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Evaluation of Anti Parkinson's Activity of Ethanolic Extract of Seeds of Perilla Frutescens in Wistar Albino Rats

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ABSTRACT

Objective: The objective of this study is to evaluate the antiparkinsonian activity of ethanolic extract of the seeds of *Perilla frutescens* in Wistar albino rats. **Methods**: Catalepsy was induced in rats using haloperidol (4 mg/kg i.p.). Treatment groups received bromocriptine (4 mg/kg) and *Perilla frutescens* seed extract (PFSE) at the dose of (50, 100, and 200 mg/kg) orally. Bar test for catalepsy, motor coordination test by rotarod, and locomotor activity by actophotometer were carried out to assess behavioral changes. Assays of dopamine and catalase were also carried out to assess biochemical parameters. **Results**: Bromocriptine and PFSE-treated groups showed a significant difference in behavioral and biochemical parameters as compared to the haloperidol control group in the experimental models. **Conclusion**: *Perilla frutescens* seeds exhibited significant antiparkinsonian activity in a haloperidol rat model.

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INTRODUCTION:

The progressive loss of neuron structure or function, known as neurodegeneration, causes neurodegenerative disorders. Neurological damage could result in cell death. Parkinson's disease (PD) is a central nervous system (CNS) neurodegenerative illness that primarily affects the motor system. It is also known as a slowly progressive neurodegenerative illness in which dopaminergic neurons projecting from the *Substantia nigra* pars compacta toward the neostriatum die, resulting in an imbalance of dopamine and acetylcholine (1). Parkinsonism affects patients' capacity to function and can result in a major loss of quality of life. It is defined by the presence of cardinal clinical motor symptoms such as rigidity, bradykinesia, and tremor. Parkinson's disease (PD) is the most frequent form of neurological Parkinsonism, affecting approximately 10 million people around the world and causing a considerable socioeconomic burden (2). Cell loss in the brain's basal ganglia (affecting up to 70% of dopamine secreting neurons in the *S. nigra* pars compacta at the end of life) (3) and the presence of Lewy bodies (aggregations of the protein alpha-synuclein) in many of the surviving neurons are the key pathological hallmarks of PD. The death of astrocytes (star-shaped glial cells) and a large increase in the number of microglia (another kind of glial cell) accompany the loss of neurons in the *S. nigra* (4). The clinical syndrome was first described by James Parkinson in his 1817 “Essay on the shaking palsy” and is commonly referred to as PD. It is an extrapyramidal motor disorder.

Haloperidol is a neuroleptic drug that is frequently prescribed to treat psychosis. It operates by inhibiting D2 dopamine receptors and D1 receptors, to a lesser extent in medium spiny neurons, which comprise the indirect and direct motor circuit routes, respectively (5). As a result of the blockage of striatal dopamine transmission, abnormal downstream firing within the basal ganglia circuits occurs, causing muscle rigidity, lack of locomotor activity, and catalepsy symptoms. Haloperidol should be taken with caution since it might produce extrapyramidal movement disorders such as tardive dyskinesia (TD), akathisia, dystonia, and Parkinsonism (6).

Perilla frutescens belongs to the Lamiaceae/Labiatae family and is often known as perilla. The perilla plant contains important phytochemicals such as Rosmarinic acid, Luteolin, Quercetin, Catechin, Caffeic acid, and Ferulic acid. Phytosterols, tocopherols, squalene, and polyunsaturated fatty acids have also been discovered in perilla seeds (7). According to biological analysis, the perilla plant contains antimicrobial, anti-allergic, anti-cancer, anti-

tumor, anti-depression, anti-viral, anti-asthmatic, and antioxidant characteristics (8). Perilla seed oil, which has the highest ALA content of any vegetable oil, also has a high α-linolenic acid bioavailability (9). Animal studies indicate that perilla seed oil can prevent atherosclerosis (10) and chemically cause cancer (11), as well as boost immunological and cerebral performance (12). It has been used as a natural, herbal medicine to treat a variety of problems, including depression, asthma, anxiety, tumors, coughs, allergies, intoxication, cold, fever, chills, headache, stuffy nose, and a few intestinal disorders (13).

With its prominent antioxidative property, perilla seed oil demonstrates neuroprotective (14) effects against dementia in preclinical studies. These studies suggest that *Perilla frutescens* seed extract may have a potential anti-parkinsonian activity. While some neuroprotective studies have been carried out using perilla seeds, anti-parkinsonian activity has not been reported yet. Due to that reason, perilla seeds have been taken as an experimental drug to evaluate anti-parkinsonian activity.

METHODS:

Collection and Authentication of Plant:

Fresh dried seeds of perilla were collected from Vanalaya Agencies, Madhavnagar racecourse road, Bangalore-560001, and the specimen were submitted to Alarsin Pharmaceuticals Andheri East, Mumbai, and Maharashtra-400093. And it was authenticated by Dr. Mahesh Atale.

Drying and Grinding:

The perilla seeds were cleaned manually and air-dried for 2 days, and the flour was generated by grinding the seeds in a food processor and passing them through a 35-mesh screen. The flour was stored at 4°C until its use.

Extraction:

Maceration was an easy and efficient approach for extracting active chemicals from perilla seeds. Perilla seeds were crushed to a fine powder. 100g of perilla powder was soaked in 90% ethanol and 10% water throughout 24-48hrs at 25°C. Then the macerate was collected and centrifuged at 6,000 rpm for 10 min. The remaining mass of powder was again soaked in the mixture of alcohol and water to get a better yield. The supernatant was then passed through a rotating vacuum evaporator. Ethanol was evaporated in this process and the

remaining liquid was collected. Then this liquid was kept in a deep freezer for 24hrs and later, it was transferred on Petri plates and kept in Lyophilizer. The water was evaporated by cold evaporation (15). A crystalline powder was obtained. This powder was then stored at 4°C.

Phytochemical Analysis (16) (17):

Preliminary phytochemical investigations were conducted employing various phytochemical tests and the phytochemical constituents were detected as elaborated by Khandelwal and Kokate *et al.*

IAEC Approval:

The CPCSEA acknowledged Institutional Animal Ethics Committee (IAEC) of Oriental College of Pharmacy approved the experimental protocol No.OCP/IAEC/2021-2022/03. Entitled "**Evaluation of Anti Parkinson's activity of Ethanolic extract of seeds of *Perilla frutescens* in Wistar albino rats**."

Animals:

Wistar albino rats

A total of 36 Albino Wistar rats of either sex of weight 150-200g were obtained from National Institute of biosciences, GAT No. 69, AT: Dhangawadi, Nigadewada Road, off Pune Bangalore highway, taluka abhor, district Pune, Maharashtra-411051. CPCSEA Registration no.: 1091/GO/bt/S/07/CPCSEA Dated 01/12/2021. The animals were housed in a well-ventilated, air-conditioned animal house with a constant temperature of 24±2°C, a 12:12 hour dark: light cycle, and relative humidity of 55-60%. The animals were housed in large polypropylene cages with paddy husk bedding (18). The animals were held on a standard diet with pellets from the *ad. Libitum* and filtered mineral water (18). The animals were allowed to acclimatize for 7 days before the study.

Drugs and chemicals:

1. *Perilla frutescens* (50mg/kg, 100mg/kg, and 200mg/kg)

2. Bromocriptine was used as a reference standard for Parkinson's activity. (BROM 2.5 Tablet)

3. Drug to induce catalepsy: Haloperidol (injection Seranace AMP 4mg/kg)
4. 0.1 M perchloric acid
5. Hydrogen peroxide
6. Levodopa
7. Distilled water

Experimental design:

Six groups of 36 Albino Wistar rats (n=6) were formed at random. Group- I (vehicle control) rats were given normal saline (2.0 ml/kg, p.o.) daily for 14 days; Group- II (Haloperidol control); Group- III (Bromocriptine control); Group- IV, V, and VI (*Perilla frutescens* extract-treated group) rats were given low, intermediate, and high doses of 50mg/kg, 100mg/kg, and 200mg/kg. Oral administration of bromocriptine and perilla seed extract was given. The mice were given haloperidol 4mg/kg intraperitoneal (i.p.) dosing one hour after receiving the medication.

Estimation of behavioral parameters:

Bar test (19):

The catalepsy was measured using a bar test. The animals' front paws were alternately placed on a horizontal bar of 3 cm and 9 cm above and parallel to the base in the bar test. The time at which the animal removed its paw from the bar was recorded. Catalepsy scoring was given as follows:

Step 1: The rat was removed from its cage and placed on a table. A score of 0.5 was given if the rat did not move when handled or gently pushed on the back.

Step 2: The rat's front paws were alternately placed on a 3-cm-high block. If the rat did not change its posture within 15 seconds, a score of 0.5 was added to the Step 1 score for each paw.

Step 3: The rat's front paws were alternately placed on a 9-cm-high block. If the rat did not change its posture within 15 seconds, a score of 1 was added to each paw's score in Steps 1 and 2.

Motor coordination test (20) (rotarod test):

Rotarod equipment was used to conduct a motor coordination test. Before the treatment, the rats were placed on the moving rod, and the rat that lasted on the rod for 120 seconds without falling was chosen for the study. Before and after the treatment, the time it took for the animals to fall from the rotating rod was recorded. The rotarod's starting speed was set to 4 rpm, while the acceleration rate was set to 20 rpm. The maximum speed was 40 revolutions per minute.

Test for locomotor activity (21) (Actophotometer):

A locomotor activity test is performed on rats using an actophotometer. The device includes six built-in light sources, a photosensor for detecting animal movement, and a sensor for recording locomotor activity. A count is taken and displayed on the digital counter when the animal blocks a ray of light falling on the photocells. Rats were placed in the actophotometer, and their basal activity was monitored in this study. Six lights and six photocells are strategically arranged around the bottom, such that a single rat may only block one beam at a time. Photocell is activated when the rays of light fall on photocells, the beam of light is interrupted and when an animal crosses the light beam, several cut interruptions were recorded for 10 min.

Biochemical test:

Determination of CAT (22) (Catalase principal):

Preparation of brain sample:

Each group of haloperidol-induced Parkinson's rats was euthanized using a carbon dioxide chamber after the bar test, motor coordination test, and locomotor activity was assessed; brains were rapidly removed and placed in ice-cold saline (23). In 0.1 M phosphate buffer, the tissues were weighed and homogenized (pH 8). To evaluate CAT activity, samples of rat brain homogenates were obtained in several test tubes. The CAT assay was performed using the supernatant.

CAT Assay:

In a cuvette containing 1.9ml of 50mM phosphate buffer, 0.1ml of supernatant (pH 7.0) was added. It is then treated with 1ml of freshly made 30mM H_2O_2. The color was developed in

solution. The solution is then examined under UV light in a UV spectrophotometer. The absorbance was measured at 240nm. The initial absorbance of the blank solution is measured. The absorbance of the actual solution is then measured. The absorbance was measured at various times. Catalase activity is measured in units per milligram of protein.

Procedure for estimation of brain dopamine (24):

1ml of supernatant from brain homogenate was collected. The solution was then mixed with 1ml of ferric chloride (1.5 X 10-2M) and 1ml of potassium ferrocyanide (1.5 X 10-2M). After that, 25ml of distilled water was added. After around 30 minutes, the solution began to develop color. The UV spectrophotometer was then used for its analysis. The absorbance was measured at 735nm. The absorbance is measured at various time intervals.

Statistical Analysis:

Values were presented as mean±SEM. Data were statistically evaluated by one-way analysis of variance followed by Dunnett's test for intergroup comparison using Instat software. Results were considered to be statistically significant at *$p \leq 0.05$. ***indicated $p \leq 0.001$, **indicated $p \leq 0.01$, and *indicates $p \leq 0.05$ when compared with standard. There was no statistically significant difference in the spontaneous locomotor activity of rats.

RESULTS:

Phytochemical analysis:

The perilla seeds were ground, and then the extract was prepared. The phytochemical analysis was carried out using that extract. Perilla seeds contain lipids, fixed oils such as omega-3 fatty acids, omega-6 alpha-linolenic acid, glycosides, antioxidants such as flavonoids, phenolic compounds, alkaloids, vitamin C, iron, and calcium, according to phytochemical analysis.

Phytochemicals	Observations
Amino acids	-
Fats and fixed oils	+
Antioxidants (Phenolic)	+
Glycosides	+
Carbohydrates	-
Flavonoids	+
Alkaloids	+
Vitamin C	+
Iron	+
Calcium	+

Note: - + indicates the presence of a compound

- indicates the absence of compound

Catalepsy in Rats:

Bar Test:

The haloperidol control group significantly increased cataleptic score as compared to the vehicle control group in the bar test Table 1 and Fig.1. Bromocriptine 4 mg/kg and *Perilla frutescens* treated groups were tested for substantial suppression of catalepsy at low, intermediate, and high doses of 50 mg/kg, 100 mg/kg, and 200 mg/kg, respectively. In comparison, Bromocriptine 4 mg/kg treated group, and PFSE 200mg/kg, inhibited catalepsy by decreasing cataleptic score.

Motor coordination test:

The time taken to fall from the rotarod was greatly reduced in the haloperidol-treated group when compared to the vehicle control group, and it was significantly enhanced by bromocriptine 4 mg/kg and perilla seed extract 200 mg/kg. (Table 2 Fig.2)

Test for locomotor activity (Actophotometer):

When compared to the vehicle control group, spontaneous motor activity was considerably reduced in the haloperidol-treated group. Bromocriptine 4 mg/kg and PFSE 200 mg/kg dramatically increased locomotor activity in the haloperidol treated group. (Table 3 Fig.3)

Determination of CAT by UV:

The haloperidol control group significantly decreases CAT levels as compared to the vehicle control group. Bromocriptine 4 mg/kg and PFSE100mg/kg showed a significant increase in CAT level. (Table 4 Fig.4)

Determination of dopamine:

The haloperidol control group significantly decreases dopamine levels as compared to the vehicle control group. Bromocriptine 4 mg/kg and PFSE50 mg/kg showed a significant increase in dopamine levels. (Table 5 Fig. 5)

Table No. 1: Effect of bromocriptine and PFSE on catalepsy in bar test: -

Time intervals in minutes	Mean ± SEM (cataleptic score)					
	Vehicle control	Haloperidol control (4mg/kg)	Bromocriptine control	*Perilla frutescens* (50mg/kg) low dose	*Perilla frutescens* (100mg/kg) intermediate dose	*Perilla frutescens* (200mg/kg) high dose
0	0.00±0.00**	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00	0.00±0.00
30	0.00±0.00**	2.44±0.27	2.47±0.24	3.76±0.19	2.91±0.21	1.69±0.11
60	0.00±0.00**	2.89±0.32	1.32±0.45	3.56±0.16	2.78±0.16	1.65±0.08
90	0.00±0.00**	3.78±0.82	1.53±0.64	2.99±0.12	2.64±0.11	1.56±0.09
120	0.00±0.00**	3.75±0.63	1.22±0.47	2.64±0.09	2.36±0.10	1.42±0.06
240	0.00±0.00**	3.38±0.57	0.46±0.23	2.31±0.06	2.07±0.10	1.12±0.02

Table No. 2: Effect of bromocriptine and PFSE on motor coordination test using Rotarod: -

Treatment group	Fall off time mean±SEM
Vehicle control	78.34±1.28
Haloperidol control (4mg/kg)	11.57±0.24
Bromocriptine control	86.24±1.22
Perilla frutescens(50mg/kg) low dose	26.15±1.40
Perilla frutescens(100mg/kg) intermediate dose	37.95±1.17
Perilla frutescens(200mg/kg) high dose	42.86±2.39

Table No. 3: Effect of bromocriptine and PFSE on locomotor activity using actophotometer:-

Treatment group	Ambulation counts/10min mean±SEM
Vehicle control	158.32±1.68
Haloperidol control (4mg/kg)	38.66±1.26
Bromocriptine control	167.32±2.25
Perilla frutescens(50mg/kg) low dose	62.5±1.72
Perilla frutescens(100mg/kg) intermediate dose	76.16±1.30
Perilla frutescens(200mg/kg) high dose	91.83±2.66

Table No. 4: Effect of bromocriptine and PFSE on Catalase activity: -

Treatment group	Unit/mg Mean±SEM
Vehicle control	5.013±1.247
Haloperidol control (4mg/kg)	1.81±0.042
Bromocriptine control	6.17±1.21
Perilla frutescens (50mg/kg) low dose	2.92
Perilla frutescens (100mg/kg) intermediate dose	4.97
Perilla frutescens (200mg/kg) high dose	2.54

Table No. 5: Effect of bromocriptine and PFSE on Dopamine concentration: -

Treatment Groups	Concentration of dopamine (ug/ml)
Vehicle control	13.27±0.39
Haloperidol control (4mg/kg)	11.27±0.28
Bromocriptine control	40.19±0.53
Perilla frutescens (50mg/kg) low dose	15.32
Perilla frutescens (100mg/kg) intermediate dose	16.20
Perilla frutescens (200mg/kg) high dose	34.16

Note: - All values are expressed in mean±SEM (n=6). Significance: **p≤0.01 when compared with perilla seed extract.

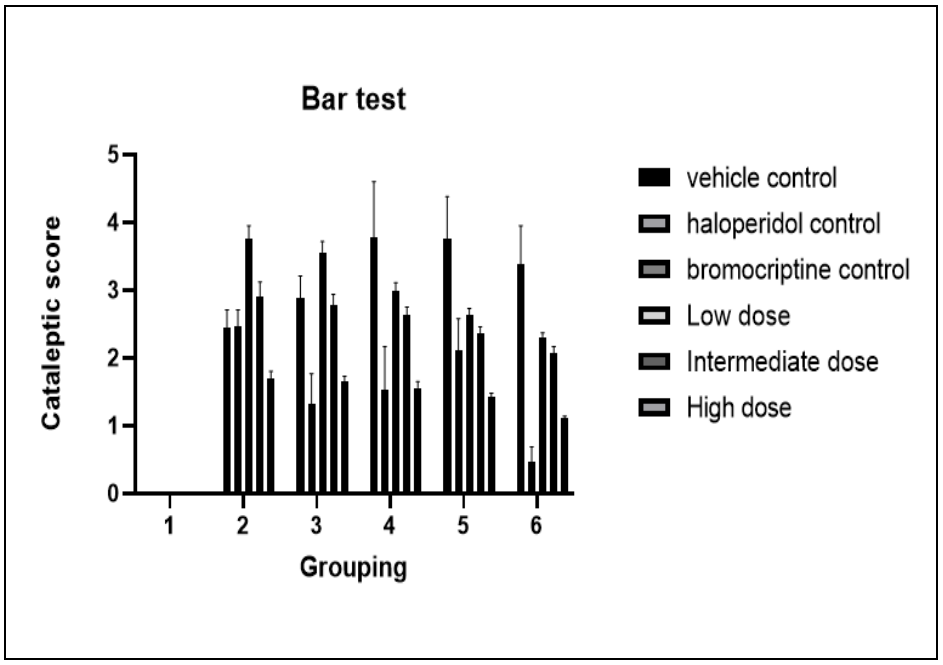
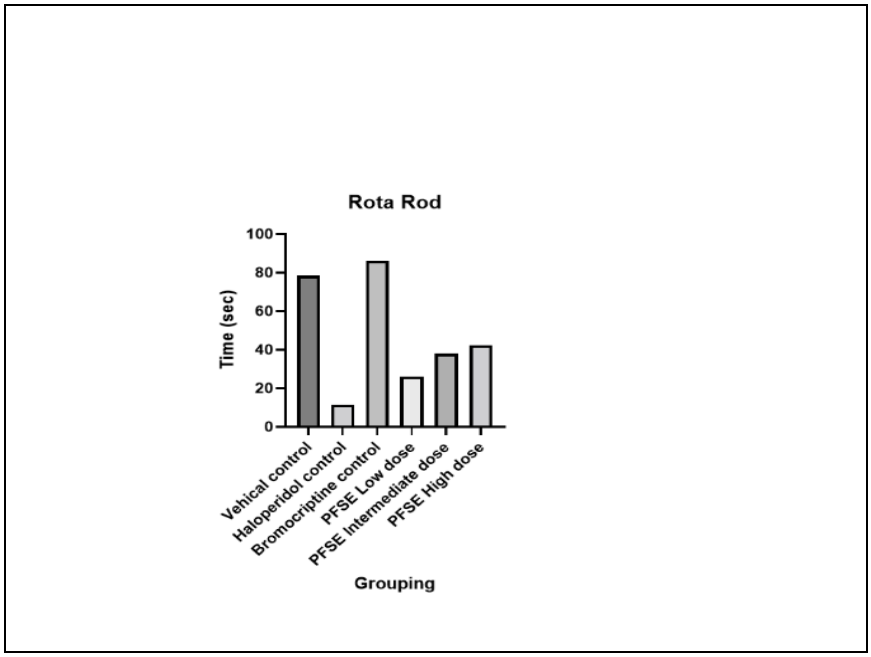


Fig. No. 1



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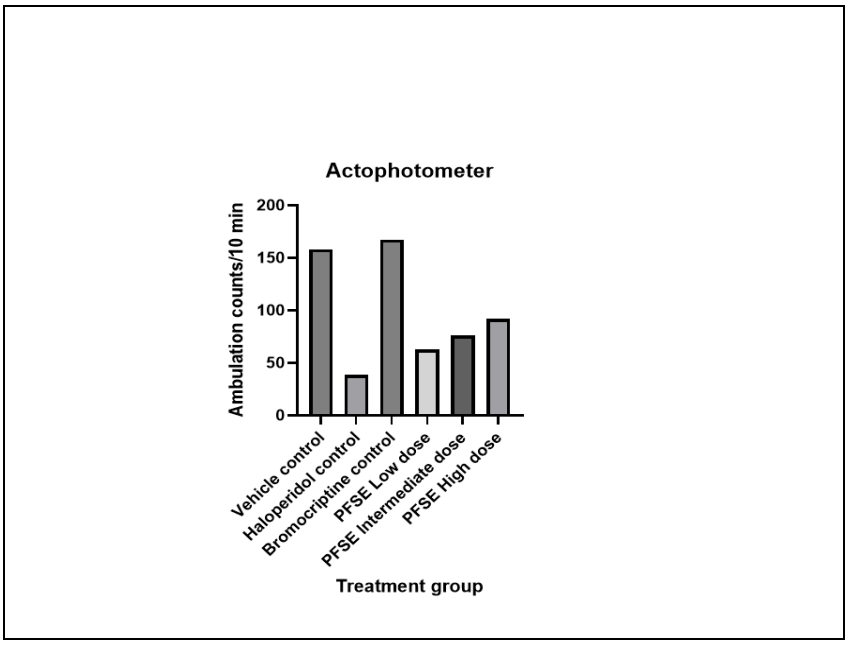

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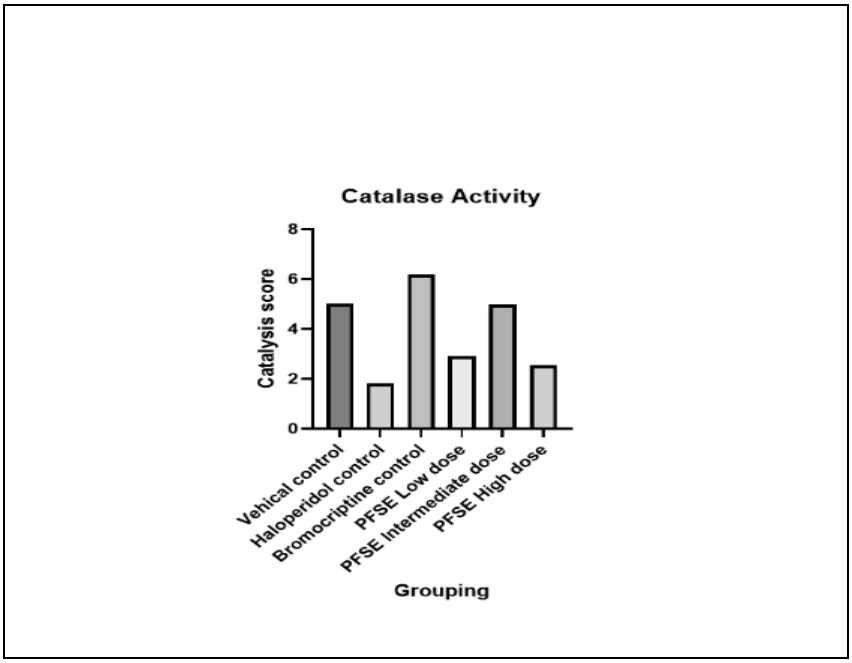

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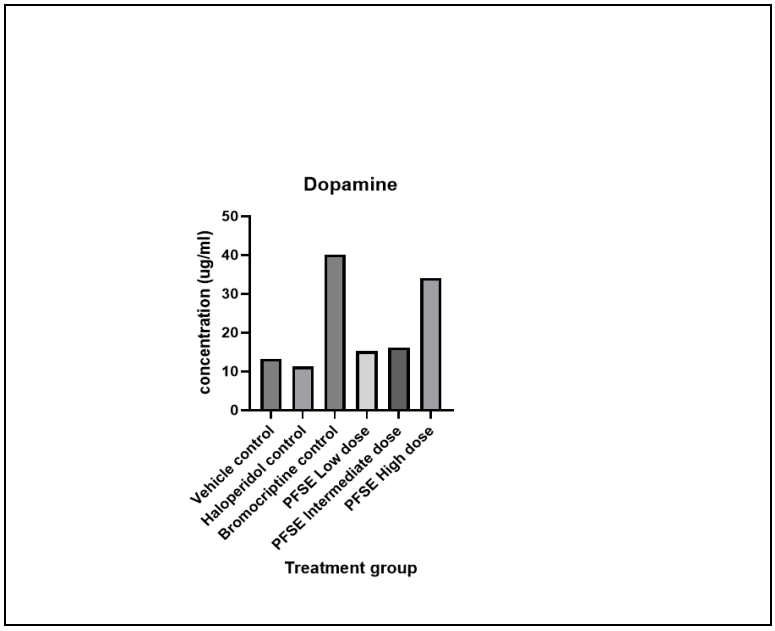

Fig. No. 5

DISCUSSION:

Haloperidol-induced catalepsy in rats:

Some of the key symptoms of PD are catalepsy (rigidity in movements), akinesia (slowing of movement), tremors, and memory loss. One of the key symptoms that makes a PD patient's life difficult is catalepsy. Bromocriptine is well-known dopamine (D2) receptor agonist that

is widely used to alleviate stiffness symptoms. Due to this reason, this medicine was utilized as a control in the current investigation.

The rats were given haloperidol (4 mg/kg) intravenously to induce catalepsy. The bar test, rotarod device, and actophotometer were used to assess the cataleptic behavior generated by haloperidol, as well as the protective impact of standard (bromocriptine) and PFSE.

Bar test:

This test determines the degree of catalepsy produced in an animal. Bromocriptine 4 mg/kg and PFSE 200 mg/kg were found to dramatically reduce cataleptic scores in a bar test, reversing the effects of haloperidol.

Motor co-ordination test by rotarod:

This test was performed to assess the imbalance, which is one of the signs of Parkinson's disease. The test consists of the animal balancing on a revolving rod. The rotarod test revealed a considerable loss of muscle coordination in haloperidol-treated rats, which could be attributable to a decrease in muscular strength. Bromocriptine 4 mg/kg and PFSE 200 mg/kg effectively reduced the motor impairment caused by haloperidol. It suggests that PFSE may include active ingredients that function as CNS stimulants.

Locomotor activity by actophotometer:

Movement limitations or, in certain cases, freezing of motions are demonstrated by PD patients due to catalepsy. As a result, a medicine that promotes locomotor activity may be able to improve the condition of PD patients. Haloperidol induced a considerable drop in locomotor counts in the actophotometer, according to the findings. When compared to haloperidol-treated rats, bromocriptine 4 mg/kg and PFSE 200 mg/kg dramatically increased locomotor activity. As measured on day 14, daily treatment with PFSE significantly reversed the reduction in locomotor activity.

Brain dopamine estimation:

Dopamine is the main important neurotransmitter in the brain responsible for control, coordination, and other important activities. Reduced dopamine in the brain due to the death of dopaminergic neurons in the reason which results in PD. Bromocriptine 4mg/kg and perilla

seed extract 50mg/kg, significantly increased the brain dopamine levels as compared to haloperidol treated animals.

Determination of CAT by UV:

CAT is an antioxidant that aids in the neutralization of hydrogen peroxide's harmful effects. The CAT enzyme converts hydrogen peroxide to water and non-reactive oxygen species, reducing the buildup of precursors for free radical production. The level of CAT is reduced as a result of oxidative stress. When compared to haloperidol-treated rats, bromocriptine 4 mg/kg and PFSE 100 mg/kg dramatically elevated CAT levels.

CONCLUSION:

Perilla frutescens exhibited significant antiparkinsonian activity in the haloperidol-induced model. It appears to be the most promising plant due to its Omega-3 fatty acid and marvelous antioxidant content and potential antioxidant activity. It contains omega-3 and omega-6 alpha-linolenic acid and also it contains antioxidants in the form of flavonoids and phenolic compounds. The predictable mode of action of this plant may be due to its neuroprotective effect. and decreased lipid peroxidation due to the presence of flavonoids and phenols. These findings provide evidence for its use as an antiparkinsonian medication, including the prevention of PD and improvement of PD symptoms. Future studies are required to investigate the phytoconstituents responsible for the activity and also to establish the exact mode of action.

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CONFLICTS OF INTEREST:

The authors have no conflict of interest.

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