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ANTIPARKINSONIAN EFFECTS AND MECHANISTIC INSIGHTS OF SOME INDIGENOUS HERBAL FORMULATIONS

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Keywords:

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ABSTRACT: **Objective**: The anti-parkinson's potential of the formulations containing roots of *Withania somnifera* family *Solanaceae*, leaves of *Ocimum sanctum* family *Lamiaceae*, and rhizome of *Curcuma longa* family Zingiberaceae, was investigated in reserpine and haloperidol induced parkinson's model. **Materials and Methods**: Parkinson's was produced by Reserpine and Haloperidol administration (1mg/kg i.p and 1mg/kg i.p. respectively). Formulation (300 mg/kg orally) was administered as pretreatment for 14 days. The animals were evaluated for Behavioral and Locomotors activity. **Results**: Pretreatment with the formulations significantly ($P < 0.01$) protected the catalepsy, rigidity from the toxic effects of reserpine and haloperidol. The hydroalcoholic extract formulation of Ashwagandha, Tulsi and Haldi was assigned to class 5 (LD_{50}> 2000mg/kg), were recommended by OECD. **Conclusion**: These results suggest an anti-parkinson's effect of *formulations* due to its nerve tonic, &increases in dopamine concentration.

INTRODUCTION: The World Health Organization (2003) has estimated that 80% of the population of developing countries are being unable to afford pharmaceutical drugs rely on traditional medicines, mainly plant based, to sustain their primary health care needs. In Ayurveda the specific properties of plants and their use as medicinal drugs has been dealt with in great detail. Neurological and psychiatric disorders together account for more chronic suffering than all other disorders combined. [1] Treating these problems however, remains a challenging field in medical science.

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Traditional therapies in the form of herbal preparations containing anticholinergics, L-dopa, and monoamine oxidase inhibitors were used in the treatment of Parkinson's disease in India, China, and the Amazon basin. No satisfactory treatment is seen in contemporary system of medicines of parkinson's disease. The conventional treatment includes levodopa preparation, anticholinergic drugs and surgery etc. which give more or less temporary relief and are expensive with the adverse effect. This needs instills a need for ayurvedic management of Parkinson's disease. [2]

Parkinson's disease (PD) is the second most common degenerative disorder of the central nervous system (CNS) surpassed only by Alzheimer's disease (AD). PD is characterized by loss of motor function, leaving its victims progressively less mobile, eventually frozen in their own bodies. PD was first described in 1817 by the English physician James Parkinson, who

summarized his observations as paralysis agitans, or the "shaking palsy". [3]

Pathologically, the hallmarks of PD are the loss of dopaminergic neurons in the substantia nigra pars compacta and the presence of cytoplasmic protein aggregates, known as Lewy bodies, in remaining dopaminergic cells. [4] Mechanistically, the death of dopaminergic neurons has been linked to mitochondrial dysfunction, oxidative stress, neuro-inflammation etc. The symptoms of bradykinesia (slowed movement), rigidity, and resting tremor are the most classic characteristics of PD and are referred to as the "diagnostic triad". Additional symptoms included masked fascies, stooped posture, shuffling gait, micrographia, microphonia (reduced speech volume) and a multitude of non-motor symptoms such as constipation, and dementia or depression. Together, these extra nigrostriatal regions may account for the observed non-motor symptoms such as sleep disturbances, depression, cognitive impairment, anosmia, constipation, incontinence, and autonomic dysfunctions.[2]

Several studieshave been used to treat the parkinson's disease. It has been reported that the reserpine due to its inhibiting the vesicular monoamine transporter hence loss of storage capacity and hence depletion of brain as well as peripheral monoamines loss of dopamine action and haloperidol due to its block of the dopamine transmission which leads to abnormal downstream firing within the basal ganglia circuits action causes parkinson's. The formulations containing herbs like *Withania somnifera, Ocimum sanctum* and *Curcuma longa* shows beneficial effects on parkinson's.

The present study shows that the anti-parkinson's effect of formulations on parkinson's induced mice by reserpine and haloperidol.

MATERIALS AND METHODS:

Plant Materials:

Roots of Ashwagandha were collected in August 2014 from local market of Mumbai. Leaves of Tulsi were collected in August 2014 from botanical garden of Dr. Bhanuben Nanavati College of Pharmacy, Mumbai. Haldi extract was collected in August 2014 from Sami labs, Bangalore. All plants were taxonomically identified and authenticated by Dr. Bindu Gopalkrishanan, Botany department at Mithibai College, Mumbai. The sample specimen was preserved in our laboratory for future reference.

Extraction Procedure:

The dried and pulverized roots of *Withania smonifera* and leaf of *Ocimum sanctum* about 100 g was extracted with 50% ethanol and 50% distilled water (60–80^{0}C) in a Soxhlet apparatus. The solvent was removed from ethanol extract under vacuum rotary dryer and a semi solid mass (17% w/w in respect of dry material) was obtained. The hydroalcoholic extracts of *Withania somnifera and Ocimum sanctum* was stored in desiccators. The extracts thus obtained were subjected to phytochemical analysis.

Preliminary Phytochemical Screening:

The phytochemical examination of the hydroalcoholic extracts of *Withania somnifera* and *Ocimum sanctum* was performed by the standard methods. [6]

Acute Oral Toxicity Testing:

Acute oral toxicity study was performed using male swiss albino mice as per OECD (Organization for economic co-operation and development) Guideline 423. The animals were fasted overnight and grouped into 4 groups with 3 animals in each group. The animals were observed continuously for 1 hour after dosing and then intermittently for further 4 hours and daily thereafter for a total 14 days. [7]

Preparation of formulations:

In order to perform psychopharmacological evaluation of formulations for anti-parkinson's activity, all three plant extracts were mixed (i.e. Ashwagandha, Tulsi and Haldi) in three different concentrations keeping final dose constant i.e. 300mg/kg (**Table 1**).

TABLE 1: PREPERATION OF FORMULATIONS

Formulation I	Ashwagandha 100mg/kg + Tulsi 100mg/kg + Haldi 100mg/kg
Formulation II	Ashwagandha 150mg/kg + Tulsi 100mg/kg + Haldi 50mg/kg
Formulation III	Ashwagandha 50mg/kg + Tulsi 150mg/kg + Haldi 100mg/kg

Animals:

Male swiss albino mice weighing between 25-30 gm were obtained from Bharat Serum, thane and used for the experiments. The animals were housed kept in polypropylene cages (32.5 × 21 × 14) cm and (24 × 14 × 12) cm lined with raw husk (renewed after 48 h), were used to accommodate rats (6/cage) and mice (4/cage) respectively. Animal house was maintained under standard laboratory conditions in 12 h light-dark cycle at 24 ± 2^0 C and humidity 30-70 %. Animals were provided with standard pellet diet and water *ad libitum.* This standard pellet diet is manufactured by Amruth Laboratory, Mumbai. Animal studies were conducted according to Institute Animal Ethics Committee regulations approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA).

***In-vivo* models for anti-Parkinson's potential:**

Reserpine induced model:

Adult male swiss albino mice (20-25 g) were used. Animals were randomly divided into 6 groups containing 6 animals each (**Table 2**). The animals were housed under standard 12hr: 12hr light/dark cycles and were provided with food and water *ad libitum* prior to experiment (**Table 2**). [8]

TABLE 2: RESERPINE INDUCED MODEL

Group	Study
I	Vehicle control
II	Negative control: treated with Reserpine (1mg/kg. i.p.)
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg + Haldi 100mg/kg.)
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg + Tulsi 100mg/kg + Haldi 50mg/kg.)
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg + Haldi 100mg/kg.)
VI	Standard (Levodopa + Carbidopa 110 mg/kg p.o.)

The Parkinsonism were induced in mice by administrating Reserpine (1mg/kg, i.p.) to all the animals of group II – VI, after 60 min of treatment. The animals were evaluated for Behavioral and Locomotors activity.

Haloperidol induced model:

Adult male swiss albino mice (20-25 g) were used. Animals were randomly divided into 6 groups containing 6 animals each (**Table 3**). The animals were housed under standard 12hr: 12hr light/dark cycles and were provided with food and water *ad libitum* prior to experiment (**Table 3**). [9]

TABLE 3: HALOPERIDOL INDUCED MODEL

Group	Study
I	Vehicle control
II	Negative control: treated with Haloperidol (1ml/kg, i.p.)
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg + Haldi 100mg/kg.)
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg + Tulsi 100mg/kg + Haldi 50mg/kg.)
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg + Haldi 100mg/kg.)
VI	Standard (Levodopa + Carbidopa 110 mg/kg p.o.)

The Parkinsonism were induced in mice by administrating Haloperidol (1ml/kg, i.p.) to all the animals of group II – VI, after 60 min of treatment. The animals were evaluated for Behavioral and Locomotors activity.

Assessment of Locomotor Activity:

Catalepsy: A cataleptic behavior was measured with a high bar test method. Catalepsy was induced by haloperidol and reserpine. Catalepsy was measured after 1 hr. injection of haloperidol and reserpine by gently placing both the forepaw of mouse over a metal bar (diameter 2-5mm suspended 6 cm above the table spot). The intensity of catalepsy was assessed by counting the time in seconds until mouse brought both forepaws down to the table top with a cut off time of 1100sec. All observations were made between 10.00 to 16.00 hrs in a quiet room at room temperature. [10]

1. Rota rod:
The speed of the rotating rod was set at 20 rpm or kept constant. Animals were placed on the rod and the fall off time before haloperidol/reserpine and for each 30 min after haloperidol/reserpine treatment was determined. Percent decrease in time compared to fall off time before haloperidol/reserpine was calculated. [11]

2. Pole test:
Briefly, mice were placed head-upward at the top of a pole (8mm in diameter and 45 cm in height), and then the time for the animal to rotate downward completely (Tturn) and to descend to the floor (Ttotal) was measured with a maximum limit of 90 s. Animals usually received trainings (3–5 min/session/day) in the pole-descending behavior for 3 or 4 days, (usually performed 2 h before the test trial) were used. Bradykinesia was evaluated as the prolongation of Tturn or Ttotal.[12]

3. Hanging wire test:
The experimental animals were suspended by its forelimbs on a wire stretched between two posts, 45 cm above a foam sheet. The time in seconds (s), until the animal fell down, was recorded. 2 min of cut off time was designated. This task was used as a measure of grasping ability and forelimb strength.[13]

Statistical Analysis:
For the in vivo study, results are expressed as mean ± SEM. Total variation present in a set of data was estimated by one way analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS:
ACUTE ORAL TOXICITY TESTING (Table 4)

TABLE 4: RESULT OF ACUTE ORAL TOXICITY TEST

Test	No. of Animals used	Test substance used	Dose mg/ kg	Acute oral toxicity study-24 h*	Acute oral toxicity study-14 days*
Limit Test	03	Hydroalcoholic extract of formulation containing *Withania somnifera*, *Ocimum sanctum* and *Curcuma longa*	300	O	O
	03		2000	O	O
	03		5000	O	O
	03		control	O	O

* (X= died, O= survival)

Signs and symptoms:

- The animals did not show any significant change in body weight.
- The animals did not show any significant changes in skin and fur, eyes and mucous membranes, and also respiratory, circulatory, autonomic, central nervous systems, somatomotor activity and behavioral pattern and failed to elicit any clinical abnormality.

Mortality:

- There was no mortality observed at any dose level.
- Hydroalcoholic extract of formulation containing *Withania Somnifera, Ocimum Sanctum* and *Curcuma Longa* was found to be safe up to 5000 mg/ kg body weight when administered orally.
- The dose of hydroalcoholic extract of formulation selected for the study was 300 mg/kg, in which each plant extract included in different concentration keeping final dose of formulation constant.

Reserpine Induced Model of Parkinson:

Effect of Formulations on Catalepsy Induced By Reserpine:

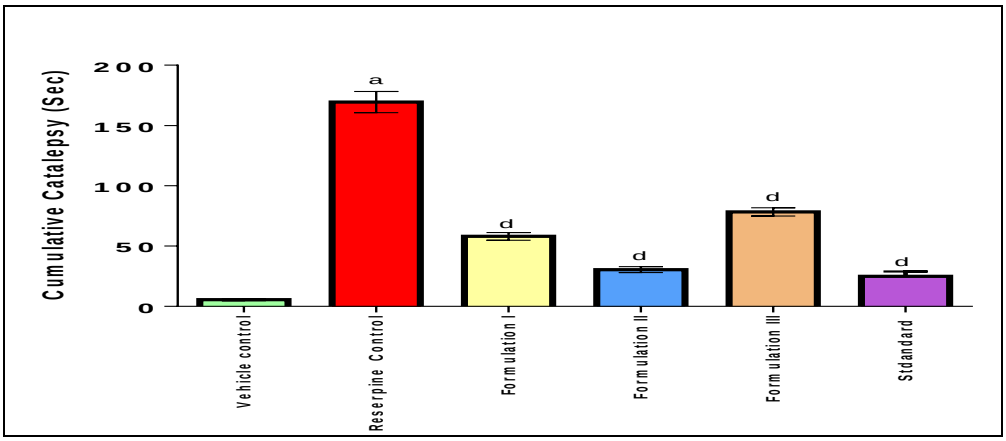

FIG. 1: EFFECT OF FORMULATIONS ON CATALEPSY INDUCED BY RESERPINE

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control [a]p< 0.001. One way ANOVA followed by Dunnett's test when compared with reserpine control [b]p<0.05, [c]p<0.01, [d]p< 0.001

TABLE 5: EFFECT OF FORMULATIONS ON CATALEPSY INDUCED BY RESERPINE

Group	Drug Treatment	Cumulative Catalepsy
I	Vehicle control	5.536± 0.821
II	Negative control: treated with Reserpine (1mg/kg, i.p.)	169.4 ± 8.795[a]
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg +Haldi100mg/kg)	58.07 ± 3.200 [d]
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg + Tulsi 100mg/kg +Haldi 50mg/kg)	30.46 ± 2.467[d]
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg + Haldi 100mg/kg)	78.34 ± 3.432[d]
VI	Standard (Levodopa + Carbidopa 110mg/kg)	24.97 ± 4.025[d]

In the present study, reserpine produced a time dependent increase in cataleptic state which was significant as compared to the vehicle treated animals. Formulation II reduced the severity of reserpine induced model (**Table 5).**

Effect of Formulations on Locomotor Activity Using Rota Rod Apparatus:

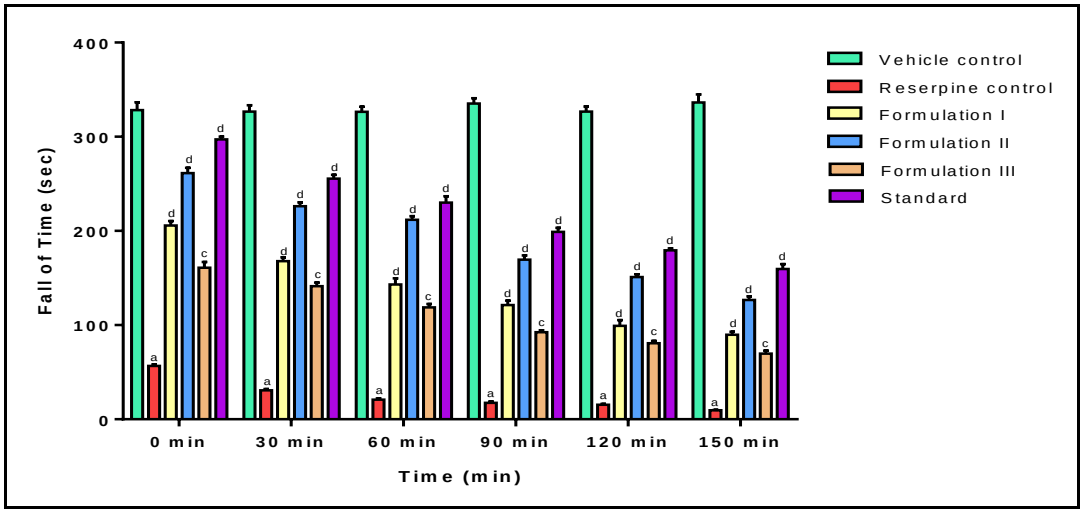

FIG. 2: EFFECT OF FORMULATIONS ON LOCOMOTOR ACTIVITY USING ROTA ROD APPARATUS

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control [a]p< 0.001. One way ANOVA followed by Dunnett's test when compared with haloperidol control [b]p<0.05, [c]p<0.01, [d]p< 0.001

TABLE 6: EFFECT OF FORMULATIONS ON LOCOMOTOR ACTIVITY USING ROTA ROD APPARATUS

Group	Drug Treatment	Counts/minutes					
		0 min	30 min	60 min	90 min	120 min	150 min
I	Vehicle control	328.22	326.78	326.53	335.23	326.60	336.26
II	Negative control: treated with Reserpinel (1mg/kg, i.p.)	56.56	30.64	20.78	17.46	15.40	9.49
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg + Haldi 100mg/kg.)	205.82	167.86	143.23	121.33	99.19	89.66
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg + Tulsi 100mg/kg + Haldi 50mg/kg.)	261.39	226.67	211.86	169.41	151.02	126.66
V	Formulation III, 300mg/kgp.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg + Haldi 100mg/kg.)	160.87	141.27	118.75	92.34	80.82	69.78
VI	Standard (Levodopa + Carbidopa 110mg/kg)	285.20	265.40	225.11	191.91	171.30	150.61

Reserpine administration significantly (P<0.01) reduced movement of mice in both models as compared to normal animals. In short term model, pretreatment with Formulation I, II & III significantly delayed and shortened the reduction in movement as compared with control

Effect of Formulations on Pole Test Induced By Reserpine:

Time taken to turn completely downward (T-turn) (Sec):

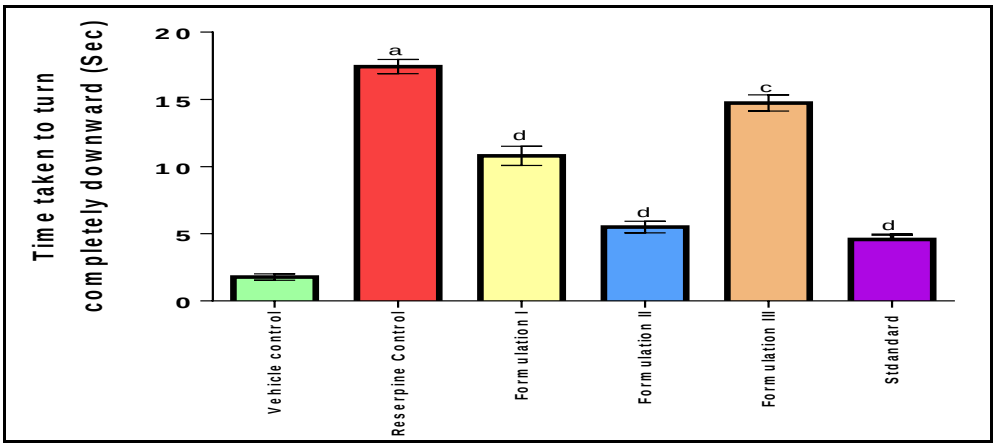

FIG. 3: TIME TAKEN TO TURN COMPLETELY DOWNWARD

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control $^{a}p<0.001$. One way ANOVA followed by Dunnett's test when compared with haloperidol control $^{b}p<0.05$, $^{c}p<0.01$, $^{d}p< 0.001$

TABLE 7: TIME TAKEN TO TURN COMPLETELY DOWNWARD

Group	Drug Treatment	Time taken to turn completely downward (Sec)
I	Vehicle control	1.780± 0.2337
II	Negative control: treated with Reserpine (1mg/kg, i.p.)	17.44 ± 0.5247[a]
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg + Haldi100mg/kg.)	10.79 ± 0.7143 [d]
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg +Tulsi 100mg/kg + Haldi 50mg/kg.)	5.50 ± 0.4277[d]
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg + Haldi 100mg/kg.)	14.73 ± 0.6032 [c]
VI	Standard (Levodopa + Carbidopa 110mg/kg)	4.576± 0.3637[d]

Time taken to reach the floor (T-total) (Sec):

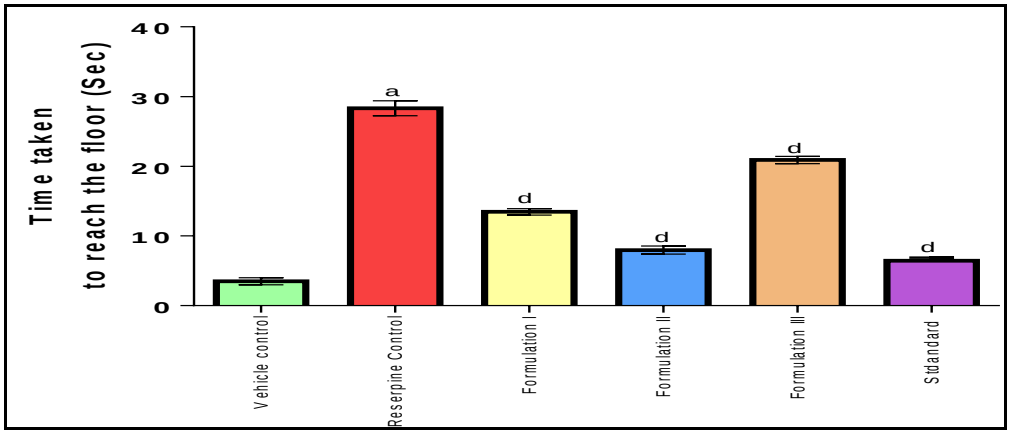

FIG. 4: TIME TAKEN TO REACH THE FLOOR

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control [a]p< 0.001. One way ANOVA followed by Dunnett's test when compared with haloperidol control [b]p<0.05, [c]p<0.01, [d]p< 0.001 (Fig. 4)

TABLE 8: TIME TAKEN TO REACH THE FLOOR

Group	Drug Treatment	Time taken to reach the floor (Sec)
I	Vehicle control	3.501± 0.5568
II	Negative control: treated with Reserpine (1mg/kg, i.p.)	28.32 ± 1.068[a]
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg+Haldi 100mg/kg.)	13.46 ± 0.4667 [d]
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg +Tulsi 100mg/kg + Haldi 50mg/kg.)	7.973 ± 0.5735[d]
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg +Haldi 100mg/kg.)	20.90 ± 0.5214[b]
VI	Standard (Levodopa + Carbidopa 110mg/kg)	6.458 ± 0.4395[d]

Vehicle Control animals placed head-upward at the top of the pole easily rotated downward and descended to the floor normally within 10 sec. Administration of reserpine(1mg/kg) increases both T-turn and T-total values, which reached about 8-9 times the control values. Standard significantly reduced the prolongation of T-turn (Table 7) and T-total Table 8) values by reserpine. When animals were pretreated with formulation before injection of reserpine, reserpine induced bradykinesia was markedly reduced.

Effect of Formulations on Hanging Wire Test

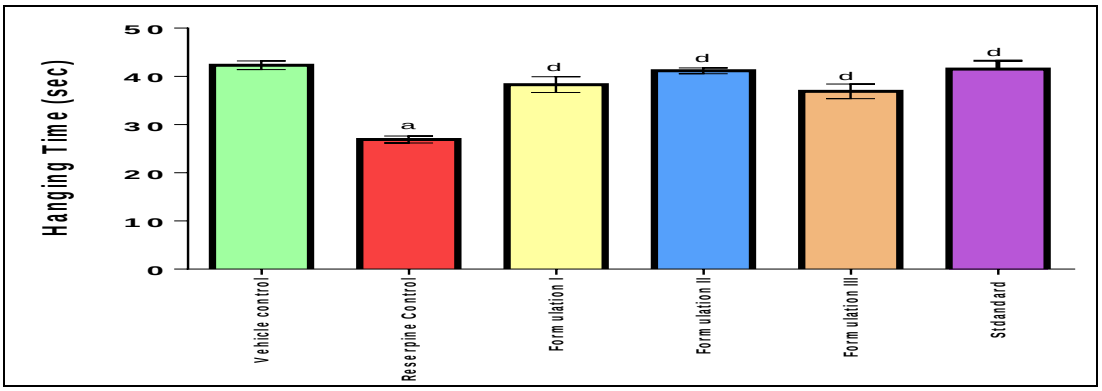

FIG. 5: EFFECT OF FORMULATIONS ON HANGING WIRE TEST

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control [a]p< 0.001. One way ANOVA followed by Dunnett's test when compared with reserpine control [b]p<0.05, [c]p<0.01, [d]p< 0.001

TABLE 9: EFFECT OF FORMULATIONS ON HANGING WIRE TEST

Group	Drug Treatment	Hanging Time (sec)
I	Vehicle control	42.30± 0.8810
II	Negative control: treated with Reserpine (1mg/kg, i.p.)	26.90 ± 8.795[a]
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg +Haldi 100mg/kg.)	38.28 ± 1.623 [d]
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg +Tulsi 100mg/kg + Haldi 50mg/kg.)	41.15 ± 0.611[d]
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg +Haldi100mg/kg.)	36.89 ± 1.528[d]
VI	Standard (Levodopa + Carbidopa 110mg/kg)	41.52 ± 1.696[d]

The treated animals showed increase in hanging time which indicates increase in muscle strength. When compared with control group, negative control showed more significant ($P < 0.001$) variation and reduction of hanging time. When compared with negative control, formulation I, II, III and standard showed significant ($P < 0.001$) increase in hanging time (Table 9).

Haloperidol Induced Model of Parkinson:

Effect of Formulations on Catalepsy Induced By Haloperidol:

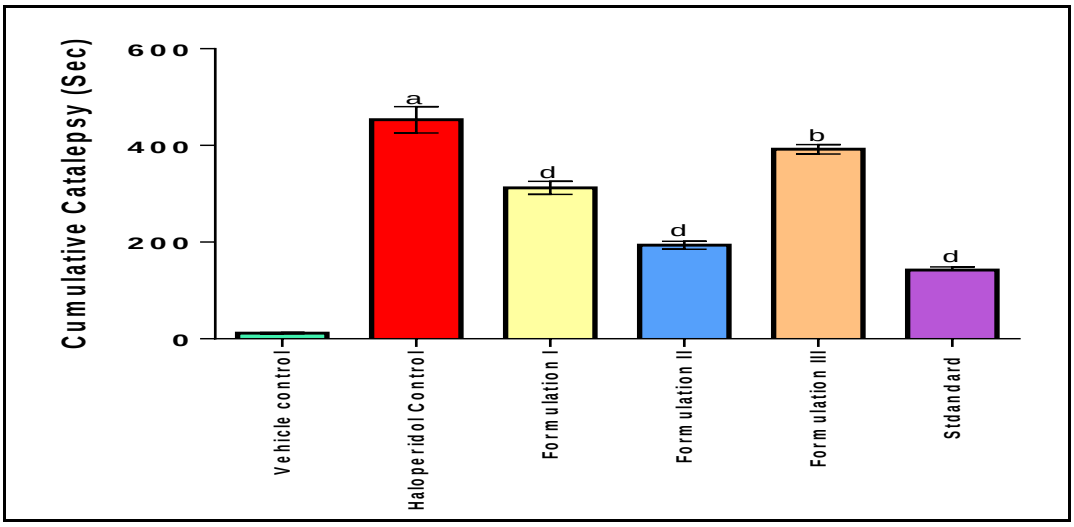

FIG. 6: EFFECT OF FORMULATIONS ON CATALEPSY INDUCED BY HALOPERIDOL

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control [a]p< 0.001. One way ANOVA followed by Dunnett's test when compared with haloperidol control [b]p<0.05, [c]p<0.01, [d]p< 0.001

TABLE 10: EFFECT OF FORMULATIONS ON CATALEPSY INDUCED BY HALOPERIDOL

Group	Drug Treatment	Cumulative Catalepsy
I	Vehicle control	11.82± 1.383
II	Negative control: treated with Haloperidol (1ml/kg, i.p.)	453.0 ± 27.15[a]
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg + Haldi 100mg/kg)	312.3 ± 13.46 [d]
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg + Tulsi 100mg/kg + Haldi 50mg/kg)	193.6 ± 8.260[d]
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg + Haldi 100mg/kg)	392.2 ± 9.716[b]
VI	Standard (Levodopa + Carbidopa 110mg/kg)	142.3 ± 6.550[d]

In the present study, haloperidol produced a time dependent increase in cataleptic state which was significant as compared to the vehicle treated animals. Formulation II reduced the severity of haloperidol induced model.

Effect of Formulations on Locomotor Activity Using Rota Rod Apparatus:

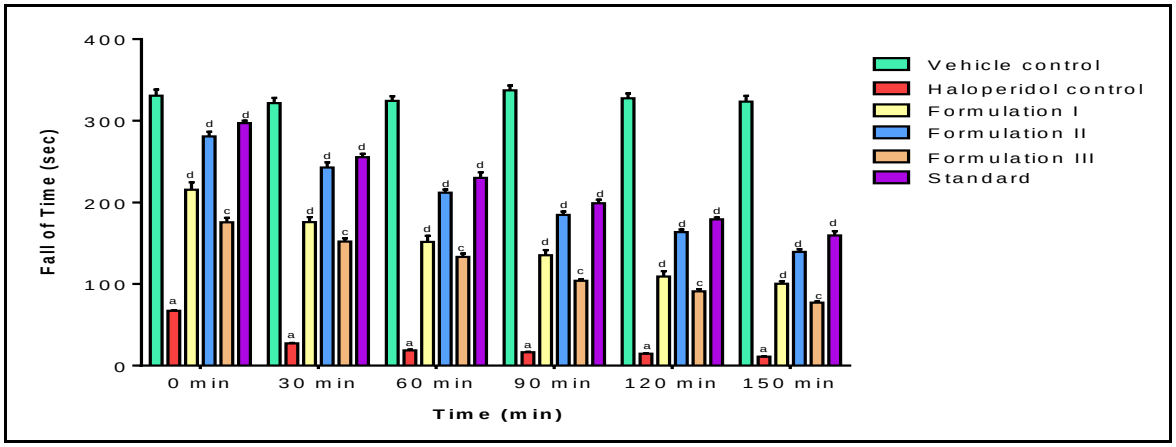

FIG. 7: EFFECT OF FORMULATIONS ON LOCOMOTOR ACTIVITY USING ROTA ROD APPARATUS

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control $^{a}p<0.001$. One way ANOVA followed by Dunnett's test when compared with haloperidol control $^{b}p<0.05$, $^{c}p<0.01$, $^{d}p< 0.001$

TABLE 11: EFFECT OF FORMULATIONS ON LOCOMOTOR ACTIVITY USING ROTA ROD APPARATUS

Group	Drug Treatment	Counts/minutes					
		0 min	30 min	60 min	90 min	120 min	150 min
I	Vehicle control	330.83	321.59	324.39	337.41	327.50	323.55
II	Negative control: treated with Haloperidol (1ml/kg, i.p.)	67.30	27.42	18.59	16.52	14.54	10.97
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg + Haldi100mg/kg.)	215.55	175.74	151.63	135.26	109.15	100.26
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg +Tulsi 100mg/kg Haldi 50mg/kg.)	280.86	242.75	211.86	184.68	163.64	139.298
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg +Haldi 100mg/kg.)	175.67	152.01	133.19	103.89	91.12	77.11
VI	Standard (Levodopa + Carbidopa 110mg/kg)	297.20	255.41	230.11	198.91	179.30	159.56

Haloperidol administration significantly (P<0.01) reduced movement of mice in both models as compared to normal animals. In short term model, pretreatment with Formulation I, II & III significantly delayed and shortened the reduction in movement as compared with control

Effect of Formulations on Pole Test:
Time taken to turn completely downward (T-turn) (Sec)

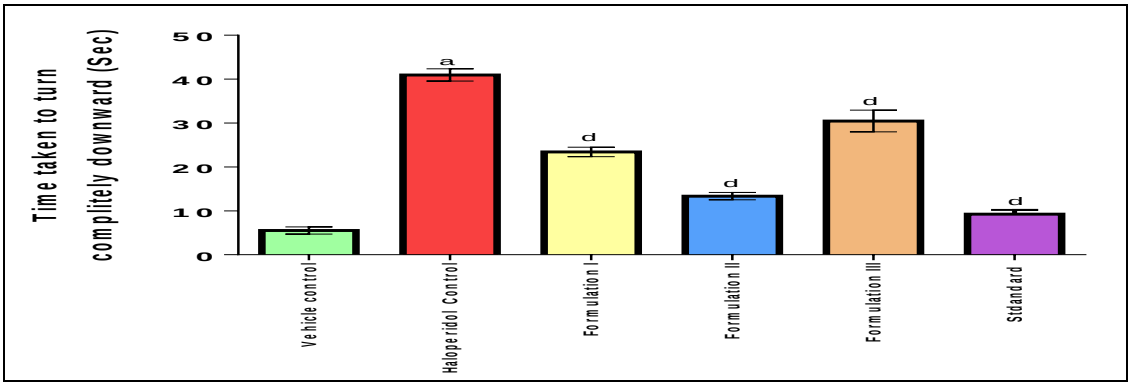

FIG. 8: TIME TAKEN TO TURN COMPLETELY DOWNWARD

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control $^{a}p<0.001$. One way ANOVA followed by Dunnett's test when compared with haloperidol control $^{b}p<0.05$, $^{c}p<0.01$, $^{d}p< 0.001$

TABLE 12: TIME TAKEN TO TURN COMPLETELY DOWNWARD

Group	Drug Treatment	Time taken to turn completely downward (Sec)
I	Vehicle control	5.536± 0.821
II	Negative control: treated with Haloperidol (1ml/kg, i.p.)	40.95 ± 1.394[a]
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg +Haldi 100mg/kg.)	23.40 ± 1.080 [d]
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg +Tulsi 100mg/kg + Haldi 50mg/kg.)	13.35 ± 0.8347[d]
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg +Haldi 100mg/kg.)	30.46 ± 2.476[b]
VI	Standard (Levodopa + Carbidopa 110mg/kg)	9.234± 0.9752 [d]

Time taken to reach the floor (T-total) (Sec):

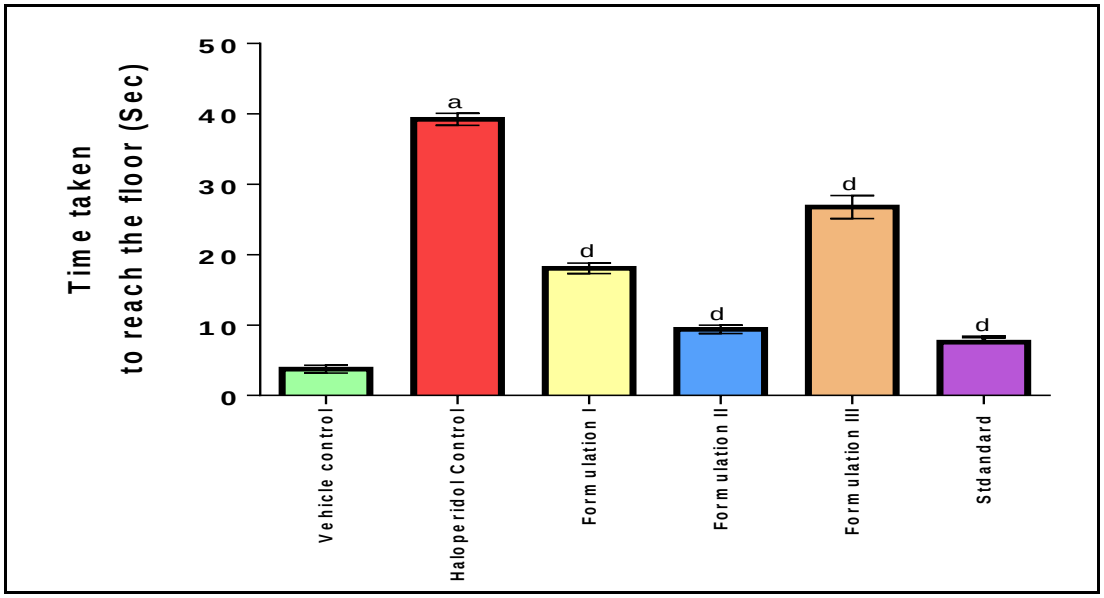

FIG. 9: TIME TAKEN TO REACH THE FLOOR

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control [a]p< 0.001. One way ANOVA followed by Dunnett's test when compared with haloperidol control [b]p<0.05, [c]p<0.01, [d]p< 0.001

TABLE 13: TIME TAKEN TO REACH THE FLOOR

Group	Drug Treatment	Time taken to reach the floor (Sec)
I	Vehicle control	3.754± 0.5568
II	Negative control: treated with Haloperidol (1ml/kg, i.p.)	39.23 ± 0.8560[a]
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg +Haldi 100mg/kg.)	18.07 ± 0.7356 [d]
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg +Tulsi 100mg/kg + Haldi 50mg/kg.)	9.384 ± 0.5859[d]
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg +Haldi 100mg/kg.)	26.76 ± 1.642[b]
VI	Standard (Levodopa + Carbidopa 110mg/kg)	7.587 ± 0.7361[d]

Administration of haloperidol (1ml/kg) increases both T-turn and T-total values, which reached about 8-9 times the control values. Standard significantly reduced the prolongation of T-turn(Table12) and T-total(Table 13) values by reserpine. When animals were pretreated with formulation before injection of haloperidol, bradykinesia was markedly reduced.

Effect of Formulations on Hanging Wire Test:

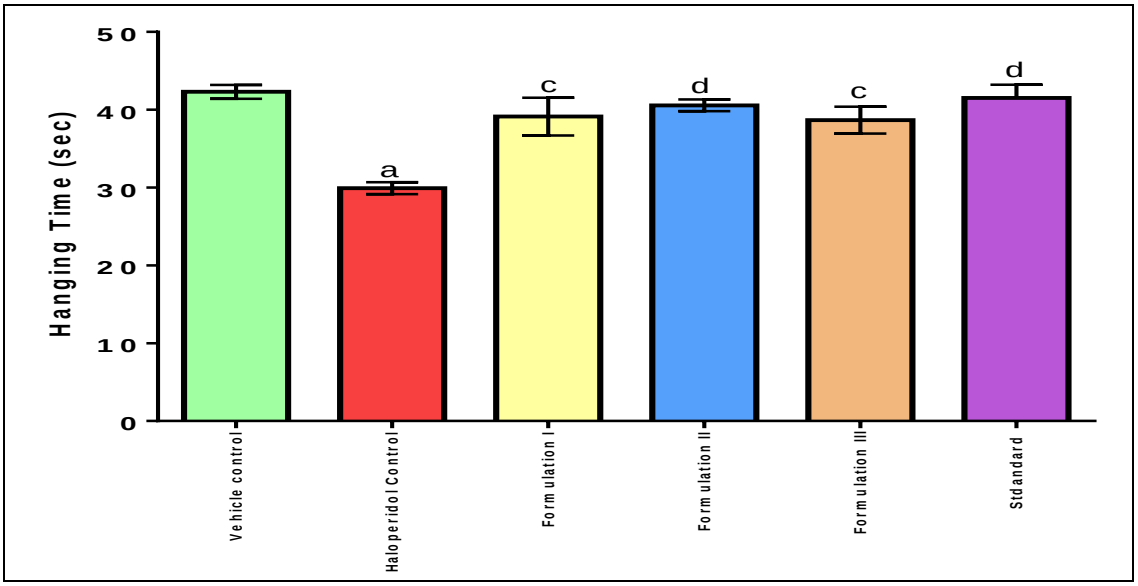

FIG. 10: EFFECT OF FORMULATIONS ON HANGING WIRE TEST

n=6 animals in each group. Values are expressed as mean ± SEM. Unpaired 't' test when compared with vehicle control [a]p< 0.001. One way ANOVA followed by Dunnett's test when compared with haloperidol control [b]p<0.05, [c]p<0.01, [d]p< 0.001

TABLE 14: EFFECT OF FORMULATIONS ON HANGING WIRE TEST

Group	Drug Treatment	Hanging Time (sec)
I	Vehicle control	42.30± 0.8810
II	Negative control: treated with Haloperidol (1ml/kg, i.p.)	29.92 ± 0.7670 [a]
III	Formulation I, 300mg/kg p.o. (Ashwagandha 100mg/kg + Tulsi 100mg/kg+Haldi 100mg/kg.)	39.12 ± 2.4230 [c]
IV	Formulation II, 300mg/kg p.o. (Ashwagandha 150mg/kg + Tulsi 100mg/kg + Haldi 50mg/kg.)	40.57 ± 0.7481 [d]
V	Formulation III, 300mg/kg p.o. (Ashwagandha 50mg/kg + Tulsi 150mg/kg + Haldi 100mg/kg.)	38.66 ± 1.7210 [c]
VI	Standard (Levodopa + Carbidopa 110 mg/kg)	41.52 ± 1.6960 [d]

Effect of formulations on haloperidol induced neuromuscular weakness was tested by wire hang test. The less latency to fall by releasing the wire soon, indicating the apathetic state in the induced animal. The latency in falling represent the improved neuromuscular strength in formulation II treated animals with the same effect as that of the standard drug. Formulation II significantly ($p<0.001$) increased the neuromuscular strength in haloperidol induced mice.

DICSUSSION& CONCLUSION: As no mortality, no adverse changes in behavior of animals as well as no abnormalities were detected in experimental mice at the dose rate of 5000mg/kg body weight, the hydroalcoholic extract formulation of Ashwagandha, Tulsi and Haldi was assigned to class 5 (LD_{50} > 5000mg/kg), were recommended by OECD.

Protective effect of formulation I, II and III against heloperidol or the vesicular monoamine transporter blocker, reserpine induced catalepsy and bradykinesia suggests that these herbal formulations influence not only dopamine receptor-mediated neurotransmission but also serotonergic receptor mediated neurotransmission.

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