

**ANTI-CATALEPTIC ACTIVITY OF THE VARIOUS EXTRACTS OF
MOMORDICA DIOICA SEED ON CLONIDINE AND HALOPERIDOL**

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Summary

The present study sought to determine, in more detail, the effects of various extracts of *Momordica dioica* seed for anti cataleptic activity as the plant is having antiasthmatic activity. The cataleptic response of mice to various extracts of *Momordica dioica* seed measured using a bar test, was enhanced by subcutaneous pretreatment with Clonidine (1 mg/kg). Methanol extract and Aqueous extract (50 mg/kg, i.p.) of the plant more significantly inhibited clonidine-induced catalepsy as compare to petroleum ether and ethyl acetate but not any one extract among these inhibited haloperidol-induced catalepsy. Thus the antihistaminic activity of *Momordica dioica* seed may be due to polar constituents.

Key words: *Momordica dioica*, Cataleptic activity, Antiasthmatic, Clonidine, Haloperidol.

Introduction

Momordica dioica climbing creeper plant fruits and leaves are traditionally used as medicinal agent of asthma, leprosy, bronchitis, fever, tridosha¹. Catalepsy is a condition in which the animal maintains imposed posture for long time before regaining normal posture. Catalepsy is a sign of extrapyramidal effect of drugs that inhibit dopaminergic transmission or increase histamine release in brain. Clonidine, α_2 -adrenoceptor agonist, induces dose dependent catalepsy in mice, which is inhibited by histamine H₁ receptor antagonists but not by H₂ receptor antagonist². They also showed that pretreatment with L-histidine, a precursor of histamine potentiated clonidine-induced catalepsy in dose dependent manner. Intracerebroventricular injection of histamine in conscious mice induced catalepsy, which was inhibited by H₁ receptor antagonist but not by H₂ receptor antagonist³. It is known that clonidine releases histamine from mast cells⁴. Histamine containing mast cells are present in the brain⁵. Clonidine-induced release of histamine from mast cells is inhibited by α_2 -adrenoceptor blocker, prazosin⁶. Neuroleptic agent also induced catalepsy, but by different mechanism. Neuroleptics inhibit dopamine D₂ receptors in the substantia nigra⁷. Out of the various extracts of *Momordica dioica* pulp of clonidine and haloperidol-induced catalepsy in mice potentially Aqueous extract of *Momordica dioica* pulp showed most potent inhibition of clonidine-induced catalepsy, so it can be concluded that polar constituents may be responsible for its antihistaminic activity⁸. Therefore it was our objective to study the effect of various extracts *Momordica dioica* seed on clonidine-induced catalepsy, as it is used traditionally in cough, asthma and inflammation⁹. Since catalepsy is a common extrapyramidal side effect of neuroleptic agents and the effect of the plant on haloperidol-induced catalepsy is not known, we also studied their effect on haloperidol-induced catalepsy in mice.

Material and Methods

Plant Material

Seed of *Momordica dioica* were collected from Therla, Ta. Patoda, Beed district of Maharashtra in September 2009 and authenticated by P.G. Diwakar, Botanical Survey of India, Pune, where a sample specimen (voucher number: RAMAM1) No. BSI/WRC/Tech/2009/593 has been deposited.

Extraction

Dried and coarsely powdered seed of *Momordica dioica* was subjected to successive solvent extraction in Soxhlet extractor using petroleum ether, ethyl acetate, and methanol as solvent and the marc left was refluxed with water. All the extracts were vacuum dried to produce Petroleum ether (12.67%), Ethyl acetate (5.2%), Methanol (19.2%) and Aqueous (4.7%) extracts respectively.

Animals

Male albino mice (Swiss strain) weighing 25-28 g were housed under standard laboratory conditions, five in each groups. The animal had free access to food and water. The ethical committee of the institute approved the protocol of the study.

Drugs and Chemicals

The following drugs and chemicals were used. Drugs: Clonidine (Neon Lab. Ltd., India), Haloperidol (Sun pharma, India), Pheniramine maleate (Unimark Remedies Ltd., India) purchased from commercial source. Chemicals: petroleum ether (60-80°C) (RFCL Ltd, India), ethyl acetate (RFCL Ltd, India), methanol (MERCK Ltd, India), and DMSO (Research Lab Industries, India).

Assessment of Anti-cataleptic Activity¹⁰⁻¹⁴

Bar test was used to study the effect of various extracts on clonidine-induced catalepsy. Clonidine (1 mg/kg, s.c.) was injected to mice (n = 5) pretreated 30 min before with vehicle (5 ml/kg, i.p.), petroleum ether, ethyl acetate, methanol, and aqueous extracts of *Momordica dioica* seed (50 mg/kg, i.p., each) or standard drug pheniramine maleate (10 mg/kg, i.p.). The dosages were selected based on preliminary studies (data not shown). The forepaws of mice were placed on 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 and the durations of catalepsy was measured at 30, 60, 90, 120 and 180 min. The standard bar test was used to determine the intensity of catalepsy. Both forelegs of a mouse were placed on a horizontal bar. The latency from paw placement until the rest complete removal of one paw from the support was measured (maximal test duration 180 s) and termed here as descent latency. If the mice did not assume the position on the bar after three attempts, it received a descent latency of 0 s.

Effect on Haloperidol-Induced Catalepsy¹⁰⁻¹³

The same Bar test was used using haloperidol. Haloperidol (1 mg/kg, i.p.) was injected to mice (n = 5) pretreated 30 min before with vehicle (5 ml/kg, i.p.), petroleum ether, ethyl acetate, methanol or aqueous extracts of *Momordica dioica* seed (50 mg/kg, i.p., each). The durations of catalepsy was measured at 30, 60, 90, 120 and 180 min.

Statistical Analysis

The data is presented as mean \pm SEM. The data was analyzed by one-way ANOVA followed by Dunnet's test. $P < 0.01$ and $P < 0.05$ was considered significant.

Results

Clonidine Induced Catalepsy

All the extracts petroleum ether, ethyl acetate, methanol and aqueous ($P < 0.05$) extracts showed significant inhibition in catalepsy (Table 1). But among them methanol and aqueous extracts shows more significant inhibition in catalepsy. Results were comparable with control drug clonidine.

Haloperidol-Induced Catalepsy

None of all the extracts petroleum ether, ethyl acetate, methanol and aqueous extracts inhibited haloperidol-induced catalepsy (Table 2). Results were comparable with control drug haloperidol.

Table 1: Effect of various extracts of *Momordica dioica* seed on clonidine-induced catalepsy in mice

Sr. No	Group/ Extract	Duration of catalepsy (sec) Mean \pm SEM at					
		0 min	30 min	60 min	90 min	120 min	180 min
1	Control	18.6 \pm 0.871	226.2 \pm 9.551	208.75 \pm 26.383	21.5 \pm 0.645	17.6 \pm 0.9798	15.25 \pm 1.315
2	Standard	16 \pm 0.316	25.5 \pm 1.756	43.5 \pm 1.436	22.75 \pm 0.853	18.2 \pm 0.200	15.25 \pm 1.109
3	Petroleum ether	37.8 \pm 4.652	60.5 \pm 9.179**	29 \pm 12.46**	22.5 \pm 8.761	20.5 \pm 3.069	14.25 \pm 8.587
4	Ethyl acetate	31.4 \pm 8.158	45 \pm 9.247**	55 \pm 13.37**	14 \pm 3.764	16.25 \pm 1.797	10.5 \pm 3.926
5	Methanol	97.4 \pm 8.18**	37 \pm 6.519**	25 \pm 3.189**	16.25 \pm 3.010	14.75 \pm 0.4787	10 \pm 2.041
6	Aqueous	63 \pm 9.13**	34.25 \pm 2.136**	22.25 \pm 2.496**	17.75 \pm 2.720	14.2 \pm 1.934	13.5 \pm 1.848

Data are expressed as Mean \pm S.E.M, n = 5 in each group, Statistical analysis done by one way ANOVA followed by Dunnett's test. **p<0.01, compared to control group.

Table 2: Effect of various extracts of *Momordica dioica* seed on haloperidol-induced catalepsy in mice

Sr. No	Group/ Extract	Duration of catalepsy (sec) Mean \pm SEM at					
		0 min	30 min	60 min	90 min	120 min	180 min
1	Control	31.75 \pm 7.157	41.5 \pm 2.398	66 \pm 12.213	48.5 \pm 8.874	49 \pm 4.546	39.75 \pm 8.350
2	Standard	21.25 \pm 7.284	51.5 \pm 5.315	57.75 \pm 9.911	136 \pm 6.976	72.25 \pm 4.479	68.75 \pm 8.901
3	Petroleum ether	125.8 \pm 3.683	91.25 \pm 1.843	81.75 \pm 9.759	81.5 \pm 3.775	78.75 \pm 10.688	63.25 \pm 7.250
4	Ethyl acetate	120.8 \pm 3.902	115 \pm 2.887	131 \pm 0.4082	117.75 \pm 6.088	61 \pm 3.697	53.25 \pm 3.945
5	Methanol	20 \pm 6.964	25.75 \pm 6.860	112 \pm 12.336	53 \pm 10.677	29.75 \pm 8.107	27.75 \pm 9.647
6	Aqueous	124 \pm 8.327	21.67 \pm 4.177	36.67 \pm 5.207	69 \pm 2.646	59.67 \pm 2.728	31.67 \pm 2.028

Data are expressed as Mean \pm S.E.M, n = 5 in each group, Statistical analysis done by one way ANOVA followed by Dunnett's test, compared to control group.

Discussion

Several drugs are known to induce catalepsy in animals. The neuroleptic agents induce catalepsy by inhibiting dopamine D₂ receptors in the substantia nigra⁷. The relative role of acetylcholine and histamine in perphenazine-induced catalepsy and suggested that anticholinergic activity of antidepressant might be due to an increase in dopamine content in brain or their ability to inhibit release of acetylcholine¹⁵. They also showed that different stages of catalepsy to be directly correlated with brain histamine content. The mast cell degranulation and its correlation with the release of histamine after administration of mast cell degranulating agent (Compound 48/80)¹⁶. Clonidine releases histamine from mast cells in a similar

manner to a selective liberator⁴. Haloperidol, a typical neuroleptic produces catalepsy in rodents and extrapyramidal side effects in human¹⁷.

Haloperidol-induced catalepsy is one of the animal models for testing the extrapyramidal side effects of antipsychotic drugs. Haloperidol, (a non-selective D₂ dopamine antagonist) induced catalepsy is primarily due to blockade of dopamine receptors in the striatum. The agents increasing dopamine transmission inhibits neuroleptic-induced catalepsy. The striatum and nucleus accumbens have been implicated as the major brain structures involved in antipsychotic induced catalepsy, which appears due to the blockade of dopamine neurotransmission¹⁸. Typical neuroleptic-induced catalepsy has been linked to a blockade of postsynaptic striatal dopamine D₁ and D₂ receptors^{7, 19}. Despite this evidence, dysfunction of several other neurotransmitters such as acetylcholine²⁰, GABA²¹ and serotonin²², have also been implicated. In addition to dysfunction of various neurotransmitters in catalepsy, many clinical and preclinical studies have suggested the involvement of reactive oxygen species in haloperidol induced toxicity^{23,24}.

The observation of this study indicated that the plant *Momordica dioica* seed having antihistaminic activity inhibited clonidine-induced catalepsy and not inhibited haloperidol-induced catalepsy. From the present study we can conclude that the cataleptic effect of clonidine in the mouse is mediated by histamine release from mast cells and the clonidine-induced catalepsy was inhibited by aqueous and ethyl acetate extract of *Momordica dioica* seed. The effect of these extracts on clonidine-induced catalepsy is probably due to their mast cell stabilizing property and the plants do not have activity on dopaminergic transmission. Aqueous extract of *Momordica dioica* seed showed most potent inhibition of clonidine-induced catalepsy so it can be concluded that polar constituents may be responsible for its antihistaminic activity.

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