Residue of *Mucuna Pruriens* Potentiates Haloperidol and Clonidine-Induced Catalepsy In Mice

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Summary

Residue of methanolic extract of *Mucuna pruriens* which was isolated from boric acid complex has cataleptic activity. Residue and synthetic L-dopa dose dependently potentiated clonidine and haloperidol-induced catalepsy. Pretreatment with methanolic extract of *Mucuna Pruriens* (MEMP) and Boric acid complex L-dopa significantly inhibited haloperidol induced catalepsy. Thus the residue and synthetic L-dopa has histaminic activity as evident by inhibition of mast cell stabilizing activity and potentiation of clonidine induced catalepsy.

Key words: Catalepsy, clonidine, haloperidol, *Mucuna pruriens*, L-dopa

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Introduction

Catalepsy is a condition in which the animal maintains an imposed posture. Catalepsy is a sign of extrapyramidal effect of drugs that inhibit dopaminergic transmission or increase histamine release in brain. Clonidine, a α_2-adrenoceptor agonist, induces dose dependent catalepsy in mice, which is inhibited by histamine H_1 receptor antagonists but not by H_2 receptor antagonist [1]. They also showed that pretreatment with L-histidine, a precursor of histamine potentiated clonidine-induced catalepsy in a dose dependent manner. It is known that clonidine releases histamine from mast cells [2]. Schwartz (1977) [3] identified histamine containing mast cells in brain. Clonidine-induced release of histamine from mast cells is inhibited by α_2-adrenoceptor blocker, yohimbine but not by α_1-adrenoceptor blocker, prazosin [4]. Neuroleptic agents also induce catalepsy by inhibiting dopamine D2 receptors in the substantia nigra [5, 6].

It was our objective to study the effect of *Mucuna pruriens* on clonidine and haloperidol-induced catalepsy. The histamine releasing activity of the Residue, synthetic L-dopa and MEMP were assessed using goat tracheal chain as described earlier by Nag Chaudhari and Lahiri, 1974 [7]and Kulshreshtha *et al*., 1983 [8]. Residue, MEMP, synthetic L-dopa were then tested for their effect on clonidine and haloperidol-induced catalepsy using bar test [9] and clonidine induced mast cell degranulation [10].

Materials and methods

Animals

Male albino mice (Swiss strain) weighing 22-25 g were housed under standard laboratory conditions, in group of five each. The animals had free access to food and water. The ethical committee of the institute approved the protocol of the study. Goat trachea was obtained from slaughterhouse.

Drugs

Clonidine (Unichem,India),histamine (Sigma,USA),haloperidol (Searle,India) were used for the study. Seeds of *Mucuna pruriens* were purchased from commercial source and was identified and authenticated. All observations were made between 10:00 hr and 16:00 hr. Residue was suspended in 0.1% carboxy methyl cellulose solution (vehicle) and synthetic L-dopa, MEMP, haloperidol and clonidine were dissolved in distilled water.

Preparation of the extract and the residue

Seeds of M.Pruriens (1 kg) were purchased locally and authenticated by Dr.S.C.Pal, MVP College of Pharmacy, Nashik. Powdered seeds were defatted with petroleum ether (60-80° C) using Soxhlet's extractor. The marc was dried and successfully extracted with methanol. The extract was concentrated under vaccum at 60° C to yield MEMP (5.6% w/w). The amount of L-DOPA in MEMP was estimated using CAMAG's HPTLC as described earlier by Sathiyanarayanan and Arulmozhi (2007) [11] and was found to be 15.14% W/W

Isolation of residue and L-dopa by complexing with boric acid from MEMP

L-dopa forms water soluble complex with boric acid or borates at a pH 7.0-7.5 .The insoluble impurities (residue) are removed by centrifugation and dried. The aqueous solution contains L-dopa as boric acid complex.

Assessment of cataleptic activity

Effect on haloperidol-induced catalepsy

The bar test was used. Haloperidol (1 mg/kg, i.p.) was injected to mice pretreated with MEMP (50,100 and 200 mg/kg, i.p.), Boric acid complex L-dopa (50,100 and 200 mg/kg ,i.p.) and Residue (50,100 and 200 mg/kg, i.p) respectively. The duration of catalepsy was measured at 30, 60, 90,120,150,180,210,240,270 and 300 min.

Effect on clonidine induced catalepsy

Bar test was used to study the effect of residue, MEMP and synthetic L-dopa on clonidine-induced catalepsy. Clonidine (1 mg/kg, i.p.) was injected to mice pretreated with residue (100 mg/kg, i.p.), MEMP (100 mg/kg, i.p.) and synthetic L-dopa (100 mg/kg, i.p.). The forepaws of mice were placed on a horizontal bar (1 cm in diameter, 3 cm above the table) and the time required to remove the paws from bar was noted for each animal. The duration of catalepsy was measured at 30, 60, 90 and 120 min.

Clonidine-induced mast cell degranulation

Four ml of normal saline was injected into the peritoneal cavity of mice. After gentle massage, the peritoneal fluid was collected and transferred into the test tube containing 3-4 ml of RPMI-1640 buffer medium (p H 7.2-7.4). Mast cells were then washed by centrifugation at low speed (400-500 rpm).Supernatant was discarded and the pellets of mast cells were suspended into buffer medium. The mast cells were treated with 1-2 drops clonidine (80 mcg/ml) and incubated at 37^{O}C in a water bath for 10 minutes. They were stained with toludine blue and observed under microscope. One hundred mast cells were observed and the mast cells showing degranulation was counted. The percentage protection against degranulation was counted. The animals were treated with vehicle and residue, MEMP and synthetic L-dopa (100 mg/kg, i.p.) for 4 days. The last dose was administered 30 minutes before collection of mast cells from the peritoneal cavity.

Statistical analysis

The data were analysed by one-way ANOVA, followed by Dunnett's multiple comparison test. $p < 0.05$ was considered significant.

Results

Haloperidol-induced catalepsy

Haloperidol (1 mg/kg, i.p.) produced catalepsy in mice which persisted for 5 hours. Maximum catalepsy was recorded 120 min after haloperidol treatment. Pretreatment with MEMP and boric acid complex L-dopa dose dependently decreased the catalepsy. A significant reduction in cataleptic score was observed with MEMP (200 mg/kg) and boric acid complex L-dopa (200 mg/kg) as compared with control group. Pretreatment with residue (50,100 and 200 mg/kg) dose dependently potentiated catalepsy.

Clonidine-induced catalepsy in mice

Clonidine (1 mg/kg, i.p.) induced catalepsy in mice which persisted for 2 hours. Maximum catalepsy was recorded 60 min after clonidine. Pretreatment with MEMP inhibited clonidine-induced catalepsy and pretreatment with residue (100 mg/kg, i.p.) and synthetic L-dopa (100 mg/kg, i.p.) potentiated clonidine induced catalepsy.

Clonidine induced mast cell degranulation

The % protection of mast cell degranulation observed with clonidine was 40%, whereas pretreatment with residue, MEMP and synthetic L-dopa significantly reduced the % protection of mast cell degranulation as compared with clonidine group.

Effect of MEMP and boric acid complex L-dopa on Haloperidol-induced catalepsy in mice

Treatment (Dose in mg/kg, i.p.)	Duration of catalepsy (MEAN ± SEM) in seconds										
	0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min
Vehicle + Haloperidol(1)	7.4 ± 0.4	171.6 ± 18.15	182.0 ± 30.35	211.4 ± 30.3	254.0 ± 27.67	218.8 ± 33.17	217.6 ± 34.72	224.8 ± 31.05	244.0 ± 31.82	247.8 ± 27.09	225.0 ± 25.0
MEMP (50) + Haloperidol(1)	4.6 ± 0.75	174.6 ± 38.99	185.2 ± 30.86	170 ± 36.52	201.6 ± 41.26	254.6 ± 27.88	250.2 ± 23.04	263.8 ± 10.2	215.2 ± 29.1	237 ± 26.2	145.60 ± 18.64
MEMP (100) + Haloperidol(1)	0.0 ± 0.0	109.5 ± 2.72	141.5 ± 13.03	188.8 ± 19.22	145.2 ± 11.3*	216.0 ± 29.52	284.2 ± 12.64	300.0 ± 0.0	242.5 ± 12.15	300.0 ± 0.0	223.2 ± 13.4
MEMP (200) + Haloperidol(1)	0.0 ± 0.0*	55.0 ± 3.8*	114 ± 4.96	82.25 ± 9.8*	99.0 ± 16.34*	118.8 ± 19.7	82.5 ± 9.24*	135.3 ± 2.96*	148.0 ± 3.51*	119.3 ± 13.37*	183.3 ± 1.453
Boric acid complex L-dopa(50) + Haloperidol(1)	3.8 ± 0.58	102.6 ± 32.38	127.2 ± 38.1	158.2 ± 47	171.6 ± 48.37	159.6 ± 52.02	153.2 ± 46.7	164 ± 40.97	136 ± 33	119.8 ± 29.58	106.4 ± 19.24
Boric acid complex L-dopa(100) + Haloperidol(1)	0.0 ± 0.0*	145.0 ± 17.64	198.0 ± 16.25	193.2 ± 21.64	171.2 ± 9.3*	173.8 ± 12.79	285.0 ± 9.57	291.5 ± 8.5	300.0 ± 0.0	238.8 ± 3.59	119.2 ± 15.6*
Boric acid complex L-dopa(200) + Haloperidol(1)	0.0 ± 0.0*	67.75 ± 8.72*	73.5 ± 14.5*	111.0 ± 6.25*	103.0 ± 8.95*	115.5 ± 3.75	106.8 ± 15.04*	132.7 ± 5.66*	49.25 ± 2.75*	75.5 ± 9.88*	51.25 ± 4.60*
F (6,28) F= P=	60.24 0.000	4.69 0.002	3.56 0.010	2.82 0.028	3.89 0.006	3.24 0.015	10.88 0.000	12.76 0.000	16.06 0.000	19.44 0.000	16.12 0.000

Effect of Residue on haloperidol-induced catalepsy in mice

Treatment (Dose in mg/kg, i.p.)	Duration of catalepsy (MEAN ± SEM) in seconds										
	0 min	30 min	60 min	90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min
Vehicle + Haloperidol(1)	7.4 ± 0.4	171.6± 18.15	182.0± 30.35	211.4± 30.3	254.0± 27.67	218.8± 33.17	217.6± 34.72	224.8± 31.05	244.0± 31.82	247.8± 27.09	225.0± 25.0
Residue(50) + Haloperidol(1)	13.0± 1.95	221.4± 40.22	199.6± 45.56	217.8± 52.35	106.6± 15.51*	193.8± 47.71	190.0± 36.09	178.6± 45.32	203.4± 47.13	212± 55.35	221.6± 49.48
Residue(100) + Haloperidol(1)	7.75± 0.62	225.8± 44.21	231.0± 27.17	142.8± 14.92	203.5± 33.39	254.0± 45.75	253.0± 28.99	196.2± 30.06	251.0± 29.68	277.5± 22.5	263.0± 21.42
Residue(200) + Haloperidol(1)	13.0± 0.58	230.8± 30.23	243.5± 23.57	166.5± 16.95	221.8± 21.19	263.8± 28.71	300.0± 0.0	209.5± 27.97	228.5± 24.84	294.2± 5.43	246.0± 22.76
F(3,16) F=	8.40	0.63	0.74	1.25	6.27	0.66	2.70	0.33	0.38	1.20	0.37
P=	0.001	0.608	0.542	0.326	0.005	0.587	0.081	0.805	0.771	0.340	0.776

N=5, *p <0.05, as compared to control (ANOVA followed by Dunnett's test

MEMP- Methanolic extract *Mucuna pruriens*

Effect of MEMP, Residue, and synthetic L-dopa on clonidine-induced catalepsy in mice

Treatment (Dose in mg/kg, i.p.)	Duration of catalepsy (MEAN ± SEM) in seconds				
	0 min	**30min**	**60min**	**90min**	**120min**
Vehicle + Clonidine (1)	0.0 ± 0.0	80.6 ± 13.98	147.2 ± 10.5	102.0 ± 30.85	56.8 ± 8.71
MEMP (100) + Clonidine (1)	2.0 ± 0.44*	86.0 ± 7.45	90.25 ± 18.25	72.75 ± 4.64	76.5 ± 20.7
Residue (100) + Clonidine (1)	6.6 ± 0.67*	252.0 ± 29.82*	177.0 ± 22.27	213.8 ± 35.08*	122.0 ± 24.86*
L-dopa (100) + Clonidine (1)	4.0 ± 0.32*	172.6 ± 27.4*	230.6 ± 28.46*	139.0 ± 24.5	152.0 ± 5.23*
F (3,16) P =	42.71 0.0001	13.97 0.0001	7.85 0.002	5.30 0.010	6.48 0.004

N=5,*p <0.05, as compared to control (ANOVA followed by Dunnett's test
MEMP- Methanolic extract of *Mucuna pruriens*

Effect of Residue, MEMP and synthetic L-dopa on Clonidine-induced mast cell degrannulation in mice

Treatment (Dose in mg/kg, i.p.)	% Protection of mast cell degranulation (MEAN ± SEM)
Vehicle + Clonidine	40.0 ± 4.95
Residue (100) + Clonidine	13.4 ± 1.29*
MEMP (100) + Clonidine	26.2 ± 2.75*
L-dopa(100) + Clonidine	19.0 ± 1.77*

N=5, *p <0.05, as compared to control (ANOVA followed by Dunnett's test
MEMP- Methanolic extract *Mucuna prurient*

Discussion

Haloperidol is a well known neuroleptic, primarily acting as a D2 receptor antagonist in the mesolimbic-mesocortical pathway. Due to its non-selective action, it also produces
blockade of post-synaptic D2 receptors in the nigrostriatal pathway leading to the development of extra pyramidal side effects in humans [12] and is observed as catalepsy in animals [5]. They also showed that different stages of catalepsy appear to be directly correlated with the brain histamine content. Jadhav *et al*., (1983) [1] noticed that clonidine, unlike haloperidol, failed to antagonize apomorphine-induce cage climbing behaviour occurring as a result of direct stimulation of post-synaptic straital dopamine receptors. It is reported that aqueous extract of *Mucuna pruriens* seeds stimulate histaminergic and muscarinic receptors [13]. The potentiation of catalepsy could be because of histaminergic and muscarinic stimulation.
Uvnas (1969) [10] studied the mast cell degranulation and its correlation with the release of histamine after administration of compound 48/80. The mast cell degranulating agents, clonidine and compound 48/80 act through the dynamic expulsion of granules without causing any damage to the cell wall [12, 14].
The observation of this study indicated that the residue and synthetic L-dopa potentiates mast cell degranulation or has histamine like action. The residue, MEMP and synthetic L-dopa potentiated clonidine-induced catalepsy. MEMP and boric acid complex L-dopa and inhibited haloperidol-induced catalepsy, whereas the residue potentiated haloperidol-induced catalepsy.
From the present study, we conclude that the cataleptic effect of clonidine in the mice is mediated by histamine release from mast cells, and the clonidine-induced catalepsy was potentiated by residue and synthetic L-dopa. The effect of residue and synthetic L-dopa on clonidine-induced catalepsy is probably due to mast cell degranulation property whereas MEMP and boric acid complex L-dopa facilitates dopaminergic transmission and therefore inhibited haloperidol induced catalepsy.

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