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Synergistic effect of *Withania somnifera* Dunal and L-dopa in the inhiition of haloperidol-induced catalepsy in mice

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ABSTRACT

The possible synergism between WS and dopamine precursor L-dopa to inhibit haloperidol-induced catalepsy was investigated by using standard bar test in mice. The effect of WS (20–200 mg/kg, oral), L-dopa (20–200 mg/kg, oral) plus carbidopa and combination of subeffective doses of WS (20 or 50 mg/kg, oral) prior to L-dopa (20, 50 or 100 mg/kg, oral) plus carbidopa was assessed in haloperidol (1 mg/kg, i.p.) induced catalepsy. L-dopa and carbidopa combination was always administered in 10:1 ratio. WS (100 or 200 mg/kg, oral) and L-dopa (50, 100 or 200 mg/kg, oral) plus carbidopa treated groups showed a dose dependent reduction in cataleptic scores. Subeffective doses of WS (20 or 50 mg/kg, oral) prior to L-dopa (20, 50 or 100 mg/kg, oral) also potentiated the anticataleptic effect of L-dopa. These results indicate that subeffective doses of WS enhance the anticataleptic actions of L-dopa and the possibility of using WS as adjunctive therapy to reduce the doses and the adverse effects of dopamine precursor in Parkinson's disease.

KEYWORDS: *Withania somnifera*, Parkinsonism, Catalepsy, L-dopa, Carbidopa

INTRODUCTION

Parkinson's disease is characterized by a motor impairment caused by the degeneration of dopaminergic neurons located in the substantia nigra pars compacta and by the reduction of dopamine levels in the striatum. Replacement therapy with the dopamine precursor L-dihydroxy-phenylalanine (L-dopa) is successful in most parkinsonian patients. In modern practice, L-dopa is always administered with peripheral decarboxylase inhibitor carbidopa or benserazide which prevents peripheral decarboxylation of L-dopa and allows maximum amount of L-dopa to reach the CNS (1). This will not only reduce the dose but also the peripheral side effects of L-dopa *per se* treatment. A principal limitation of long-term use of L-dopa therapy is that, with time there is a development of motor fluctuations such as "wearing off" and "on-off" phenomena. Increasing the dose of medication may help but this leads to development of dyskinesias (2, 3). To avoid such problems, there has been an increasing thrust worldwide to opt for safer and effective herbal drugs mentioned in the traditional medical systems.

Withania somnifera Dunal (WS) (family, Solanaceae), known as ashwagandha in Ayurveda, the ancient Hindu system of medicine, has been in use for more than 2500 years. Historically, WS, or its major active principles, has been used as an antioxidant, adaptogen, anxiolytic, antidepressant, memory enhancer, antiinflammatory,

antiulcerogenic, antiparkinsonian and anticarcinogenic agents. The active principles of WS, consisting of sitoindosides VII–X and withaferin-A, have been shown to exhibit significant antistress and antioxidant effect in rat brain frontal cortex and striatum (4).

A decoction containing cow's milk powdered *Mucuna pruriens* seeds and WS has been reported to be effective in 18 clinically diagnosed parkinsonian patients (5). Moreover, haloperidol induced catalepsy has been blocked by WS and a polyherbal preparation, BR-16A (Mentat), which has WS as a one of its ingredient (6, 7). WS is also reported to be safe in long term use with no adverse effects (4). Therefore, the present study was undertaken to investigate the effects of WS as an adjunct to L-dopa against haloperidol-induced catalepsy in mice.

MATERIALS AND METHODS

Animals

Male, Swiss albino mice weighing 25 to 30 g were housed in group of 4 per cage under controlled light (12:12 light: dark cycle, light on at 0700 h) and temperature (25 ± 20C) environment and behavioral assessment was conducted during the light cycle. Food (Rat chow, Lipton, India) and water was provided ad libitum. All procedures were carried out under strict compliance with ethical principles and guidelines of the Institutional Animal Ethical Committee constituted as per the direction of the Committee for the Purpose of Control and Supervision of Experimental Animals; Madras (Reg. No. 870/ac/05/CPCSEA).

Drugs

Commercial ethanolic WS root extract (Dabur, New Delhi, India), haloperidol (RPG Life Sciences Ltd, Ankleshwar, India), L-dopa plus carbidopa in 10:1 ratio (Sun Pharmaceuticals, Mumbai, India) were obtained from respective sources. WS root extract, l-dopa plus carbidopa was suspended in 0.5% w/v carboxy methyl cellulose (CMC) in distilled water and administered via oral route. The stock solution contained 100 mg/ml of WS. Haloperidol was obtained in an injectable form and diluted with water for injection I.P. Haloperidol was injected via i.p. route.

Bar test

In this test, front paw of the mice was gently placed on a horizontal metal bar with 2 mm diameter and placed 5 cm above ground level. The length of time the mouse maintain this abnormal posture with atleast one paw was measured. The test was stopped when the paw of animal touch the ground or 180 sec pass. If the animals do not hold on to the bar after three attempts, it receive the score of 0 seconds (8).

Experimental design

All subjects were experimentally naïve at the beginning of each study. The behavioral observations were made during the light cycle between 0900 and 1600 h to minimize variations due to diurnal fluctuations. Overnight-fasted mice were used in order to increase the drug absorption of orally administered drugs. The degree of catalepsy was assessed at different time intervals (30, 60, 120, 180 or 240 min) after haloperidol injection by subjecting individual mouse to the bar test.

(a) Effect of haloperidol on catalepsy

Mice were randomly divided in different groups (n=6 in each) and vehicle (0.2 ml of water for injection, I.P., n=6) or haloperidol (0.3, 1, 5 or 10 mg/kg, n=6 per group) was injected via i.p. route. The degree of catalepsy was assessed at different time intervals (30, 60, 120, 180 or 240 min) after haloperidol injection by subjecting individual mouse to the bar test.

(b) Effect of WS on haloperidol-induced catalepsy

Vehicle or WS (20-200 mg/kg, n=6 per group) was administered through oral route, 60 minutes before haloperidol (1 mg/kg, i.p., n=6 per group) and 30 min thereafter individual mouse was subjected to the bar test at different time intervals (30, 60, 120, 180 or 240 min after haloperidol injection).

(c) Effect of L-dopa plus carbidopa on haloperidol-induced catalepsy

To assess the influence of L-dopa plus carbidopa on haloperidol-induced catalepsy, separate groups of mice administered with L-dopa at different doses 20, 50, 100 or 200 mg/kg, oral, n=6 per group. Haloperidol (1 mg/kg, i.p., n=6 per group) was administered 30 min after L-dopa plus carbidopa combination and 30 min thereafter individual mouse was subjected to the bar test for different time intervals (30, 60, 120, 180 or 240 min after haloperidol injection). L-dopa and carbidopa combination was always administered in 10:1 ratio.

(d) Effect of combined treatment of WS and L-dopa plus carbidopa on haloperidol-induced catalepsy

To assess the influence of WS in the effect of L-dopa plus carbidopa on haloperidol-induced catalepsy, separate groups of mice administered with subeffective doses of WS (20 or 50 mg/kg, n=6 per group) 30 min prior to L-dopa (20, 50 or 100 mg/kg, oral, n=6 per group). Haloperidol

(1 mg/kg, i.p., n=6 per group) was administered 30 min after L-dopa plus carbidopa combination and 30 min thereafter individual mouse was subjected to the bar test for different time intervals (or 30, 60, 120, 180 or 240 min after haloperidol injection).

TLC study of WS root extract

Thin-layer chromatography (TLC) was used to identify the steroidal lactones (withanolides) present in WS. The solvent system used was chloroform:methanol:water (64:50:10, v/v) and spots were finally identified with vanillin–phosphoric acid (9).

Statistical analysis

The data are presented as Mean ± SEM. Statistical significance was determined by one-way analysis of variance (ANOVA) followed by post hoc Dunnett's multiple comparison test. Differences were considered to be significant at $P<0.05$.

RESULTS

Effect of haloperidol on cataleptic behavior on bar test

In the present study, haloperidol (0.3, 1, 5 or 10 mg/kg, i.p.) produced significant and dose dependent increase in cataleptic score on bar test as compared to vehicle treated group during whole assessment time, except 30 and 60 min ($P>0.05$) after haloperidol (0.3 mg/kg, i.p.) injection (Table 1).

Effect of WS on haloperidol-induced catalepsy

WS (20 or 50 mg/kg) showed insignificant protection against haloperidol induced catalepsy during whole assessment time, as compared to respective control group in haloperidol-induced catatonia ($P>0.05$). On the other hand, WS (100 or 200 mg/kg) significantly and dose dependently decreased cataleptic score on the bar test as compared to respective control group during whole

Table 1: Effect of haloperidol on cataleptic behavior as tested on bar test in mice

Groups (mg/kg)	Cataleptic score at different time points (Sec)				
	30 min	60 min	120 min	180 min	240 min
Control	2.66 ± 1.358	4.33 ± 2.140	3.83 ± 2.257	2.83 ± 2.007	5.66 ± 2.906
Haloperidol (0.3)	12.16 ± 3.410	17.33 ± 4.356	31.83 ± 6.002*	43.83 ± 6.554*	55.83 ± 7.968*
Haloperidol (1)	62.83 ± 5.776*	93.83 ± 5.570*	139.83 ± 4.658*	171.50 ± 3.170*	177.17 ± 1.641*
Haloperidol (5)	114.67 ± 5.869*	154.67 ± 8.464*	Cataleptic*	Cataleptic*	Cataleptic*
Haloperidol (10)	138.67 ± 8.381*	168.83 ± 5.375*	Cataleptic*	Cataleptic*	Cataleptic*
One-way ANOVA					
F	120.18	185.39	557.76	641.81	463.22
df	4,29	4,29	4,29	4,29	4,29

All values are Mean ± SEM over 180 sec duration of 6 mice in each group;

**P<0.01 [Compared with respective control], Dunnett's multiple comparison test;*

Cataleptic:- Animal holds the bar during whole assessment time (180 Sec).

Table 2: Effect of WS and L-dopa plus carbidopa on haloperidol-induced catalepsy as tested on bar test in mice

Groups (mg/kg)	Cataleptic score at different time points after haloperidol administration (Sec)				
	30 min	60 min	120 min	180 min	240 min
Control	62.83 ± 5.776	93.83 ± 5.570	139.83 ± 4.658	171.50 ± 3.170	177.17 ± 1.641
WS (20)	59.66 ± 7.093	91.16 ± 3.016	136.33 ± 4.958	164.33 ± 4.208	175.33 ± 1.453
WS (50)	53.83 ± 4.045	89.00 ± 5.774	125.50 ± 6.864	155.83 ± 5.269	170.33 ± 2.906
WS (100)	45.50 ± 7.446	39.83 ± 7.231**	80.66 ± 3.783**	86.66 ± 6.130**	120.83 ± 8.220**
WS (200)	44.16 ± 4.729	28.33 ± 3.029**	64.16 ± 2.786**	68.66 ± 5.783**	90.66 ± 3.116**
L-dopa (20)	50.16 ± 3.655	84.16 ± 3.229	124.67 ± 5.283	156.33 ± 4.716	169.17 ± 3.692
L-dopa (50)	43.50 ± 3.170*	31.83 ± 3.250**	90.66 ± 5.116**	135.50 ± 8.401**	147.50 ± 4.689**
L-dopa (100)	19.66 ± 2.777**	Reversed+	Reversed+	98.66 ± 6.479**	137.67 ± 5.445**
L-dopa (200)	Reversed+	Reversed+	Reversed+	91.83 ± 5.263**	132.83 ± 3.487**
One-way ANOVA					
F	17.414	88.027	157.85	47.431	46.186
df	8,53	8,53	8,53	8,53	8,53

All values are Mean ± SEM over 180 sec duration of 6 mice in each group;

**P<0.05.*

***P<0.01 [Compared with respective control (1 mg/kg, i.p. of haloperidol)], Dunnett's multiple comparison test;*

+: Reversed, animal did not hold the bar in three attempts; In all experiments L-dopa and carbidopa was used as 10:1 ratio; WS - Withania somnifera.

Table 3: Effect of combined treatment of WS and L-dopa plus carbidopa on haloperidol-induced catalepsy as tested on bar test in mice

Groups (mg/kg)	Cataleptic score at different time points after haloperidol administration (Sec)				
	30 min	60 min	120 min	180 min	240 min
WS (20) + L-dopa (20)	32.33 ± 4.047**	38.33 ± 3.703**	64.83 ± 4.847**	131.67 ± 10.33*	158.33 ± 7.342
WS (50) + L-dopa (20)	25.50 ± 2.705**	29.16 ± 5.787**	48.33 ± 6.254**	110.83 ± 7.743**	137.67 ± 6.776**
WS (20) + L-dopa (50)	17.66 ± 2.459**	20.16 ± 3.995*	42.66 ± 4.208**	64.33 ± 5.998**	98.83 ± 8.677**
WS (50) + L-dopa (50)	11.83 ± 3.027**	13.50 ± 3.481**	31.16 ± 6.145**	55.16 ± 5.431**	85.50 ± 5.156**
WS (20) + L-dopa (100)	Reversed+	Reversed+	Reversed+	Reversed+	40.16 ± 9.257*
One-way ANOVA					
F	31.429	60.677	85.362	57.781	40.872
Df	7,47	7,47	7,47	7,47	7,47

All values are Mean ± SEM over 180 sec duration of 6 mice in each group;

**P<0.05.*

***P<0.01 [compared with respective L-dopa control (Table 2)], Dunnett's multiple comparison test;*

+: Reversed, animal did not hold the bar in three attempts; In all experiments L-dopa and carbidopa was used as 10:1 ratio; WS - Withania somnifera.

assessment time, except 30 min (P>0.05) after haloperidol injection (Table 2).

Effect of L-dopa plus carbidopa on haloperidol-induced catalepsy

L-dopa (20 mg/kg) plus carbidopa showed insignificant protection against haloperidol-induced catatonia during whole assessment time (P>0.05) while higher doses of L-dopa (50, 100 or 200 mg/kg) plus carbidopa significantly and dose dependently decreased cataleptic score on the bar test as compared to respective control group (Table 2).

Effect of combined treatment of WS and L-dopa plus carbidopa on haloperidol-induced catalepsy

Prior administration of subeffective doses of WS (20 or 50 mg/kg) potentiated the effect of subeffective dose of L-dopa (20 mg/kg) plus carbidopa by significantly decreasing cataleptic score on the bar test as compared to subeffective dose of L-dopa treated control group. The enhancement of anticataleptic effect was significant during whole assessment time except at dose WS (20 mg/kg) with L-dopa (20 mg/kg) at 240 min (Table 3). On the other hand, prior administration of subeffective doses of WS (20 or 50 mg/kg) also potentiated anticataleptic effect of L-dopa (50 or 100 mg/kg) plus carbidopa by significantly decreasing descent time on the bar test as compared to respective dose of L-dopa treated group during whole observation period (Table 3). WS (20 mg/kg) prior to L-dopa (100 mg/kg) plus carbidopa showed complete reversal of catatonia in the bar test upto 180 min after haloperidol injection and also showed significant decrease in descent time on bar test at 240 min after haloperidol injection (Table 3).

TLC study of WS root extract

TLC showed the presence of four blueish violet spots (R_f value: 0.8–0.9).

DISCUSSION

In the present study, WS and L-dopa showed synergistic effect in the inhibition of haloperidol-induced catalepsy in mice. Haloperidol is a well known neuroleptic for the treatment of schizophrenia and other affective disorders (10), primarily acting as a D_2 receptor antagonist in the mesolimbic-mesocortical pathway. Due to its non-selective action, it also produces blockade of post-synaptic D_2 receptors in the nigrostriatal pathway leading to the development of extrapyramidal side effects in humans (11) and catalepsy in animals (12). Catalepsy has been defined as "an inability to correct an imposed abnormal posture while maintaining the righting reflex". Haloperidol induced catalepsy is also associated with an increase in oxidative stress in the brain (13). In this study, haloperidol (0.3, 1, 5 or 10 mg/kg, i.p.) produced dose dependent increase in cataleptic state, this was significant as compared to vehicle treated group. For further studies, haloperidol (1 mg/kg, i.p.) was chosen to produce a moderate degree of catalepsy, so that attenuation or potentiation of the phenomenon could be detected.

The anticataleptic effect of WS has already been reported (6, 7). The anticataleptic effect of WS could be attributed to the direct scavenging of free radicals by polyphenols present in it and also by inhibition of lipid peroxidation in the central nervous system. In our study also, WS (100 or 200 mg/kg, oral) significantly and dose dependently reduced cataleptic score. The standard drug L-dopa (50, 100 or 200 mg/kg, oral) plus carbidopa showed significant protection against haloperidol-induced catatonia. Prior administration of subeffective dose of WS potentiated the effect of sub-effective dose of L-dopa plus carbidopa as indicated by decreased cataleptic score.

When compared with the standard drug L-dopa (100 or 200 mg/kg, oral), WS at the dose of 100 and 200 mg/kg

was found to be less effective but significant in reversing haloperidol-induced catalepsy. WS (20 mg/kg) prior to L-dopa (100 mg/kg) plus carbidopa showed complete reversal of catatonia in the bar test after haloperidol injection.

The anticataleptic effect of WS and potentiation of the effect to the different doses of L-dopa may be due to the antioxidant or neuroprotective action, in stress induced dopamine neuron degeneration (14). A polyherbal preparation, EuMil, which has WS as a one of its ingredient, significantly increased the stress-induced decrease in dopamine level in striatal, hypothalamal region of the rat brain (15). However, further investigations are needed to determine the active compound(s) and correlation between dopamine levels with the effect of WS.

These results indicate that subeffective doses of WS enhance the anticataleptic action of L-dopa and leave open the possibility of using WS as adjunctive therapy to reduce the doses and the adverse effects on long term use of dopamine precursor in Parkinson's disease.

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