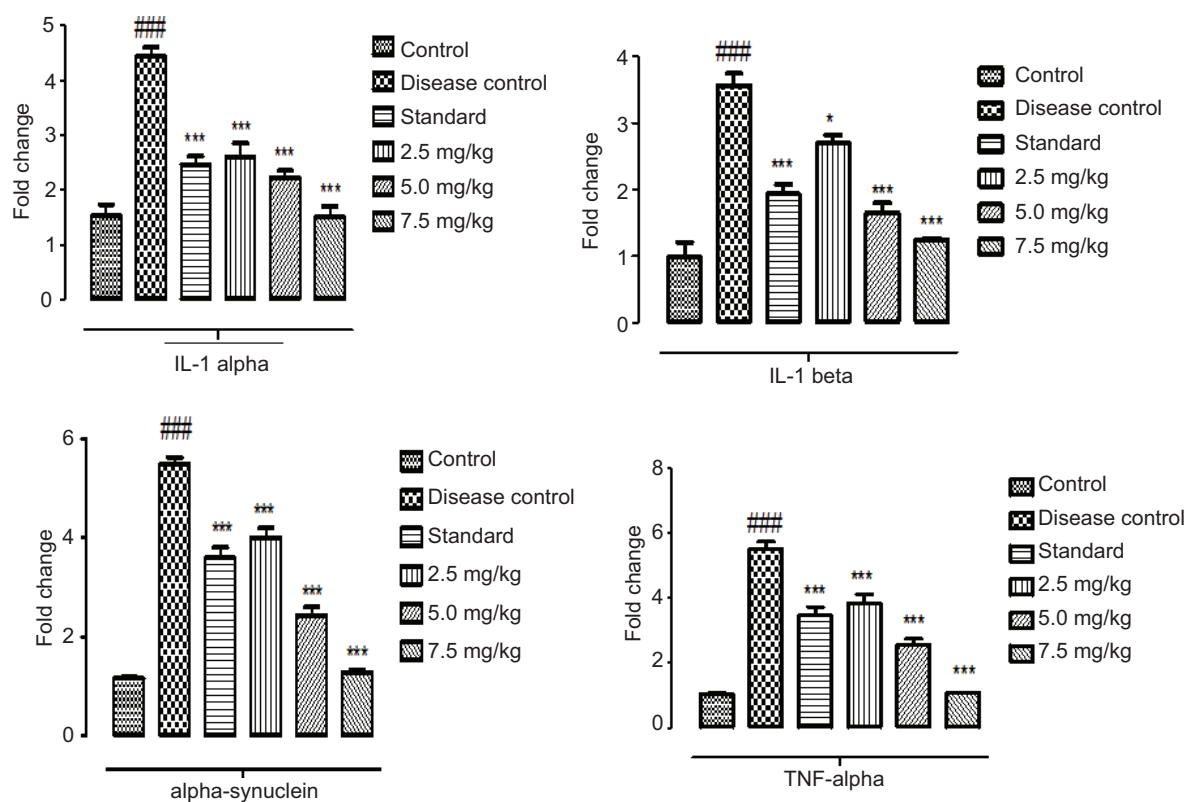


Figure 6. Effect of CERBO on RT-PCR analysis. Mean±SEM of all figures with sample size (n = 6) is presented. Control group difference to disease control group ### $p < 0.001$ and disease control group difference to other treatment groups * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$.

neurofibrillary tangles, infiltration of inflammatory cytokines, pigmentation, and formation of Lewy's bodies. However, treatment with CERBO, similar to a standard drug (L-dopa+carbidopa) in a dose-dependent manner recovered pathological signs, neurofibrillary tangles, neuronal loss, and neurodegeneration (Figure 7).

Histopathological examination of vital organs

CERBO treatment did not produce any notable sign of toxicity or adverse effects on the histopathology of vital organs. Similar to the control group, CERBO-treated group showed the healthy architecture of myocardial fibers. Anyhow, mild degree of vascular congestion in myocardial fibers was observed in the disease control group. Similarly, it was revealed that CERBO did not produce any lethal or harmful effect on the histology of hepatocytes and nephrons. Identical to the control group, chamuangone-rich extract treatment groups showed normal renal parenchyma. However, in the disease control group, moderate degree of congestion and necrotic changes were observed in the renal parenchyma and nuclei (Figure 8).

The liver in the control group showed normal-shaped hepatocytes, which were connected to the portal vein, a

compact structure with no fibrosis. Liver cells in the disease control group showed moderate degree of vascular congestion and vacuolization in the parenchyma cells. The standard group showed an abundance of hepatic vacuolization in the parenchyma cells. Chamuangone-rich extract at 7.5-mg/kg dose showed normal epithelial cells and hepatocytes with mild degree of congestion (Figure 8).

The lungs of the control group showed normal bronchi, alveoli, blood vessels, and normal epithelium. The disease control group showed congestion in the lungs. The standard group showed normal bronchi and alveoli. Chamuangone-rich extract at 7.5-mg/kg dose showed no histopathologically harmful manifestations on lung tissues (Figure 8).

Discussion

The active compounds of *G. cowa* chamuangone show remarkable pharmacological pursuits and therapeutic potential through the modulation of multiple underlying mechanistic pathways. Vegetable oil and rice bran oil as alternative green solvents were used for extraction of

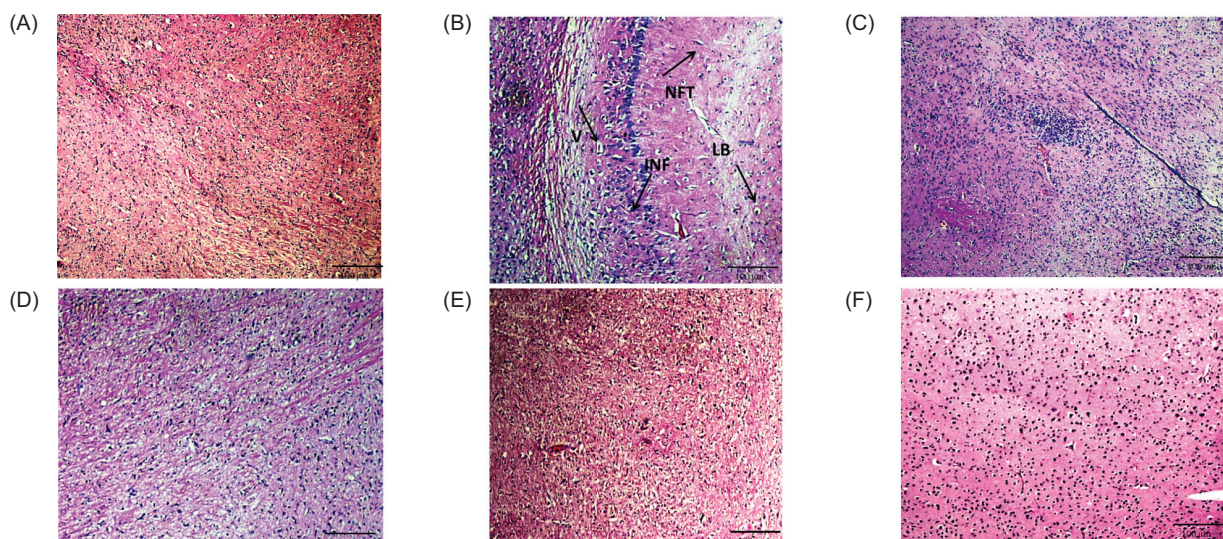


Figure 7. Effect of chamuangone-enriched rice bran oil treatment on histopathology of brain tissues. (A) Control; (B) Disease control; (C) Standard; (D–F) Treatment groups with 2.5-, 5-, and 7.5-mg/kg doses, respectively. INF: infiltration; LB: Lewy's bodies; V: vacuolization; NFT: neurofibrillary tangles.

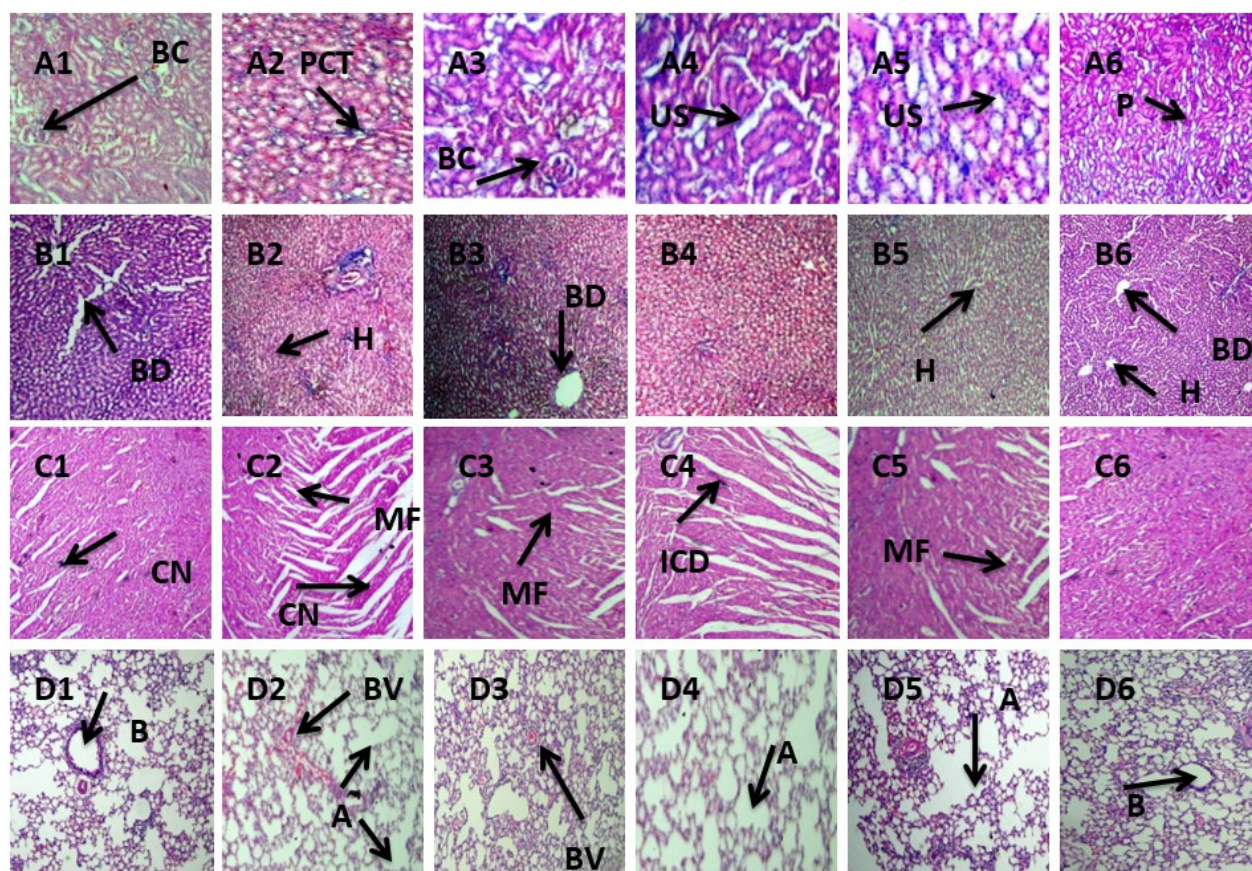


Figure 8. Effect of chamuangone-enriched rice bran treatment on the histopathology of vital organs. (A) kidney; (B) liver; (C) heart; (D) lungs. 1: control; 2: disease control; 3: standard; 4–6: treatments groups at 2.5-, 5-, and 7.5-mg/kg doses, respectively. BC: Bowman's capsule; PCT: proximal convoluted tubules; US: urinary space; P: podocytes; BD: bile duct; H: hepatocytes; CN: central nuclei; MF: myocardial fibrils; ICD: intercalated disc; B: bronchioles; BV: blood vessels; and A: alveoli.

this compound using microwave. This green method of extraction was adopted to yield enriched extract of chamuangone. This rich extract was investigated in previous studies that explored its anticancer activities against human lung adenocarcinoma, human colorectal adenocarcinoma cell lines, and human breast carcinoma, but was noninvasive to healthy cells. However, the therapeutic aptitude of this compound led to the designing of the current work to evaluate the neuroprotective potential of this compound in rat models. In previous studies, the anticancer, anti-inflammatory, and antibacterial cytotoxic activity against *Leishmania major* were reported (Sae-Lim *et al.*, 2019a, 2020; Sakunpak and Panichayupakaranant 2012; Sakunpak *et al.*, 2017).

The second-most prevalent neurodegenerative condition is PD, followed by rigidity, bradykinesia, tremor, loss of body movement, and postural instability. Patients with PD may develop non-motor symptoms, such as sleep problems, psychopathic conditions, sensory disorders, mood swings, and intellectual disabilities. The occurrence of both intracellular clumps of misfolded alpha-synuclein and diminished dopaminergic neurons in the substantia nigra are related to PD (Chopade *et al.*, 2023). The incidence of PD increases with increase in age. The factors implicated in the prognosis of PD are due to neuroinflammation and oxidative stress, leading to the reduction of inlaying antioxidant contents.

Many toxicity profiles of the currently available anti-Parkinson drugs are reported. Therefore, modern and ongoing perspectives are studied for the treatment challenges of new agents which either inhibit and overcome the progression of ailment and are also inexpensive. Therefore, the necessity for developing new compounds of plant origin having preventive effects against PD with minimum adverse effects is increasing. Plants with renowned antioxidant activities are documented. These plants have a great source of possible bioactive moieties with neuropharmacological activities (Wang, 2010).

Chamuangone-rich extract in rice bran oil from *Garcinia cowa* leaves exhibited robust pharmacological activities, especially anticancer, antioxidant and anti-inflammatory properties. In light of the previously reported studies, the current study reported the neuroprotective potential of chamuangone-rich extract (Sae-Lim *et al.*, 2020). Chamuangone-rich extract has remarkable potential to reduce and prohibit free radical production in HT22 cells (Nguyen *et al.*, 2022). The lipid membranes of the brain are very vulnerable to lipid peroxidation and are key characteristics in the development of neurodegenerative disorders, such as PD and Alzheimer's disease (Singh *et al.*, 2019). The accumulation of lipid peroxide was discovered to lead toward amyloidosis and neurofibrillary

tangles in PD. Antioxidant-like flavonoids reinforced antioxidant enzymes and repletion of GSH contents in neurodegenerative disorders (Angelova, 2021).

Haloperidol belongs to a typical group of antipsychotics. Because of its highly potent nature, it is used in the management of psychosis, mania, hyperactivity, aggressiveness, and delirium. Haloperidol serves as a high affinity D2 receptor antagonist in basal ganglia and causes destruction of dopaminergic neurons (Andreassen *et al.*, 2001). It is confirmed that a long administration of haloperidol leads to Parkinsonian symptoms, such as bradykinesia, tremor, cognitive impairment, and ataxia. Consecutive administration of haloperidol leads to the reduction of dopaminergic neurons. There are two potential ways for haloperidol to cause catalepsy. The oxidation of haloperidol by itself or by oxidase results in a considerable amount of oxyradicals and a toxic metabolite that resembles pyridinium and attacks the complex 1 process. Neuroleptics that block D2 receptors in the nigrostriatal neurons of the brain over time cause an increase in basal ganglia dopamine turnover, which may lead to an abundance of free radicals, such as hydrogen peroxide and dopamine quinone, via monoamine oxidase activity (Pathan *et al.*, 2022).

Catalepsy, a key behavioral feature of PD, was found to be attenuated by treatment with chamuangone-rich extract and showed improvement in the muscular strength of experimental rats. It was manifested in the hanging test that chamuangone-rich extract enhanced muscularity, which was reduced due to the persistent administration of haloperidol. Forced swimming test, open field test, and grip walk test showed improvement in latency time, physical strength, motor coordination, cognitive abilities, and balance because of administration of chamuangone. These neurobehavioral studies collaborated with previous study conducted on TTME extract at 100-, 300-, and 1,000-mg/kg doses, indicating the neuroprotective effect of different doses (Saleem *et al.*, 2020). Current results revealed that chamuangone-rich extract improved cognitive abilities, motor coordination, and neuromuscular strength.

It was documented that numerous mental disabilities were interlinked with CAT deficiency. Antioxidant enzymes, such as CAT, which function as the body's first line of defense, neutralized the toxic effects of hydrogen peroxide by converting it in water and inert oxygen. CAT levels dropped because of oxidative stress caused by haloperidol injection (Bhangale and Acharya, 2016). Therefore, in the current study, the CAT level declined in the disease control group compared to the control group, whereas in the treatment group, CAT level increased in a dose-dependent manner, showing the antioxidant effect of chamuangone.

The crucial antioxidant enzyme GPx, which is found in the mitochondria and cytoplasm, suppresses oxidative stress and lipid peroxidation, and its absence was related to neurotoxic consequences. Another antioxidant enzyme, GSH, serves as a store for reduced GSH as well as a scavenger of singlet oxygen and hydroxyl radicals. Reduced levels of GSH are associated with neurodegeneration and ageing (Asaduzzaman Khan *et al.*, 2010; Chabory *et al.*, 2009; Santambrogio *et al.*, 2015).

Superoxide dismutase is a crucial enzyme that catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, thus preventing the formation of highly reactive and damaging oxygen species. Studies have shown that CERBO significantly upregulates the activity of SOD. This increase in SOD activity enhances cellular defense against oxidative stress, thereby preserving cellular integrity and functioning (Law *et al.*, 2017).

Similarly, reduced GSH plays a crucial role in cellular antioxidant defense by scavenging free radicals and regenerating other antioxidants. CERBO has been observed to augment intracellular GSH levels. This augmentation enhances the cell's capacity to neutralize ROS and counteract oxidative damage to biomolecules (Law *et al.*, 2017).

Lipid peroxidation and protein oxidation are characteristic processes associated with oxidative stress, leading to cellular damage and dysfunction. MDA is a commonly used biomarker to assess lipid peroxidation. Research findings suggest that CERBO exhibits a remarkable ability to attenuate lipid peroxidation, as evidenced by reduced MDA levels. This inhibition of lipid peroxidation contributes to the maintenance of membrane integrity and function, thus preserving cellular homeostasis (Law *et al.*, 2017).

Furthermore, protein peroxidation, characterized by the oxidative modification of proteins, can compromise their structural and functional integrity. CERBO has been shown to mitigate protein peroxidation, thereby preserving the functionality of key cellular proteins and enzymes.

Chamuangone-enriched rice bran oil demonstrates significant modulation of the first-line antioxidant enzymes, such as SOD, GSH, and CATs, leading to enhanced cellular antioxidant defense mechanisms. Moreover, it effectively attenuates lipid and protein peroxidation, as indicated by the reduced levels of MDA and protein oxidation products. These findings underscore the potential therapeutic applications of CERBO in mitigating oxidative stress-related pathologies and promoting overall health and well-being.

Moreover, levels of neurotransmitters (dopamine, serotonin, and noradrenaline) were decreased remarkably in the disease control group in comparison to the control group. On the other hand, the standard group (L-dopa+carbidopa) exhibited a remarkable increase in serotonin, noradrenaline, and dopamine levels against the disease control group. Moreover, all the treatment groups having chamuangone-rich extract exhibited dose-dependent improvement in dopamine, serotonin, and noradrenaline, which showed the strong neuroprotective effect of CERBO for treating neurodegenerative disorders.

Acetylcholinesterase is primarily responsible for hydrolyzing acetylcholine to stop cholinergic neurotransmission at synapses. It was well documented in earlier studies that elevated levels of a synaptic homologous of AChE mRNA cause apoptosis in a variety of cell types. In the PD model, the neurotoxic effects of haloperidol increased AChE level and its mRNA expression (Ben-Shaul, *et al.*, 2006). By activating microglial cells in the striatum body and cerebral spinal fluid (CSF), the early research observed that PD etiopathogenesis was closely linked to the increased progression of pro-inflammatory cytokines, such as TNF- α and IL-1 α . It was proposed that the death of dopaminergic neuronal cells in PD brain was the result of persistently elevated levels of the pro-inflammatory mediator, IL-1 α . According to reports, elevated levels of TNF- α lead to neurodegeneration and akinesia in forelimbs (Godoy *et al.*, 2010; Saleem *et al.*, 2020). Furthermore, the histopathological examination revealed a significant improvement in brain tissues of treatment groups. Interestingly, chamuangone-rich extract's neuroprotective properties become apparent at a higher dosage of 7.5 mg/kg. Therefore, CERBO and its formulations could be considered as a tonic for neuronal health and a therapeutic choice to develop novel medicament to treat PD.

Conclusion

Parkinson's disease is widely recurring neurodegenerative disorder in people aged >60 years. In spite of having effective treatment strategies, the basis for the development of remedies for both chronic and acute conditions has been established by plants. The current study was established to observe the therapeutic efficacy of chamuangone-rich extract, the active compound of *Garcinia cowa* leaves, in PD. It is revealed that CERBO treatment markedly recovers the motor and non-motor manifestations of PD. CERBO treatment improved both antioxidant defense system and level of central neurotransmitters and regained antioxidant enzymes. Therefore, considering all these findings, we concluded that CERBO could be considered an alternative to develop modern therapeutics for treating PD.

Author Contributions

Uzma Saleem, Muhammad Ajmal Shah, Pharkphoom Panichayupakaranant, and Ana Sanches Silva conceived and designed the research. Badriyah S. Alotaibi, Aqsa Ahmad, Zunera Chaudhary, Maryam Farrukh, Rana O. Khayat, and Ifat Alsharif performed the experiments. Tourki A.S. Baokbah, Aishah E. Albalawi, Norah A. Althobaiti, Khairul Anam, Yasmene F. Alanazi, and Renald Blundell analyzed and interpreted the data. All authors equally participated in preparing, revision, and final approval for submission of the manuscript.

Conflict of Interest

All authors declared no conflict of interest.

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