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Original Article

Evaluation of Antiparkinsonism Activity of *Manilkara zapota* (L.) P. Royen Leaf Extract in Haloperidol-Induced Parkinsonism in Swiss Albino Mice

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ABSTRACT

Background: *Manilkara zapota*, a plant with a rich history in traditional medicine, holds potential as a therapeutic agent. However, its neuroprotective properties remain largely unexplored, highlighting the need for further scientific research to understand its potential in this regard. **Objectives:** This study aimed to explore the neuroprotective effects of *Manilkara zapota* ethanolic extract against haloperidol-induced Parkinsonism in Swiss albino mice. **Materials and Methods:** The *M. zapota* leaf powder was extracted using 70% ethanol, followed by a qualitative and quantitative investigation. In this study, the effects of extract at 200 and 400 mg/kg (p.o.) against haloperidol in mice were assessed using various *in vivo* behavioural parameters including catalepsy, grid hang, horizontal bar, and parallel bar tests. **Results:** The qualitative analysis of ethanolic extract identified phenols, alkaloids, carbohydrates, flavonoids, tannins, proteins, and saponins. Furthermore, the quantitative assessment indicated total flavonoid and phenol contents of 64.52 mg RTE/g and 17.6 mg GAE/g, respectively. The extract showed significant and dose-dependent enhancements in behavioural activity, motor function, muscle strength, and motor coordination. Moreover, its administration dose-dependently elevated antioxidant enzyme levels, such as glutathione, superoxide dismutase, and catalase in haloperidol-treated mice, suggesting its ability to alleviate oxidative stress. Additionally, the histopathological analysis indicated that ethanolic extract treatment restored normal architecture. **Conclusion:** These findings suggest that *M. zapota* extract has significant neuroprotective properties against haloperidol-induced Parkinsonism, possibly *via* its antioxidant properties. Further research is needed to understand its mechanisms and therapeutic potential for Parkinson's disease.

Keywords: *Manilkara zapota* (L.) P. Royen, Haloperidol, Ethanolic extract, Histological studies.

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INTRODUCTION

Parkinson's Disease (PD) is an age-related, progressive neurodegenerative condition. Its hallmark pathology involves continuous and dynamic cellular damage within the substantia nigra, primarily affecting the ventral portion of the pars compacta. This results in a decrease in dopamine levels in the striatum.[1,2] Consequently, there's a decrease in the function of dopaminergic neurons, while cholinergic neuron activity becomes relatively dominant, contributing to the development of movement disorders. Clinically, PD manifests through primary motor symptoms like resting tremors, bradykinesia, rigidity, and postural instability, alongside non-motor symptoms such as neuropsychiatric manifestations, dysautonomia, gastrointestinal issues, and sensory complaints.[3] Additional cognitive and behavioural complexities may arise, potentially leading to dementia as the disease progresses. Emotional disturbances sleep disturbances, and sensory symptoms may also manifest alongside these cognitive and behavioural changes. The second most common cause of Parkinsonism in older individuals is drug-induced Parkinsonism. Neuroleptic medications such as haloperidol are prominent contributors to drug-induced Parkinson's worldwide. Haloperidol is an antipsychotic drug frequently prescribed for schizophrenia and is known for its tendency to induce extrapyramidal symptoms like Parkinsonism and tardive dyskinesia. Despite its widespread usage, the specific pathophysiology behind haloperidol-induced extrapyramidal symptoms remains unclear.[4-7] One proposed mechanism revolves around oxidative stress, arising from an increase in Reactive

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Oxygen Species (ROS) production alongside a decline in antioxidant defence mechanisms. Haloperidol treatment blocks dopamine receptors, leading to a rise in dopamine turnover rates, potentially resulting in ROS generation as metabolic by-products. Additionally, Haloperidol administration is linked to a significant reduction in glutathione levels, which further exacerbates oxidative stress.[8,9]

The synthetic drug levodopa, often paired with a carbidopa, has been considered the gold standard for managing Parkinson's Disease (PD).[10] However, prolonged use of these medications frequently leads to debilitating side effects, including respiratory disturbances, nausea, vomiting, mania, hallucinations, dyskinesia, convulsions, and anxiety, among others.[11,12] Current pharmacological treatments for PD are associated with numerous side effects and do not address the underlying degeneration of dopaminergic neurons.[13] Consequently, there's been a surge in demand for natural products with antiparkinsonian properties, driven by their perceived lower side effect profiles and cost-effectiveness.

Manilkara zapota (L.) P. Royen, commonly known as Sapodilla or chikoo, is a noteworthy medicinal plant belonging to the Sapotaceae family and is indigenous to India. *M. zapota* has been employed for treating various conditions such as fever, haemorrhage, arthritis, wound healing, ulcers, rheumatism, pulmonary diseases, and as an antifungal agent. Additionally, the fruits of *M. zapota* are utilized in traditional medicine for their antioxidant properties, attributed to their polyphenolic content.[14,15] Different parts of this plant have been reported for their antioxidant, anticancer, antidiarrheal, antimicrobial, analgesic, antipyretic, anti-inflammatory, hypocholesterolemic, antifungal, CNS depressant, antiarthritic, and wound healing properties.[16-18] However, to date, no research has been conducted on the neuroprotective activity of *M. zapota* leaves. Thus, the objective of this study was to investigate the neuroprotective effects of *M. zapota* leaves in a haloperidol-induced Parkinson's disease model.

MATERIALS AND METHODS

Plant Materials

In March 2023, the leaves of *Manilkara zapota* (L.) Royen (*M. zapota*) were gathered from the vicinity of Gungal village, located in the Yacharam Mandal of Ranga Reddy district, Telangana State, India. The plant was authenticated by Dr. L Rasingam, Scientist-E at the BSI, Deccan Regional Centre, Hyderabad, with the Identification number BSI/DRC/2022-23/911.

Preparation of extract

Initially, the plant leaves were cleaned under running tap water to remove adhering dust/foreign particles and dried under the shade. Once dried, the leaves were finely ground into a coarse powder and sieved. The resulting dried powdered *M. zapota* (100 g) leaves were then placed into the thimble of a Soxhlet apparatus and subjected to extraction with ethanol (500 mL) at 65°C. The resultant extract was filtered and then evaporated to dryness using a rotary evaporator. The final dried extract sample was preserved at -19°C for further analysis.

Qualitative phytochemical analysis

The Ethanolic Extract of *M. zapota* (EEMZ) leaves underwent several qualitative chemical tests following standardized protocols to ascertain the presence or absence of secondary metabolites. *viz.*, alkaloids, flavonoids, glycosides, carbohydrates, proteins and amino acids, tannins, phenols and terpenoids.[19]

Quantitative analysis

Total Phenolic Content (TPC)

The Folin-Ciocalteu reagent method was used to assess the concentration of phenolic compounds in the *M. zapota* extracts. In test tubes, standard gallic acid solutions (10, 20, 40, 60, 80, 100 µg/mL) were prepared. To each tube, 5 mL of distilled water and 0.5 mL of Folin-Ciocalteu's reagent were added. After incubating for 5 min, 1.5 mL of 20% sodium carbonate solution was introduced, followed by the addition of 10 mL of distilled water. The mixture was then allowed to incubate at room temperature for 2 hr, resulting in a deep blue coloration. After incubation, a UV-visible spectrophotometer was used to measure the absorbance at 750 nm. Triplicate extractions were performed, with a blank prepared using a reagent blank and solvent. Gallic acid served as the control, and a calibration curve was constructed. The total phenolic content of the extract was expressed as milligrams of Gallic Acid Equivalent weight (GAE) per g of dry mass.[20]

Total Flavonoid Content (TFC)

The Aluminium chloride colorimetric method was employed to estimate the total flavonoid content. In test tubes, 1 mL aliquots of various concentrations of standard rutin solution (20, 40, 60, 80, 100, 120 µg/mL) were combined with 4 mL of distilled water and 0.3 mL of 5% sodium nitrite solution. After incubating for 5 min, 0.3 mL of 10% aluminum chloride solution was added to each tube. Following another 5 min incubation, 2 mL of 1 M sodium hydroxide was introduced. Finally, the volume was brought up to 10 mL with distilled water and thoroughly mixed. The resulting orange-yellowish color was measured using a UV-visible spectrophotometer at 510 nm. A blank was prepared using distilled water, and rutin served as the standard. Triplicate analyses were performed for each sample, and a calibration curve was generated using rutin. The total flavonoid content of the extract was expressed as mg of Rutin Equivalent Weight (RTE) per g of dry mass.[21]

Experimental animals

Male Swiss albino mice, weighing between 20 and 30 g, were selected and procured from Mahaveera Enterprises located in Hyderabad, Telangana. The mice were acclimated to the laboratory environment for 1 week before the start of the experiment. During this period, they were provided with a standard laboratory diet and had unlimited access to water. A 12:12 hour light-dark cycle and a room temperature of 25-27°C were maintained. Institutional guidelines were strictly followed throughout the study, and the animals were handled in accordance with CCSEA guidelines. The study was approved by the Institutional Animal Ethics Committee (RBVRR 1328/05/2023).

Experimental Design

Male Swiss albino mice were divided into five groups, each consisting of six animals (Table 1).

The test drugs EEMZ (200 mg/kg and 400 mg/kg, p.o.) and the standard drug levodopa+carbidopa (100 mg/kg+25 mg/kg, i.p) were administered 30 min before the infusion of haloperidol for 21 days.

Neurobehavioral studies

Catalepsy

Catalepsy is a measure of muscle rigidity that can arise through various reasons such as Parkinson's disease or pharmacological exposure to antipsychotics or cannabis. Catalepsy bar tests are commonly used to assess stiffness. The mice's arms are placed on a horizontal bar lifted off the ground, and the time it takes for the subjects to remove themselves from this imposed posture is measured (Figure 1). Traditionally, this has been measured by an experimenter using a stopwatch or by prohibitively expensive commercial apparatus that has its difficulties. The maximum cut-off time is 30 sec.[22]

Grid Hang Test

The grid-hang test detects muscle strength deficiencies. A wire cage lid is utilized for this test, closing the sides with the help of cello tape to keep the mouse from going off the rim. The animal is placed on the cage lid's top. The lid is then rocked moderately to drive the mouse to hold the wire of the lid. The cover is then progressively rotated upside down (Figure 1). The lid is secured at a height of around 50 cm from the ground above a soft underlayment, which is high enough to keep the mouse from plunging but not high enough to inflict harm during the fall. The time it takes for the animal to fall in seconds is recorded. The maximum time limit is set at 30 sec.[3]

Horizontal Bar Test

This test evaluates the motor coordination and muscle strength of the forelimbs. Grasp the mouse by its tail, then position it on the bench in front of the apparatus. Swiftly slide it backwards around 20 cm, ensuring it's perpendicular to the bar. Lift the mouse quickly and allow it to grasp the horizontal bar at the midpoint using only its forepaws (Figure 1). At the same time, start the stopwatch. Executing this procedure effectively can be

Table 1: Experimental design.

Sl. No.	Groups	No. of Mice	Drug treatment
1	Vehicle control	6	Distilled water x 21 days.
2	Haloperidol (Halo)	6	Haloperidol (1 mg/kg; i.p., once/day)x21 days.
3	Halo + (L-dopa+carbidopa)	6	L-dopa+carbidopa (100+25 mg/kg; i.p., once/day)+Haloperidol x 21 days.
4	Halo+EEMZ-1	6	EEMZ-1 (200 mg/kg; p.o., once/day)+Haloperidol x 21 days.
5	Halo+EEMZ-2	6	EEMZ-2 (400 mg/kg; p.o., once/day)+Haloperidol x 21 days.

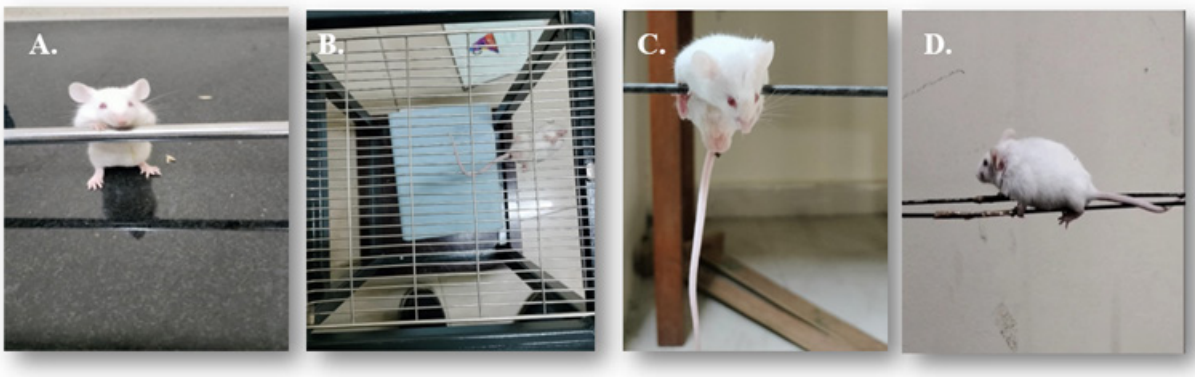

Figure 1: Behavioural studies A. Catalepsy Test B. Grid Hang Test C. Horizontal Bar Test D. Parallel Bar Test.

Table 2: Effect of various doses of *M. zapota* on various endogenous antioxidant enzymes like GSH, SOD, and CAT in haloperidol treated mice.

Treatment Groups	GSH (µM/mg of brain tissue)	SOD (U/mg of brain tissue)	CAT (U/mg of brain tissue)
Vehicle Control	1.810±0.002	2.592±0.068	3.011±0.214
Haloperidol (Halo)	0.774±0.032###	0.551±0.008###	1.195±0.063###
Halo+(L-dopa+Carbidopa)	1.299±0.063***	2.020±0.066***	2.578±0.150***
Halo+EEMZ-1	1.064±0.010**	1.118±0.052**	1.821±0.113**
Halo+EEMZ-2	1.127±0.021**	1.588±0.077**	2.051±0.237**

Values are mean±SEM; n=6 in each group Statistical significance is denoted as **p<0.01, and ***p<0.001 compared to the Haloperidol-treated group. ###p<0.001 compared with the normal control.

demanding; some mice grip better when their tails are suddenly released. If they sense support, they might fail to grasp it. The standard endpoint is either the mouse falling from the bar before reaching either end or reaching one of the ends of the horizontal bar. The maximum cut-off time is 30 sec.[3]

Parallel Bar Test

Position the mouse at the centre of the two bars, ensuring its longitudinal axis is perpendicular to them. Have both front paws on one bar and both hind paws on the other. Record the duration it takes for the mouse to reach one of the end supports, also noting any instances of the mouse flipping upside down. Normally, reasonably healthy mice take longer than 5 sec to fall; if they do so in under 5 sec, it may suggest a medical issue. The maximum duration allowed for this test is 120 sec.[23]

Biochemical Parameter Estimation

The biochemical parameters were evaluated in brain homogenate to measure levels of reduced Glutathione (GSH) as well as Superoxide Dismutase (SOD) and Catalase (CAT) enzyme activities, following established protocols.[24,25]

Histopathological Studies

Brains from all groups were fixed in 10% formalin, then embedded in paraffin wax and longitudinally sectioned into thin slices of 5 µm thickness. Following this, the sections were stained with hematoxylin and eosin dyes before undergoing histopathological examination.[26]

Statistical Analysis

All values are reported as mean±SEM. The data was subjected to statistical analysis using one-way ANOVA to compare control and pharmacological treatments, followed by the Tukey test for multiple comparisons. GraphPad Prism version 10.0 was employed for this analysis. p<0.05 was considered a significant difference between the groups.

RESULTS

Percentage Yield of Ethanolic Extract of *M. zapota* (EEMZ) Leaves

The percentage yield was calculated and was found to be 39.41%

$$\%\ \text{Dry weight} = \frac{\text{weight of dry sample}}{\text{weight of sample}} \times 100$$

$$\% = \frac{39.41}{100} \times 100 = 39.41\%$$

Qualitative analysis

Table 3: Phytochemical Screening of *M. Zapota* leaves extract.

Sl. No.	Phytochemical Test	Observation	Results
1	Test for Alkaloids		
	Dragendroff's Test	Orange Brown ppt	++
	Wagner's	Reddish Brown ppt	++
	Hager's	Yellow ppt	++
2	Test for Carbohydrates		
	Fehling's Test	Brick Red ppt	+++
	Molisch's Test	Violet ring at the junction	-
3	Test for Tannin and phenols		
	Ferric Chloride Test	Green Black ppt	+++
4	Test for Saponin		
	Froth Test	Constant Foam	+++
5	Test for Flavonoids		
	Ammonium Test	Yellow Colour	++
6	Test for proteins		
	Millon's Test	Brick Red Colour	++
	Biuret Test	Violet Colour	++
7	Test for Cardiac Glycosides		
	Legal's Test	Pink Colour	-

(++)=Moderately Present, (+++)=Highly Present, (-)=Absent.

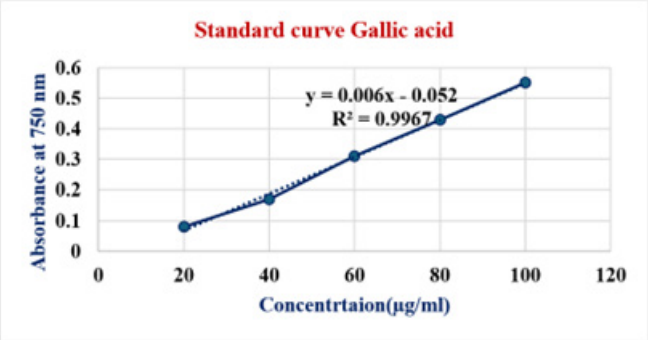

Figure 2: Total phenolics standard curve with gallic acid.

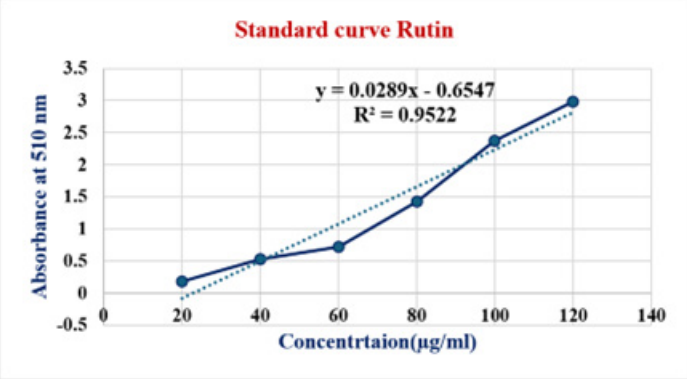

Figure 3: Total flavonoid standard curve with rutin.

Quantitative analysis

Total phenolics

Total phenolics were quantified using a gallic acid-mediated standard curve (Figure 2). The total phenolic contents of EEMZ were determined to be 17.6 mg GAE/g.

Total flavonoids

Total flavonoids were quantified using a rutin-mediated standard curve (Figure 3). The total flavonoid contents of EEMZ were determined to be 64.52 mg RTE/g.

Neurobehavioral Studies

Catalepsy bar test

The results, as presented in Table 4 and Figure 4, demonstrate the impact of EEMZ on haloperidol-induced catalepsy in mice.

Grid Hang Test

The grid-hanging test was used to assess muscle strength and coordination. Results are presented in Figure 5 and Table 5.

Horizontal Bar Test

The horizontal bar test was used to assess grip strength and motor coordination in mice. Results are presented in Figure 6 and Table 6.

Parallel Bar Test

The parallel bar test evaluated motor coordination, balance, and strength. Results are presented in Figure 7 and Table 7.

Estimation of antioxidant (GSH, SOD and CAT) enzyme levels

Administration of haloperidol led to significant changes in biochemical parameters compared to the vehicle control group. Haloperidol caused oxidative stress in the brain, demonstrated by lower activities of the antioxidant enzymes Catalase (CAT) and Superoxide Dismutase (SOD), as well as decreased levels of Glutathione (GSH). However, treatment with EEMZ significantly ($p<0.01$) increased the activities of SOD and CAT enzymes and raised GSH levels compared to the group treated with haloperidol alone as shown in Table 2.

Histopathological studies

The histopathological examination of the brain tissue from the vehicle-treated mice group reveals normal morphology of the cerebral cortex, with the typical appearance of glial cells. The haloperidol-treated mice group showed moderate hyperplasia, proliferation of neurons in the hippocampus and accumulation of glial cells in the cerebral cortex. Reversal of neuronal alterations and proliferation was observed in mice treated with L-dopa+Carbidopa (125 mg/kg) and EEMZ at doses of 400 mg/kg. However, treatment with EEMZ at a dose of 200 mg/kg did not demonstrate substantial recovery of neuronal damage as shown in Figure 8.

The vehicle control group exhibited normal morphology of the cerebral cortex and hippocampus architecture. Haloperidol-treated mice showed accumulation of glial cells in the cerebral cortex and moderate hyperplasia, with proliferation of neurons in the hippocampus. Mice treated with Haloperidol and (L-dopa+Carbidopa) exhibited minimal changes in the cerebral cortex and hippocampus. Mice treated with Haloperidol and EEMZ (200 mg/kg) showed focal necrosis of neurons in the cerebral cortex with infiltration of lymphocytes, along with proliferation of glial cells in the hippocampus. Mice treated with Haloperidol and EEMZ (400 mg/kg) showed a decrease in the accumulation of glial cells in the cerebral cortex and reduced proliferation of glial cells in the hippocampus.

DISCUSSION

Parkinson's Disease (PD) is the most common form of a group of progressive neurodegenerative disorders. The pathogenesis of Parkinson's Disease (PD) involves several factors, including protein accumulation (such as α-synuclein), oxidative stress, apoptosis, mitochondrial dysfunction, and neuronal excitotoxicity. Among these, oxidative stress stands out as a crucial pathological mechanism in PD.[3,25]

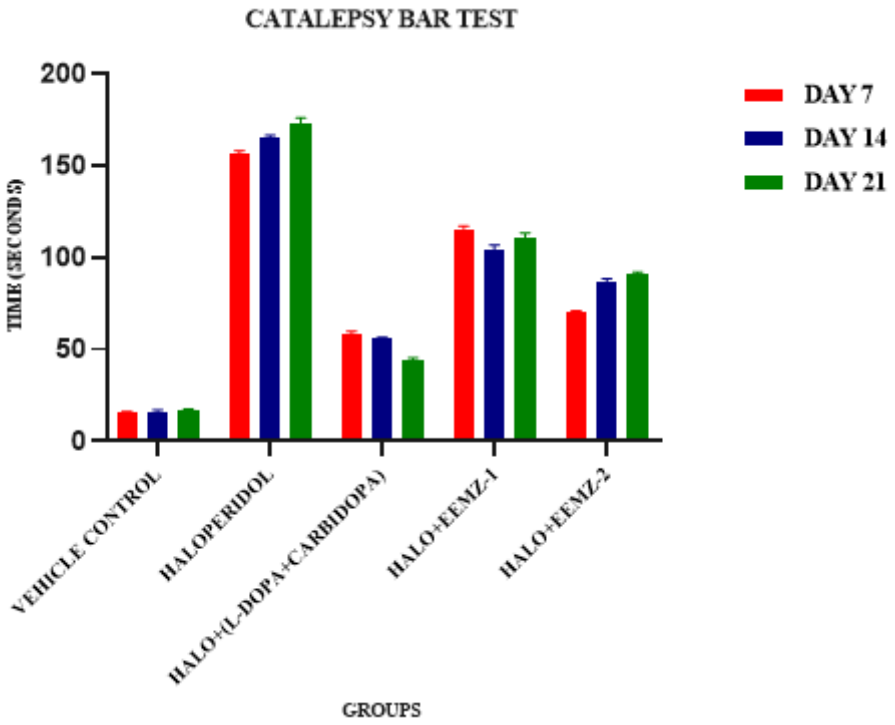

Figure 4: Catalepsy bar test. Values are mean±SEM; *n*=6 in each group, ###*p*<0.001 compared with the normal control and ***p<0.001 compared with the haloperidol-treated group.

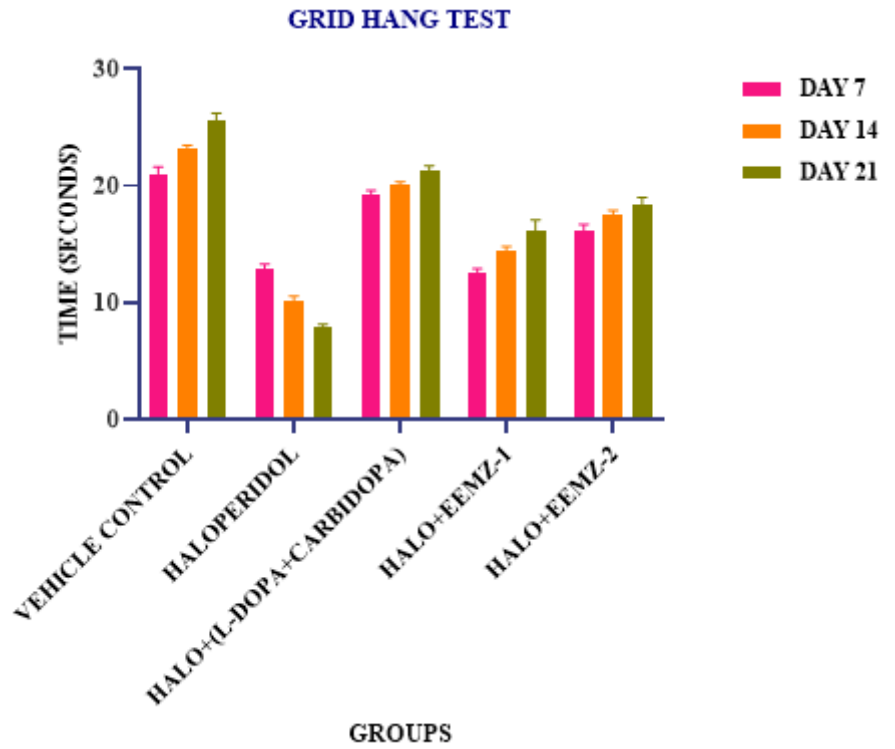

Figure 5: Grid hang test. Values are mean±SEM; *n*=6 in each group, ###*p*<0.001 compared with the normal control and ***p<0.001 compared with the haloperidol-treated group.

Phenolic and flavonoid compounds are major secondary metabolites with strong therapeutic potential. These plant-derived compounds offer a range of health benefits, including the reduction of inflammation, prevention of heart disease, and lowering the incidence of diabetes and cancers. They also help to prevent neurodegenerative diseases such as Parkinson's and Alzheimer's disease.[27] The qualitative phytochemical analysis of the Ethanolic Extract of *M. zapota* (EEMZ) leaves revealed the presence of carbohydrates, alkaloids, phenols, flavonoids, proteins, tannins, and saponins as shown in Table 3. Additionally, quantitative estimation was conducted for total phenolics and flavonoids. The TPC in the EEMZ was determined using the regression equation of the standard gallic acid calibration curve ($y=0.006x-0.052$, $R^2=0.9967$) and measured at 17.6 mg GAE/g of extract. Similarly, the TFC in the EEMZ was determined using the regression equation of the standard rutin calibration curve ($y=0.0289x-0.6547$, $R^2=0.9522$) and measured at 64.52 mg RTE/g of extract. The findings in this study revealed that the *M. zapota* leave extract contains significant levels of phenolic and flavonoid compounds. These substances could potentially engage with free radicals, offering a defence against oxidative stress-induced cellular damage.

In this study, we evaluated the potential of Ethanolic Extract of *M. zapota* (EEMZ) leaves against haloperidol-induced parkinsonism in Swiss albino Mice. Haloperidol, a D_2 dopamine antagonist, can induce a model of Parkinsonism by interfering with the intracellular storage of Catecholamines (CAs), leading to dopamine depletion in nerve endings.

In this experimental study, four behavioural evaluation parameters were used to examine haloperidol-induced Parkinson's Disease (PD) in mice: the catalepsy metal bar test, grid hang test, horizontal bar test, and parallel bar test. In the catalepsy test, the disease group (haloperidol) showed significant catalepsy as evidenced by a notable increase in the time spent on the block compared to vehicle-treated animals. Treatment with EEMZ exhibited a dose-dependent reduction in catalepsy in haloperidol-treated mice. EEMZ at doses of 200 and 400 mg/kg demonstrated a protective effect ($p<0.001$) against haloperidol-induced catalepsy, suggesting that this plant possesses the ability to protect dopaminergic neurotransmission in the striatum as shown in Figure 4 and Table 4. The grid-hanging test was used to measure muscle strength and coordination. The disease group demonstrated a significant decrease in hanging time compared to the vehicle control group, indicating muscle weakness. Treatment with EEMZ resulted in a dose-dependent

Table 4: Effect of *M. zapota* on catalepsy bar test.

Sl. No.	Treatment Groups	7th Day	14th Day	21st Day
1.	Vehicle Control	15.33±0.962	16.33±0.769	17.00±0.471
2.	Haloperidol (Halo)	156.6±1.407###	165.3±1.018###	172.8±3.067###
3.	Halo + (L-dopa+Carbidopa)	58.50±1.196***	55.50±0.993***	44.33±1.018***
4.	Halo+EEMZ-1	115.5±1.522***	104.0±2.757***	110.6±2.704***
5.	Halo+EEMZ-2	70.66±0.384***	86.50±1.775***	90.83±1.232***

Table 5: Effect of *M. zapota* on Grid hang test.

Sl. No.	Treatment Groups	7th Day	14th Day	21st Day
1.	Vehicle Control	21.00±0.623	23.16±0.280	25.50±0.696
2.	Haloperidol (Halo)	12.83±0.435###	10.16±0.366###	7.833±0.280###
3.	Halo+(L-dopa+ Carbidopa)	19.16±0.435***	20.00±0.333***	21.33±0.384***
4.	Halo+EEMZ-1	12.50±0.390***	14.33±0.451***	16.16±0.894***
5.	Halo+EEMZ-2	16.16±0.597***	17.50±0.390***	18.33±0.652***

Table 6: Effect of *M. zapota* on horizontal bar test.

Sl. No.	Treatment Groups	7th Day	14th Day	21st Day
1.	Vehicle Control	15.16±0.641	18.83±0.435	20.16±0.280
2.	Haloperidol	8.833±0.548###	10.75±0.743###	11.33±0.693###
3.	Halo +(L-dopa+ Carbidopa)	14.33±0.509***	15.16±0.280***	16.66±0.304***
4.	Halo+EEMZ-1	9 .333 ±0.384***	11.33±0.652***	12.50±0.696***
5.	Halo+EEMZ-2	11.83±0.723***	13.50±0.565***	14.16±0.435***

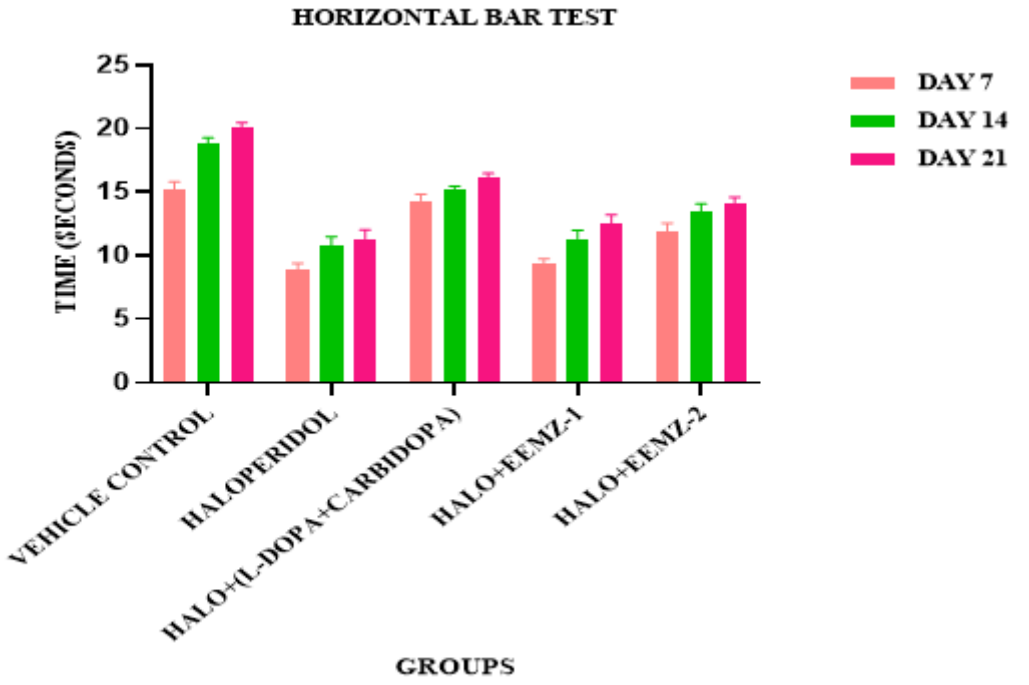

Figure 6: Horizontal bar test. Values are mean±SEM; *n*=6 in each group, ###*p*<0.001 compared with the normal control and ***p<0.001 compared with the haloperidol-treated group.

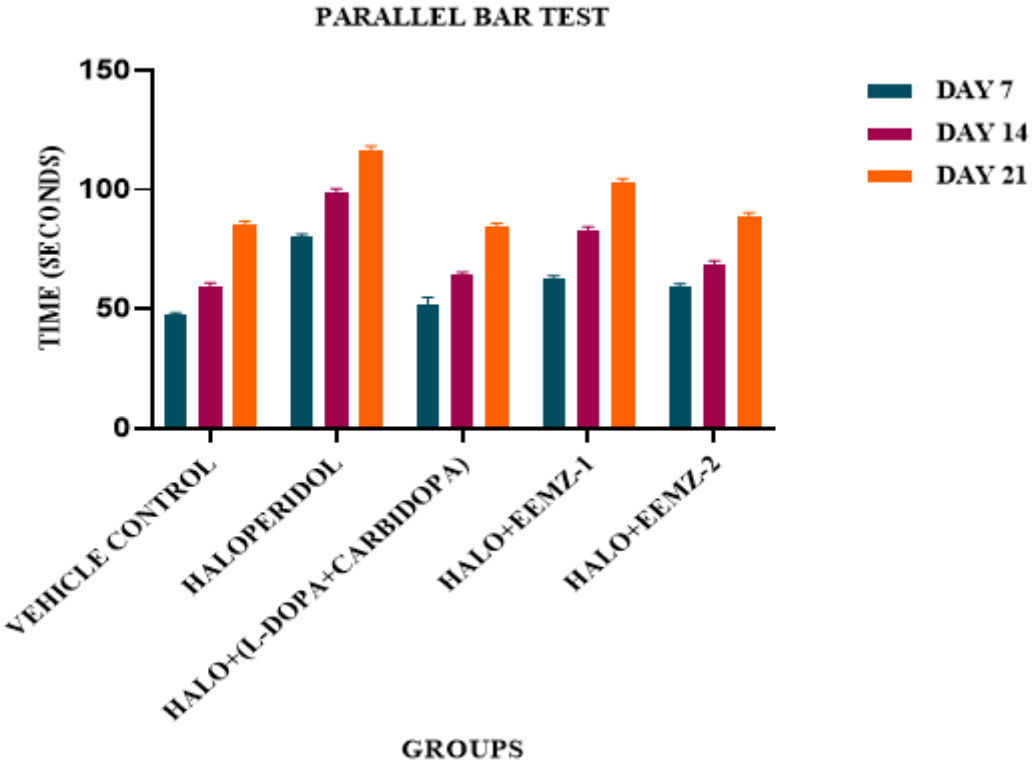

Figure 7: Parallel bar test. Values are mean±SEM; *n*=6 in each group, ###*p*<0.001 compared with the normal control and ***p<0.001 compared with the haloperidol-treated group.

increase in hanging time in haloperidol-treated mice. EEMZ at doses of 200 and 400 mg/kg significantly increased (p<0.001) hanging time, which is interpreted as evidence of enhanced muscle strength as shown in Figure 5 and Table 5. The horizontal bar test is used to assess grip strength and motor coordination in mice. The disease group (haloperidol) showed a decreased time on the bar, indicating motor impairment when compared to the normal control group. Treatment with EEMZ resulted in a dose-dependent improvement in performance on the horizontal bar test in haloperidol-treated mice. EEMZ at doses of 200 and 400 mg/kg significantly increased (p<0.001) the time on the bar, suggesting a protective or restorative effect on motor function and dopaminergic neurotransmission as shown in Figure 6 and Table 6. The parallel bar test is used to evaluate motor coordination, balance, and strength in mice. In this test, the group that received only haloperidol exhibited a significant decrease in the time of transverse movement (p<0.001) compared to the vehicle control group. In the EEMZ-treated groups (200 and 400mg/kg), a significant increase in the time of transverse movement (p<0.001) was observed compared to the haloperidol-treated group.

Table 7: Effect of *M. zapota* on parallel bar test.

Sl. No.	Treatment Groups	7th Day	14th Day	21st Day
1.	Vehicle Control	47.33±1.146	59.66±1.287	85.33±1.539
2.	Haloperidol (Halo)	80.00 ±1.490###	98.83±1.656###	116.5±1.798###
3.	Halo+(L-dopa+ Carbidopa)	52.00±2.934***	64.16±1.334***	84.50±1.532***
4.	Halo+EEMZ-1	62.66±1.334***	82.83±1.570***	103.0±1.627***
5.	Halo+EEMZ-2	59.33±1.283***	68.83±1.382***	88.83±1.570***

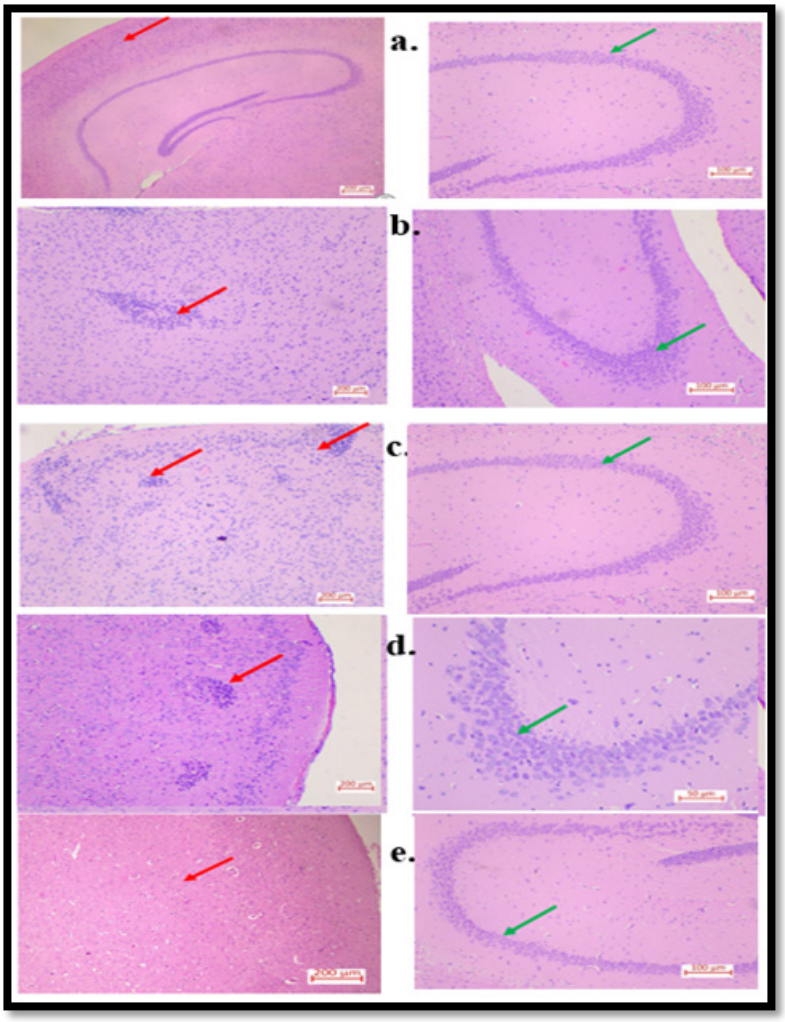

Figure 8: Effect of EEMZ on histopathological alteration in the cortex and hippocampus of brain tissue.

Enhanced performance, characterized by prolonged time spent on the bars and fewer instances of slips and falls in EEMZ-treated mice, suggests potential augmentation of motor coordination and strength as shown in Figure 7 and Table 7.

Glutathione (GSH) is a potent antioxidant that plays a crucial role in the development of Parkinson's Disease (PD). Decreased levels of GSH in the substantia nigra, a brain region affected by PD, may result from neuronal loss. This reduction in GSH was also observed in haloperidol-treated animals, indicating increased oxidative stress in their brains. However, treatment with EEMZ dose-dependently elevated the GSH levels, suggesting its antioxidant properties in these animals (Table 2).

Superoxide Dismutase (SOD) is another important enzyme that protects cells from oxidative damage by converting superoxide radicals into less harmful molecules like hydrogen peroxide. In the haloperidol-treated group, SOD levels decreased, signalling oxidative stress. Treatment with EEMZ at both doses similarly increased SOD levels, indicating its protective, antioxidant effect.

Catalase (CAT) is an enzyme that helps neutralize the toxic effects of hydrogen peroxide, another reactive oxygen species. Like GSH and SOD, CAT levels were reduced in the brains of haloperidol-treated animals, highlighting oxidative stress. EEMZ treatment at both doses (200 and 400 mg/kg) restored CAT levels, further supporting its role as an antioxidant in mitigating oxidative stress in these animals.

Histopathological findings revealed that treatment with an ethanolic extract of *M. zapota* at a dose of 400 mg/kg resulted in a decrease in the accumulation of glial cells in the cerebral cortex and a reduction in the proliferation of glial cells in the hippocampus. Additionally, the normal architecture of these brain regions was restored in the treated animals as shown in Figure 8. This study has limitations, as it concentrates only on behavioral assessments and antioxidant levels. Future research should explore molecular pathways, long-term effects, and interactions with other neuroprotective mechanisms.

CONCLUSION

Parkinson's Disease (PD) is a common neurodegenerative disorder characterized by motor symptoms such as bradykinesia, tremors, and muscle stiffness. Oxidative stress is a key pathological mechanism in PD, contributing to neuronal damage and loss. Phenolic and flavonoid compounds, known for their therapeutic potential, exhibit antioxidant properties that can counteract oxidative stress and potentially alleviate neurodegenerative diseases like PD. The Ethanolic Extract of *M. zapota* (EEMZ) was found to contain significant levels of these compounds, suggesting its potential as a neuroprotective agent. In this study, EEMZ demonstrated protective effects against haloperidol-induced Parkinsonism in Swiss albino mice. It improved motor function, muscle strength, and coordination while increasing antioxidant enzyme levels in the brain. Additionally, histopathological analysis revealed structural improvements in brain regions affected by PD-like pathology.

Overall, these findings highlight the potential of *M. zapota* as a natural neuroprotective agent against Parkinson's disease and warrant further investigation into its therapeutic mechanisms and clinical applications. Future research would focus on investigating the molecular mechanisms and characterizing the active constituents responsible for the neuroprotective effects of *M. zapota*, as well as conducting long-term efficacy and safety studies across diverse Parkinson's disease models.

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CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

PD: Parkinson Disease; **EEMZ:** Ethanolic extract of *Manilkara zapota*; **Halo:** Haloperidol; **L-dopa:** Levodopa; **GSH:** Glutathione; **SOD:** Superoxide dismutase; **CAT:** Catalase.

ETHICAL APPROVAL

Project proposal no: RBVRR 1328/05/2023 was approved on 08-02-2023 held at RBVRR Women's College of Pharmacy.

SUMMARY

The Ethanolic Extract of *M. zapota* (EEMZ), rich in phenolic and flavonoid compounds, demonstrated neuroprotective effects against haloperidol-induced Parkinsonism in Swiss albino mice. The ethanolic extract of *M. zapota* improved motor function, increased antioxidant enzyme levels in the brain, and revealed structural improvements in affected brain regions. These findings highlight the potential of *M. zapota* as a natural therapeutic agent for Parkinson's disease.

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