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Phytochemical Evaluation and Pharmacological Screening of Antiparkinson's Activity of Allium Sativum In Swiss/Albino Mice

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Abstract: Objective: To evaluate the Antiparkinson activity of *Allium sativum* in Haloperidol induced Parkinson's disease in mice. **Method:** Parkinson's disease was induced by administering haloperidol (2.0 mg/kg i.p.) Daily for a week. The mice were divided into 5 group (n=6). Group II received haloperidol (2mg/kg body weight).Group III received combination of levodopa and carbidopa (100mg+ 10mg/kg by i.p along with haloperidol) and Group IV and V received Allium sativum extract (200 and 400mg/kg by p.o), respectively for 7 days along with haloperidol. To evaluate the antiparkinson effect of Allium sativum, catalepsy bar test, rotarod test, hang test and horizontal bar test were used. One way ANOVA was used to test statistical significance followed by Bonferroni multiple comparison tests .**Results:** Allium sativum extract (200 and 400mg/kg by p.o) was found to decrease the duration of catalepsy significantly ($P<0.001$) in catalepsy bar test as compared to haloperidol group, and significantly increases ($P<0.001$) fall off time in, rotarod test, hang test and horizontal bar test respectively as compared to haloperidol group. **Conclusion:** The result of the present study conclusively shows the Antiparkinson's activity of *Allium sativum* in haloperidol induced Parkinson's disease in mice.

Keywords: *Parkinson's disease, Allium sativum, Haloperidol, Levodopa.*

I. INTRODUCTION

Parkinson's disease (PD) is a slowly progressive neurodegenerative disease caused when a small group of brain cells that control body movements die. This disease was first described by James Parkinson in 1817. It is characterised clinically by bradykinesia, resulting tremor, rigidity and postural instability. [1] Pathological features of PD include loss of dopamine neurons in substantia nigra and presence of intracytoplasmic inclusions known as Lewy bodies in surviving dopamine neuron. It is not clear why Lewy body formation causes neuronal cell death. [2]Among the available antiparkinson drugs, levodopa remains the most efficacious and still the mainstay of therapy. However, long term use of levodopa leads to wearing off phenomenon, on- off phenomenon, motor fluctuations and dyskinesia, which limit its further usage. Even though antiparkinson drugs are highly effective in alleviating the symptoms of Parkinsonism, but they do not give complete cure. Moreover, these drugs are often associated with frequent side effects like nausea, vomiting, depression, hallucinations, dizziness, dry mouth, sore throat, postural hypotension, diarrhea, mydraiasis, anxiety etc. The significance of many indigenous medicinal plants and their phytoconstitutents in the management of Parkinsonism with minimal side effect profile arise in this context. [3]There has been an enormous demand for further scientific development of animal models that can mimic the progressive motor impairment as in PD. One such model is Haloperidol induced catalepsy i.e., a state of akinesia with muscular rigidity in animals. It is an established model for screening the drugs for antiparkinson effect. [1]

Garlic, Allium sativum is a member of the Amaryllidaceae family, has been widely recognized as a valuable spice and a popular remedy for various ailments and physiological disorders. The name garlic may have originated from the Celtic word 'all' meaning pungent. Garlic has been used for thousands of years for medicinal purposes. Sanskrit records show its medicinal use about 5,000 years ago, and it has been used for at least 3,000 years in Chinese medicine. In the recent decades, researchers, have published over 2000 scientific works on the therapeutic potential of garlic (Allium Sativum), one of the most used plants in traditional medicines and wider cited in the literature for its medicinal properties.[4]Garlic is stated to possess many therapeutic benefits. Its strong odour is largely due to sulphur containing compound (e.g.: S-allycysteine, sulphoxise), which are believed to account for most of its medicinal properties. One of the most biologically active compounds, allicin (diallyl thiosulfinate or diallyl disulfide) does not exist in garlic until it is crushed or cut; injury to the garlic bulb activates the enzyme allinase, which metabolizes alliin to allicin. Allicin is further metabolized to vinyldithiines.[4]Numerous preclinical studies proved that this plant possess antihypertensive, anticancer, wound healing, antidiabetic, antifungal, antifibrinolytic, antimicrobial, antioxidant, immunomodulatory activities.[5]

This research work was conducted to evaluate the efficacy of an ethanolic extract of Allium Sativum in the treatment of Haloperidol induced catalepsy, for antiparkinson activity. To verify the potential of plants with scientific approach for antiparkinson activity.

II. MATERIALS AND METHODS

2.1 Collection of plant material

The garlic cloves were obtained from Central Market of Telangana State and identified by Ahmedullah, Scientist in charge at Botanical survey of India, as cultivated garlic *Allium Sativum.*

2.2 Preparation of extract:

The transparent covering of garlic was removed manually and chopped and dried in shade to ensure that the volatile components are not lost. It was then subjected to size reduction by mechanical grinder. The powdered material was subjected to soxhlet extraction with ethanol; the extract obtained was dried and used for preclinical studies. The % yield of the extract was found to be 10%.

2.3 Preliminary phytochemical screening:

The preliminary phytochemical investigation was carried out with ethanolic extract of cloves of *Allium sativum* for quantitative identification of photochemical constituents. Photochemical tests were carried out by standard methods [6]

2.4 Experimental animals:

The animal care and handling will be done according to the guidelines set by the CPCSEA. All animals will be housed in a polypropylene cage containing sterile paddy husk as bedding throughout the experiment. Eight to ten week old Male Swiss / Albino Mice weighing 25- 30g will be selected from an inbred colony maintained under the controlled conditions of temperature (23± 2ºC) and 12 hr light/dark cycle with food and water provided *ad libitum.*

III. ACUTE TOXICITY

The acute oral toxicity study would be carried out as per the guidelines set by Organization for Economic Cooperation and Development (OECD) received from Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).The acute toxicity will be determined on Female Swiss /Albino Mice by **fixed dose method** of OECD **Guide line no. 420.**

3.1 Method:

Female Swiss /Albino Mice were accommodated in polypropylene cages and temperature was maintained between 22°C with 12 h each of dark and light cycle. The mice were fed with standard laboratory pelleted feed. The mice were fasted overnight before and 3 h after the administration .A single dose of plant extract 2000 mg/kg orally was administered to one of the animals included in the study. Based on whether the animal survived or died, suitable tests were performed. The drug treated animals were carefully observed for mortality and clinical signs for first 10 min, 30 min, 1 h, 2 h, 4 h, and 6 h after dosing and thereafter twice daily for mortality and once a day for clinical signs, for 14 days. Animals were sacrificed at the end of the study period of 14 days.

IV. EXPERIMENTAL DESIGN

4.1: Haloperidol induced Parkinson's disease Model:

4.1.1 Animal required:

a) Species : Adult Swiss Albino Mice.
b) Age/Weight/Size : 3 months, 21-30g
c) Gender : Male.
d) Numbers to be used : Mice: 30.
e) Number of days each animal will be housed: 07days.

4.1.2 Preparation and mode of administration of drugs:

All drug solutions are freshly prepared and suspended in saline solution

- **Drug for induction of Parkinson's Disease**

Haloperidol (2.0 mg/kg i.p.) Daily for a week.

- **Standard drug:**

Levodopa and carbidopa (10mg/kg, i.p, once per day x 1 week)

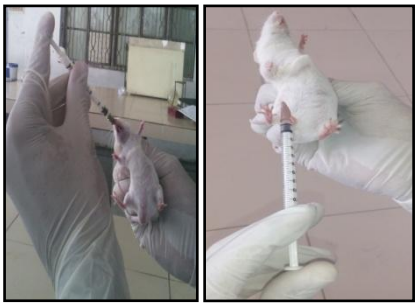

Fig 1: Drug Administration by Oral and i.p. route.

TABLE I: Group Classification:

Sl. No	Groups	No. of Mice	Inducing PD
I	**Normal Control**	06	Distilled water + Feed x 1 week
II	**Haloperidol Control**	06	Haloperidol (2mg/kg,b.w,i.p.once/day x 1 week)
III	**Standard**	06	Levodopa+ carbidopa (10mg/kg,b.w, i.p, once/day x 1 week) +Haloperidol.
IV	**Test low dose**	06	Allium sativum extract (200 mg/kg, b.w, orally x1 week)
V	**Test high dose**	06	Allium sativum extract (400 mg/kg, b.w, orally x1 week)

4.1.3 Experimental procedure:

Male Swiss / Albino mice weighing 20-25 g were divided into five groups of six animals each (n=6). Group I received the drinking water and feed and served as normal control. Group II received haloperidol (2mg/kg body weight) alone and served as negative control without any drug treatment. Group III received combination of levodopa and carbidopa (100mg+ 10mg/kg by intraperitoneal administration) and served as positive control and Group IV and V received Allium sativum extract at doses of 200 and 400mg/kg body weight, respectively for 7 days. The standard (L-dopa) drug was administered by intraperitoneal route and test drug was administered by oral route, half an hour prior to the Haloperidol administration for 07 days of experimental period.

4.1.4 Investigation of antiparkinson activity:

4.2 Behavioural studies test

4.2.1 Measurement of catalepsy:

It was assessed in terms of the time for which the mouse maintained an imposed position with both front limb extended and resting on a 4cm high bar (1cm diameter). The end point of catalepsy was considered to occur when both front paws were removed from the bar or if the animal moved its head in an exploratory manner.

A cut off time 300 seconds was applied. Between the determinations, the animals were returned to their individual home cages. All the observations were made between 9.00 and 15.00hrsina quiet room at 23-25^{0}C.

Scoring Method

If the animal maintained the imposed posture for at least 30seconds.It was considered to be cataleptic and the time was recorded in seconds. The animals were tested on every first, fourth and seventh day of the drug treatment and only the greater duration of the immobility were considered.

Fig 2: Showing Catalepsy Model

4.2.2 Hang test:

Neuromuscular strength was determined in the grid hang test. Mice were lifted by their tail and slowly placed on a horizontal grid and supported until they grabbed the gird with both their fore and hind paws. The grid was then inverted so that the mice were allowed to hang upside down. The grid was mounted 20 cm above a hard surface, to discourage falling but not leading to injury in case of animal fall. Start a stop clock and note the time when the mice fall off or remove it. When the criterion time of 30 seconds is reached.

Fig 3: Showing Hang Test Model

4.2.3 Rotarod:

The main symptom of the Parkinsonism disease is muscle rigidity. This effect can be easily studied in animal by using rotarod apparatus. Turn on the rotarod. Select the speed (20-25 rpm is ideal). Before the test, each animal was given 1 min exposure to the moving rod. The animal was placed on the rotating rod for 3 mins. Latency to fall off from the rotating rod of animal in control and the treated group was recorded. Movement impairement was indicated by the inability of the animal to remain on the rotating rod for a 3 min test period.

Fig 4: Showing Rotarod Model

4.2.4 Horizontal bar test:

Hold the mouse by the tail; place it on the bench in front of the apparatus. Slide it quickly backwards about 20 cms, rapidly raise it and let it grasp the horizontal bar at the central point with its fore paws only and release the tail simultaneously starting the stop clock. The criterion point is either a fall from the bar before the mouse reaches one of the end columns of the bar, or the time till one forepaw touches a column Maximum cut off time is 30 seconds.

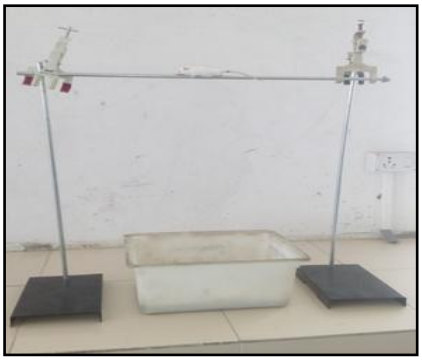

Fig 5: Showing Horizontal Bar Test

4.3 Statistical analysis:

Statistical analysis was performed using one way analysis of variance (ANOVA) followed by Bonferroni multiple comparisons test using statistical package PRISM 5.0 version. Results values were expressed as Mean ± SEM for six mice in each group. The significant difference between and within various groups was determined. Difference were considered to be extremely significant when P<0.001.

V. RESULTS

5.1 Yield calculation

Calculate the % dry weight as follows:

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

Extract of Allium sativum: % dry weight= $\frac{20}{200}$ x 100 = 10%

5.2 Phytochemical evaluations:

The ethanolic extract of Allium Sativum was subjected to preliminary phytochemical screening for the presence of different phytoconstituents. The phytochemical result shows the presence of Alkaloids, Steroids, Glycoside, Flavanoids, Phenolic compounds, Carbohydrate, Proteins and aminoacids. As, the ethanolic extract shows the presence of most of these compounds, so the extract was selected for the study

TABLE II: Phytochemical investigation of *Allium sativum* extract

S.No	Test	Results
1.	Alkaloids	+
2.	Steroids	+
3.	Glycoside	+
4.	Flavonoids	+
5.	Phenolic compounds	+
6.	Protein and aminoacids	+
7.	Carbohydrates	+
8.	Tannins	-

"+" represents Present; "-"= represent absent.

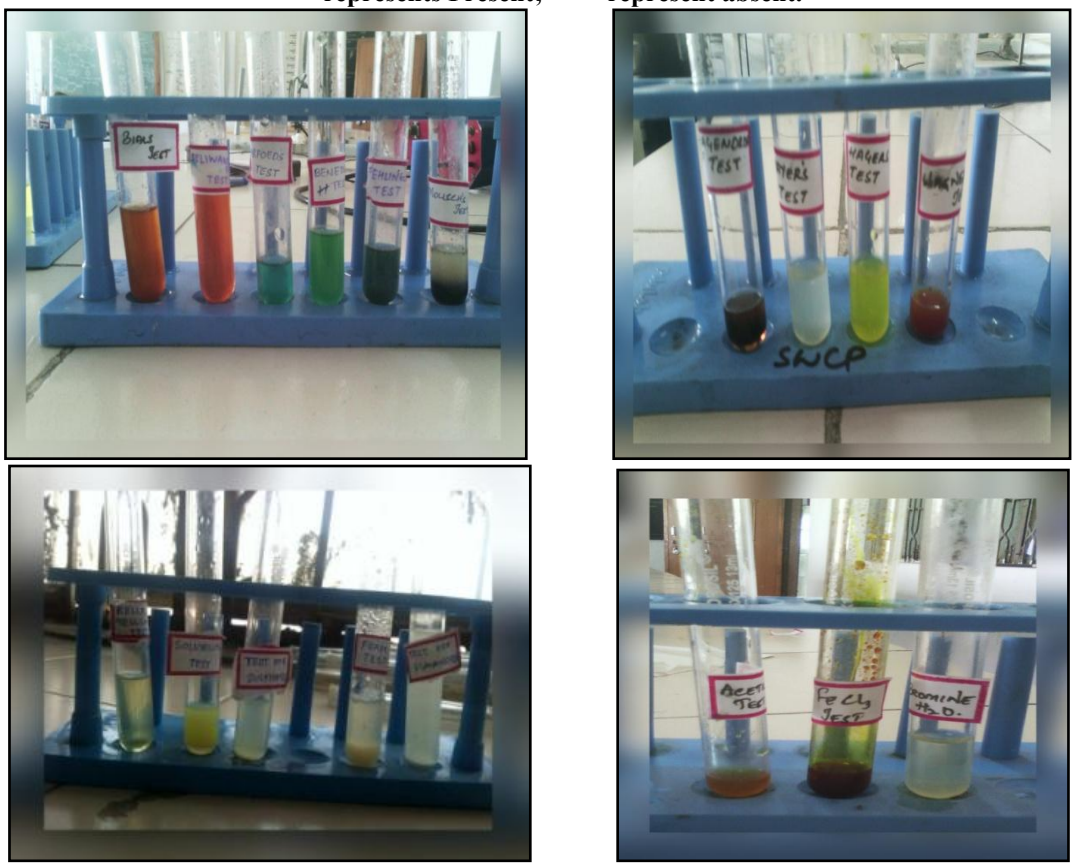

Fig 6: Test for Carbohydrates, Alkaloids, Proteins, Fats and oils, Steroids and other specific Test.

5.3 Acute toxicity

The behavioural pattern of animals were observed first 6h and followed by 14 h, after the administration and the animal in both vehicle- extract treated groups were normal and did not display significant changes in behaviour ,skin effects, breathing, impairment in food intake and water consumption, postural abnormalities and hair loss.

TABLE III: General appearance and behavioural observation for control and treated group

Observation	Control Group		Treated Group		Sign of Toxicity	Duration	Death
	6h	14h	6h	14h			
Awareness/ Alertness	N	N	N	N	NIL	14 Days	NIL
Stereotype	N	N	N	N	NIL	14 Days	NIL
Grooming	N	N	N	N	NIL	14 Days	NIL
Vocalization	N	N	N	N	NIL	14 Days	NIL
Irritability	N	N	N	N	NIL	14 Days	NIL
Touch response	N	N	N	N	NIL	14 Days	NIL
Pain response	N	N	N	N	NIL	14 Days	NIL
Visual placing	N	N	N	N	NIL	14 Days	NIL
Motor in coordination	N	N	N	N	NIL	14 Days	NIL
Body position	N	N	N	N	NIL	14 Days	NIL
Limb position	N	N	N	N	NIL	14 Days	NIL
Grip strength	N	N	N	N	NIL	14 Days	NIL
Pinna reflexes	N	N	N	N	NIL	14 Days	NIL
Abdominal gait	N	N	N	N	NIL	14 Days	NIL
Skin colour/Fur	N	N	N	N	NIL	14 Days	NIL

N = NORMAL

TABLE IV: Gross observation of systemic organs from control and extract treated mice

S.NO	ORGANS	ORGAN WEIGHT	
		Normal Control Group	Extract Treated Group
1.	Brain	0.3g	0.4g
2.	Heart	0.1g	0.2g
3.	Lungs	0.18g	0.28g
4.	Liver	1.4g	1.4g
5.	Stomach	0.6g	0.7g
6.	Kidney	0.28g	0.38g
7.	Spleen	0.1g	0.1g

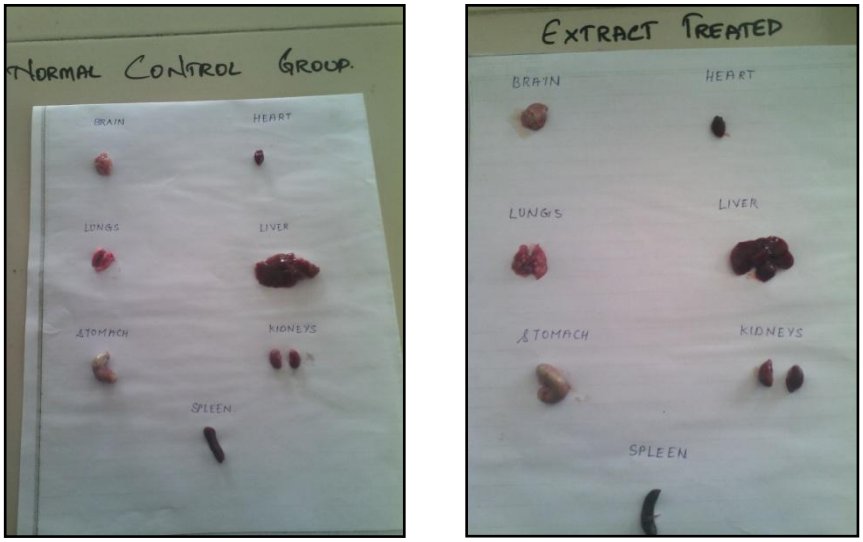

Fig 7: Systemic Organs from Control and Extract Treated Mice

5.4 Antiparkinson's activity

TABLE V: Effect of Allium sativum on catalepsy bar test in mice

S.NO	GROUPS	CATALEPSY (SEC)		
		DAY = 1st	DAY= 4th	DAY= 7th
I.	Normal	7.833 ± 0.703	6.833 ± 0.600	7.167 ± 1.138
II.	Haloperidol	132.5 ± 11.98*	142.6 ± 14.54*	154.3 ± 17.89*
III.	Standard	64.16 ± 5.425***	67.16 ± 5.192***	70.83 ± 5.003***
IV.	Test Low Dose	81.33 ± 5.536**	86.66 ± 5.512**	92.83± 5.588**
V.	Test High Test Dose	72.83 ± 4.094**	74.66 ± 4.014**	78.33 ± 3.921**

Values were expressed as Mean ± SEM (n= 6)

*P < 0.001 extremely significant on comparing group II vs. group I, **P < 0.001 extremely significant on comparing group III, IV, V vs. group II, ***P < 0.001 extremely significant on comparing group III vs. group IV, V.

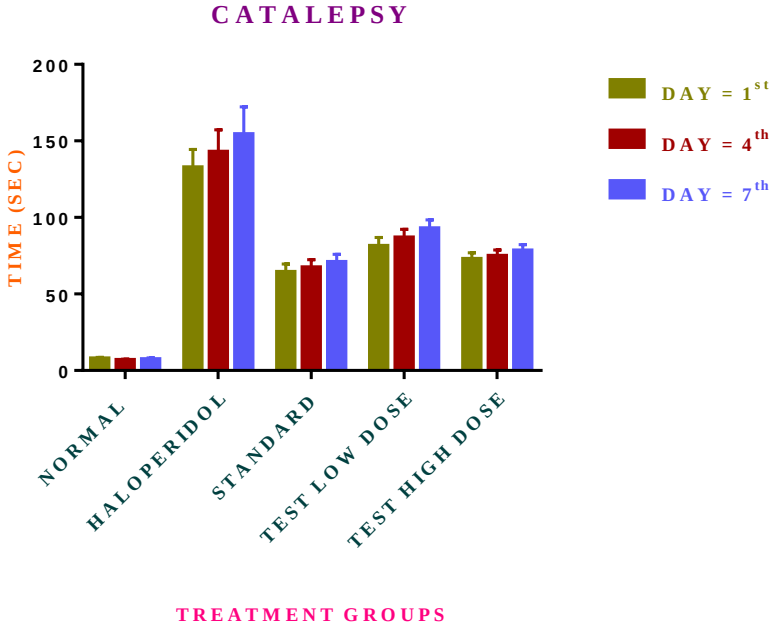

Fig 8: Effect of Allium Sativum on Catalepsy in Mice

TABLE VI: Effect of Allium sativum on hang test in mice

S.NO	GROUPS	HANGING TIME (SEC)		
		DAY = 1st	DAY= 4th	DAY= 7th
I.	Normal	20.000 ± 1.155	22.333 ± 2.828	24.0 ± 4.195
II.	Haloperidol	12.000 ± 1.713*	9.333 ± 1.978*	7.667 ± 2.216*
III.	Standard	18.667 ± 1.430***	17.667 ± 0.955***	18.333 ± 0.14***
IV.	Test Low Dose	11.667 ± 4.967**	13.000 ± 5.177**	14.0 ± 3.578**
V.	Test High Test Dose	15.000 ± 3.742**	16.333 ± 4.082**	15.667 ± 2.944**

Values were expressed as Mean ± SEM (n= 6)
*P < 0.001 extremely significant on comparing group II vs. group I, **P < 0.001 extremely significant on comparing group III, IV, V vs. group II, ***P < 0.001 extremely significant on comparing group III vs. group IV, V.

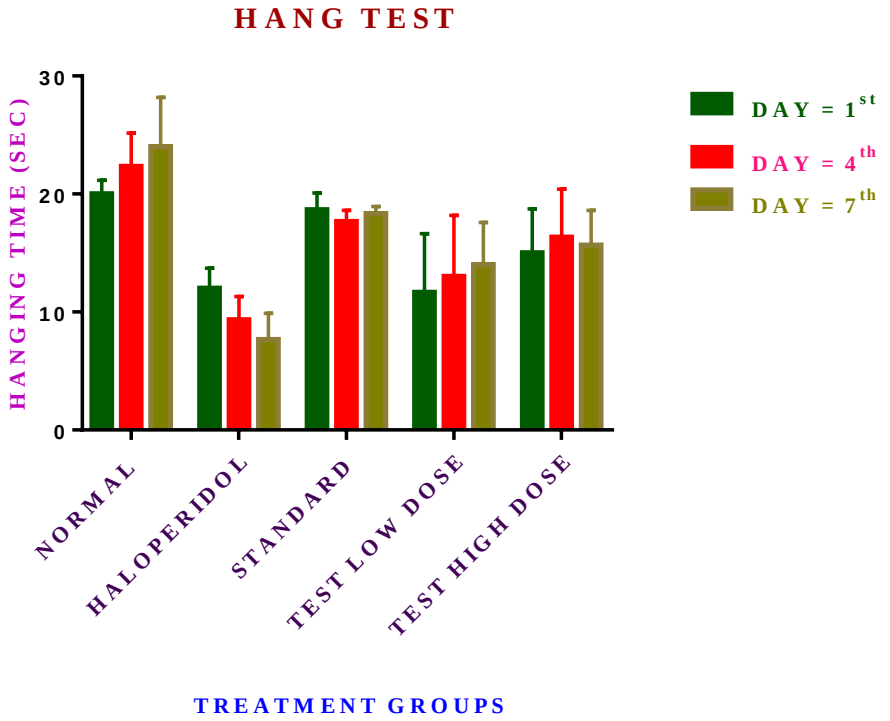

Fig 9: Effect of Allium Sativum on Hang Test in Mice.

TABLE VII: Effect of Allium sativum on rotarod test in mice

S.NO	GROUPS	ROTAROD TEST (SEC)		
		DAY = 1st	DAY= 4th	DAY= 7th
I.	Normal	71.667 ± 3.712	70.500 ± 2.487	71.000 ± 2.620
II.	Haloperidol	26.833 ± 2.522*	23.833 ± 2.522*	22.833 ± 2.386*
III.	Standard	50.000 ± 3.000***	52.667 ± 2.716***	54.833 ± 1.990***
IV.	Test Low Dose	26.000 ± 3.130**	30.500 ± 2.540**	32.167 ± 1.922**
V.	Test High Test Dose	40.167 ± 1.376**	44.500 ± 1.996**	46.333 ± 2.201**

Values were expressed as Mean ± SEM (n= 6)
*P < 0.001 extremely significant on comparing group II vs. group I, **P < 0.001 extremely significant on comparing group III, IV, V vs. group II, ***P < 0.001 extremely significant on comparing group III vs. group IV, V.

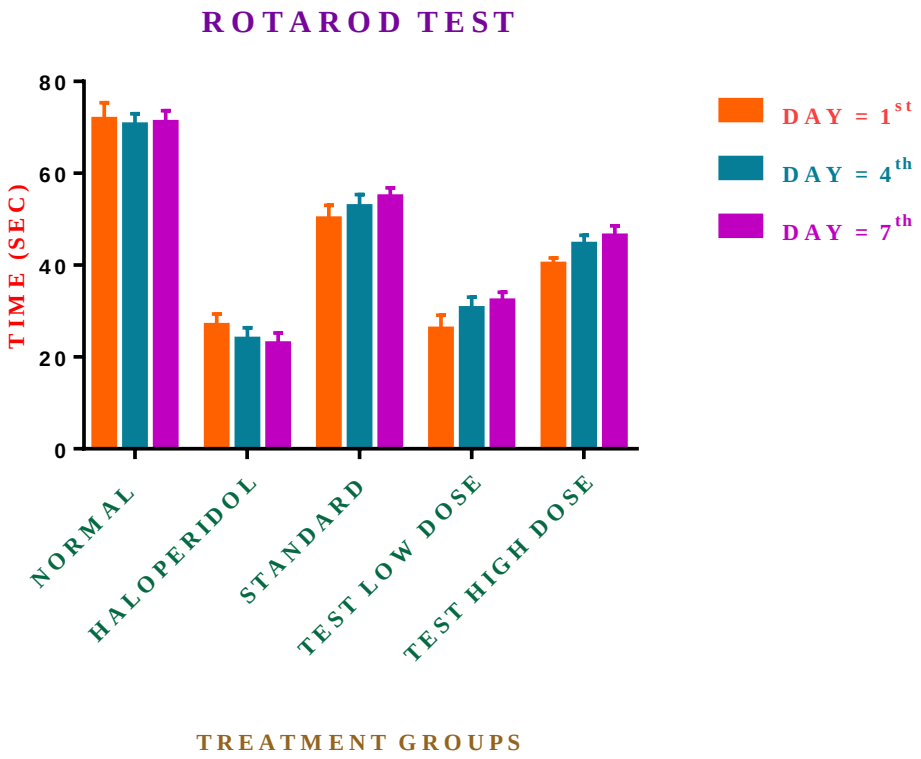

Fig 10: Effect of Allium Sativum on Rotarod Test in Mice.

TABLE VIII: Effect of Allium sativum on horizontal bar test in mice

S.NO	GROUPS	HORIZONTAL BAR TEST (SEC)		
		DAY = 1st	DAY= 4th	DAY= 7th
I.	Normal	14.833 ± 1.740	18.167 ± 1.740	19.500 ± 1.384
II.	Haloperidol	7.000 ± 1.366*	8.667 ± 1.116*	9.833 ± 0.792*
III.	Standard	13.167 ± 1.195***	13.000 ± 1.390***	13.667 ± 1.282***
IV.	Test Low Dose	7.500 ± 1.285**	10.333 ± 1.256**	11.000 ± 1.633**
V.	Test High Test Dose	10.333 ± 0.882**	12.500 ± 0.764**	12.833 ± 0.872**

Values were expressed as Mean ± SEM (n= 6)
*P < 0.001 extremely significant on comparing group II vs. group I, **P < 0.001 extremely significant on comparing group III, IV, V vs. group II, ***P < 0.001 extremely significant on comparing group III vs. group IV, V.

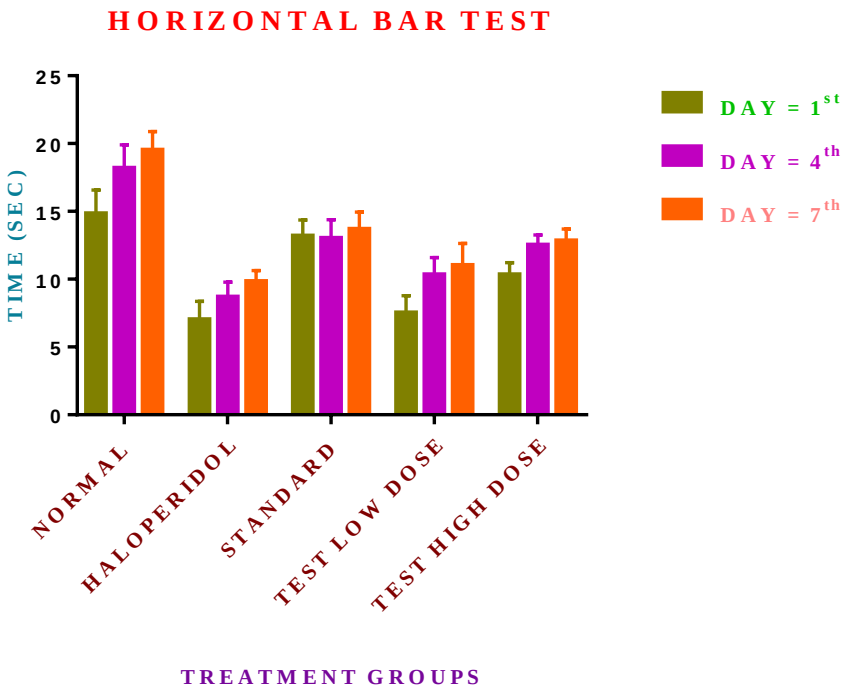

Fig 11: Effect of Allium Sativum on Horizontal Bar Test in Mice.

VI. DISCUSSION

In the present study, the cloves of *Allium sativum* were shade dried, powdered and then extracted with ethanol by soxhlet extraction method. The % yield of the extract was found to be 10%. The ethanolic extract, then subjected to preliminary phytochemical screening. The phytochemical result shows the presence of Carbohydrate, Proteins, Alkaloids, Steroids, Glycoside and Flavanoids.

The ethanolic extract of *Allium sativum* was analysed for their acute toxicity in Swiss/ Albino mice. In this study, the acute toxicity is carried out by "Fixed Dose Method" of OECD guideline no.420. The mice which are orally administered with ethanolic extract of *Allium sativum* at single dose of 2000mg/kg were monitored daily until day 14, showed no signs of toxicity and deaths. All the mice gained weight and displayed no significant change in behaviour, this indicate that the administration of the crude extract has negligible level of toxicity on growth of the animal. Organ weight is also important factor of physiological and pathological status in animal. The relative organ weight is fundamental to diagnose whether the organ was exposed to injury or not. The Brain, Heart, Lung, Liver, Kidneys, Spleen are the essential organs affected by metabolic reaction caused by toxicant. The result revealed that the essential organs were not adversely affected throughout the treatment as seen in the gross observation of systemic organ of both control and treated groups. It is also observed that the organ weight of treated group when compared to control group was increased significantly which shows, that the extract nurtures the organ. It indicates that the ethanolic extract of *Allium sativum* was not lethal to the mice at 2000mg/kg doses. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of the doses were selected for the further study.

The present study uses four behavioural parameter such as Catalepsy bar test, Hang test, Rotarod test and Horizontal bar test to assess Haloperidol Induced Parkinson's Disease in mice. In Catalepsy test, the group which received only Haloperidol (Group II), significantly, increases catalepsy ($P<0.001$) which was seen on 1^{st}, 4^{th} and 7^{th} day as compared to the Normal group (Group I).In standard treated group (Group III), a significant decrease in catalepsy ($P<0.001$) was seen on 1^{st}, 4^{th} and 7^{th} day as compared to the Haloperidol treated group (Group II). In extract treated group (Group IV & V), a significant decrease in catalepsy ($P<0.001$) was seen on 1^{st}, 4^{th} and 7^{th} day as compared to the Haloperidol treated group (Group II).Whereas no significant difference in catalepsy was seen when 400mg/kg treated group (Group V) compared to standard treated group (Group III).

In Hang test, the group which received only Haloperidol (Group II), significantly, decreases hanging time ($P<0.001$) which was seen on 1^{st}, 4^{th} and 7^{th} day as compared to the Normal group (Group I). In standard treated group (Group III), a significant increase in hanging time ($P<0.001$) was seen on 1^{st}, 4^{th} and 7^{th} day as compared to the Haloperidol treated group (Group II). In extract treated group (Group IV & V), a significant increases in hanging time ($P<0.001$) was seen on 1^{st}, 4^{th} and 7^{th} day as compared to the Haloperidol treated group (Group

II).Whereas no significant difference in hanging time was seen when 400mg/kg treated group (Group V) compared to standard treated group (Group III).

In Rotarod test, the group which received only Haloperidol (Group II), significantly, decreases fall off time (P<0.001) which was seen on 1st, 4th and 7th day as compared to the Normal group (Group I). In standard treated group (Group III), a significant increase in fall off time (P<0.001) was seen on 1st, 4th and 7th day as compared to the Haloperidol treated group (Group II). In extract treated group (Group IV & V), a significant increases in fall off time (P<0.001) was seen on 1st, 4th and 7th day as compared to the Haloperidol treated group (Group II).Whereas no significant difference in fall off time was seen when 400mg/kg treated group (Group V) compared to standard treated group (Group III).

In Horizontal bar test, the group which received only Haloperidol (Group II), significantly, decreases fall off time (P<0.001) which was seen on 1st, 4th and 7th day as compared to the Normal group (Group I). In standard treated group (Group III), a significant increase in fall off time (P<0.001) was seen on 1st, 4th and 7th day as compared to the Haloperidol treated group (Group II). In extract treated group (Group IV & V), a significant increases in fall off time (P<0.001) was seen on 1st, 4th and 7th day as compared to the Haloperidol treated group (Group II).Whereas no significant difference in fall off time was seen when 400mg/kg treated group (Group V) compared to standard treated group (Group III).

VII. CONCLUSION

Parkinson's disease is a progressive neurodegenerative disorder characterised by the selective loss of dopaminergic neuron of substantia nigra pars compacta. Haloperidol is commonly used to induce Parkinson's disease in mice. It blocks dopamine receptors present in the straitum, thus induces Parkinson's disease Toxicity of haloperidol leads to the generation of free radicals leading to oxidative stress.

The results of the present study conclusively showed that the *Allium sativum* has antioxidant activity and presence of major phytoconstituents like Flavanoids, Polyphenol, and Organo sulphur compound in the plant is believed to have neuroprotective effect. The ethanolic extract of *Allium sativum* was also found to be effective in increasing Rotarod, Hang, Horizontal bar test performance and decreasing the duration of the cataleptic score in haloperidol experimental model of Parkinson's disease in mice.

Thus, the result of the present study conclusively shows the Antiparkinson's activity of *Allium sativum* in haloperidol induced Parkinson's disease in mice.

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