

**EFFECT OF *AGERATUM CONYZOIDES* LINN ON CLONIDINE AND HALOPERIDOL INDUCED CATALEPSY IN MICE**

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**Summary**

The leaves of *Ageratum conyzoides* are used in traditional medicine for the treatment asthma. Thus the hydroalcoholic extract of leaves *Ageratum conyzoides* Linn was investigated for its anti-cataleptic property in albino mice to substantiate folkloric claim. Catalepsy was induced by clonidine (1 mg/kg, s.c.) and haloperidol (1 mg/kg, i.p) in clonidine and haloperidol-induced catalepsy in mice respectively. The phytochemical analysis revealed that tannins and flavonoids are the major components of the extract. At the doses of 250, 500 & 1000 mg/kg per oral, hydroalcoholic significantly ( $P < 0.01$ ) inhibited clonidine-induced catalepsy but not inhibited haloperidol-induced catalepsy. These results substantiate the efficacy of the extract in the treatment of asthma and the anticataleptic activity exhibited by hydroalcoholic extract of *Ageratum conyzoides* Linn leaves can be attributed to the antihistaminic activity; however *Ageratum conyzoides* Linn does not possess antidopaminergic activity.

**Key words:** *Ageratum conyzoides*, asthma, anti-cataleptic property, Clonidine, Haloperidol.

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### Introduction

*Ageratum conyzoides* L. is an erect, herbaceous annual plant from the family Asteraceae (Compositae), native to tropical America, but with a distribution range in tropical and subtropical areas around the world. *Ageratum conyzoides* Linn known commonly as “mentrasto”, has been used in Brazilian folk medicine to treat various ailments (metrorrhagia, fevers, dermatitis, inflammation, rheumatism, diarrhea and diuretics)<sup>1</sup>. In Central Africa the plant is used to treat particularly wounds caused by burns, while in Kenya East Africa the plant is used in traditional medicine for its antiasthmatic, antispasmodic and haemostatic effects<sup>2</sup>. The plant is reported to have many biological properties it includes Anti-inflammatory, analgesic & antipyretic activity<sup>3</sup>; Anti-inflammatory & antioxidant Activity<sup>4</sup>; Bronchodilating & uterine relaxant activity<sup>5</sup>; Gastroprotective activity<sup>6</sup>; Antibacterial activity & wound healing property<sup>7</sup>.

Catalepsy is a condition in which the animal maintains imposed posture for long time before regaining normal posture. Catalepsy is a sign of extrapyramidal side effects of drugs that inhibit dopaminergic transmission or increase histamine release in brain. Clonidine, a  $\alpha_2$  adrenoreceptor agonist, induces dose dependent catalepsy in mice, which is inhibited by histamine H1 receptor antagonists but not by H2 receptor antagonist<sup>8</sup>. Clonidine causes mast cell degranulation in brain, causing histamine release in higher amount. This will ultimately leads to cataleptic condition. Drugs having antihistaminic property will reduce the cataleptic duration<sup>9</sup>.

Neuroleptic agents also induce catalepsy, but different mechanism. Neuroleptics inhibit dopamine D<sub>2</sub> receptors in the substantia nigra<sup>10, 11</sup>. Dopamine receptors in the striatum are involved in this neuroleptic induced catalepsy<sup>12</sup>. Dopamine receptors have been shown to be equally distributed postsynaptically on striatal neurons and presynaptically on corticostriatal terminals<sup>13</sup>. There is considerable evidence that blockade of dopamine transmission produces catalepsy<sup>10, 14</sup> in rats and extrapyramidal side effects in humans<sup>15</sup>. Since catalepsy is a common extra pyramidal side effect of neuroleptic agents and the effect of test drugs on haloperidol induced catalepsy was also studied in this study.

Thus hydroalcoholic extract (HAE) of *Ageratum conyzoides* was investigated for its anticataleptic effects on clonidine and haloperidol induced catalepsy.

## **Materials and Methods**

### **Plant material**

Aerial parts of *Ageratum conyzoides* Linn were collected in months of September and October from the Empress botanical garden of Pune, India. The plant was authenticated by Botanical survey of India, Pune (Voucher number: BB680414).

### **Preparation of extract**

Air-dried and powdered leaves of *A. conyzoides* (100 g) were extracted with 1500 ml of Ethanol: water (70:30 v/v) at room temperature for 48h using Soxhlet apparatus. After extraction, the dark green solution was concentrated to dryness and kept in a freezer. A fresh dilution of dried extract in vehicle (1% Tween 80) was prepared on the day of the experiments, and the employed doses were expressed relative to dried extract.

### **Drugs and chemicals**

Clonidine – Unichem, India, Haloperidol – Searle, India, Chlorpheniramine maleate - Research Lab Fine Chem. Industries, India, Ethanol AR grade.

### **Animals**

Swiss albino mice (20–25 g) of either sex were kept under standard environmental conditions (i.e.12:12 hour light and dark sequence; at an ambient temperature of 25±2.c; 35-60% humidity). They were housed in cages and fed with standard pellet diet and water ad libitum. For experimentation, the animals were fasted overnight and 5–10 animals were included in each group. The principles of Laboratory Animal Care (NIH, 1985) were followed and instructions given by our institutional animal ethical committee were maintained throughout the experiment.

### **Effect on clonidine induced catalepsy in mice<sup>16,17</sup>**

Bar test was used to study effect of extracts on clonidine induced catalepsy, to determine indirect antihistaminic activity. Mice were divided in five groups, six animals each. Clonidine (1 mg/kg, s.c) was injected to mice pretreated with vehicle (10 ml/kg, i.p.), Chlorpheniramine maleate (10 mg/kg, i.p), *Ageratum conyzoides* Linn (250mg/kg, 500mg/kg and 1000mg/kg, p.o each) respectively. The forepaws of mice were placed on a horizontal bar (1 cm in diameter, 3 cm above the table). The time required to remove the paws from bar was noted for each animal. Duration of catalepsy was measured at 15, 30, 60, 90, 120, 150 and 180 min interval.

### **Effect on haloperidol induced catalepsy in mice<sup>16,17</sup>**

The Bar test was used to study the effect of test drugs on the haloperidol induced catalepsy in mice. Mice were divided into five groups, six animals each.

Animals belonging to group I served as control and were administered the vehicle (10 ml/kg, i.p.). The animal belonging to Group II received standard drugs Chlorpheniramine maleate (10 mg/kg, i.p.). Animals belonging to groups III, IV and V received hydroalcoholic extract of *Ageratum conyzoides* Linn in doses 250, 500 and 1000 mg/kg, p.o respectively.

All the groups received Haloperidol (1 mg/kg, i.p) 1 hr after the drug administration and the duration of catalepsy was measured at 15, 30, 60, 90, 120, 150 and 180 min.

### **Preliminary phytochemical screening**

Analysis of the hydroalcoholic extract was carried out for various constituents like alkaloids, saponins, flavonoides, tannins, terpenoides/steroids, quinines, polyphenols, anthocyan, amino acids, glucides.etc<sup>18</sup>.

### **Statistical analysis**

All observations were presented as mean  $\pm$  SEM. The data was analyzed by one-way ANOVA followed by Dunnett's test.  $P < 0.05$  and  $0.01$  was considered as significant. Prism graph Pad 5 was used for statistical analysis.

## **Results**

### **Clonidine induced catalepsy in mice**

Clonidine (1 mg/kg, s.c) produced catalepsy in mice, which remained for 2 hr. As shown in table 1, the vehicle treated group showed maximum duration of catalepsy ( $281 \pm 2.91$  sec.) at 60 minute after the administration clonidine. There was significant inhibition ( $p < 0.01$ ) of clonidine induced catalepsy in the animals pretreated with *Ageratum conyzoides* Linn extract (250 mg/kg, p.o, 500 mg/kg, p.o, 1000 mg/kg, p.o) and the duration of catalepsy was found to be  $233.33 \pm 7.71$ ,  $175.38 \pm 1.59$  and  $156.66 \pm 3.42$  seconds respectively at 60 minute after the administration clonidine. Chlorpheniramine maleate (10 mg/kg, i.p.) significantly inhibited ( $p < 0.01$ ) clonidine induce catalepsy in mice at 120 minute after the administration clonidine.

### **Haloperidol induced catalepsy in mice**

Haloperidol (1 mg/kg, s.c) produced catalepsy in mice, which remained for 2 hr. As shown in table 2, the vehicle treated group showed maximum duration of catalepsy ( $188.83 \pm 2.90$  sec.) at 120 minute after the administration haloperidol. But there was no significant inhibition of Haloperidol induced catalepsy in the animals pretreated with *Ageratum conyzoides* Linn extract (250 mg/kg, p.o, 500 mg/kg, p.o, 1000 mg/kg, p.o).

**Table 1: Effect of *Ageratum conyzoides* Linn on clonidine induced catalepsy in mice.**

Groups	Duration of catalepsy (sec) at Mean $\pm$ SEM						
	15 Min	30 min	60 Min	90 min	120 min	150 min	180 Min
Group I	14.16 $\pm$ 1.16	148.66 $\pm$ 3.18	281 $\pm$ 2.91	174.33 $\pm$ 7.54	115.05 $\pm$ 1.58	91.66 $\pm$ 1.76	75.16 $\pm$ 3.71
Group II	7.16 $\pm$ 0.74**	63.33 $\pm$ 4.41**	141.66 $\pm$ 2.17**	108.33 $\pm$ 5.31**	87.05 $\pm$ 1.70**	65.66 $\pm$ 1.64**	30.66 $\pm$ 3.77**
Group III	12.83 $\pm$ 1.3	126.83 $\pm$ 2.50**	233.33 $\pm$ 7.71**	163.33 $\pm$ 3.20*	105 $\pm$ 2.63**	85.33 $\pm$ 2.60	61.66 $\pm$ 4.84**
Group IV	10.5 $\pm$ 0.84*	75.16 $\pm$ 2.50**	175.38 $\pm$ 1.59**	148.66 $\pm$ 5.85**	91.83 $\pm$ 1.86**	74 $\pm$ 1.84**	54.66 $\pm$ 4.08**
Group V	9.5 $\pm$ 0.42**	65.83 $\pm$ 2.76**	156.66 $\pm$ 3.42**	127.33 $\pm$ 6.80**	88.66 $\pm$ 1.92**	68 $\pm$ 2.60**	42.83 $\pm$ 3.60**

Values are mean  $\pm$  SEM; n = 6 in each group, \*p<0.05, \*\* p<0.01, compared to control group (One way ANOVA followed by Dunnett's test).

Figure 1: Effect of Chlorpheniramine (10 mg/kg, i.p.) on clonidine – induced catalepsy in experimental animals.

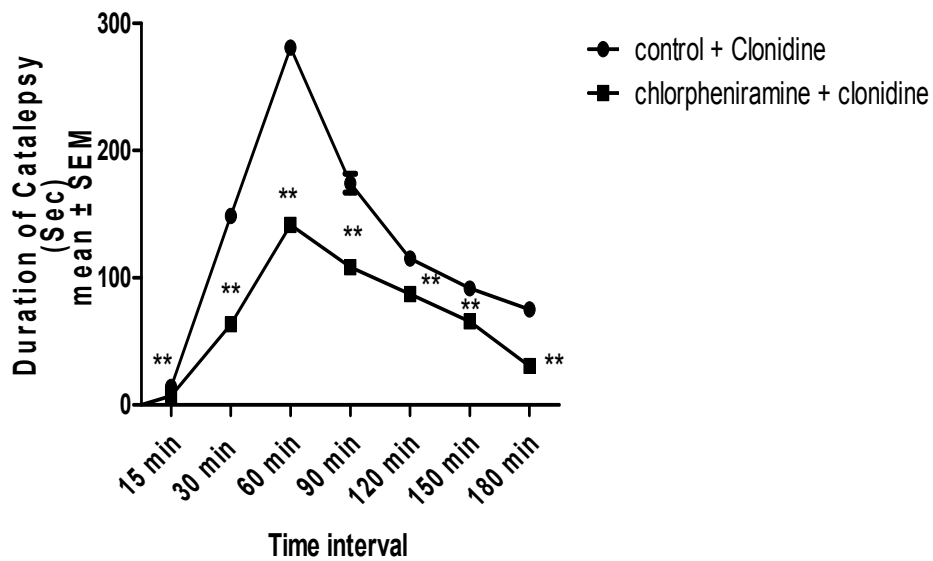
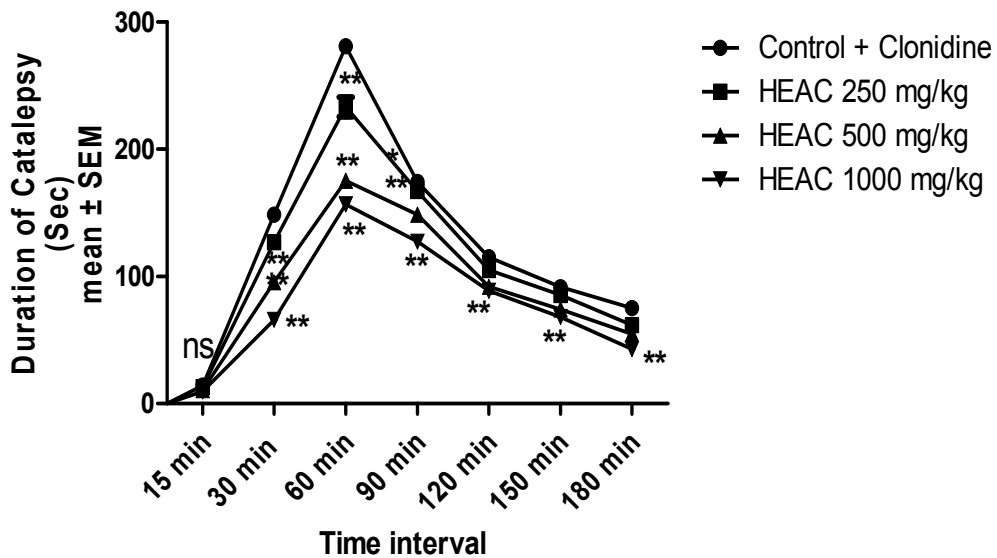


Figure 2: Effect of *Ageratum conyzoides* Linn on Clonidine – induced catalepsy in experimental animals.



**Table 2: Effect of *Ageratum conyzoides* Linn on Haloperidol induced catalepsy in mice.**

Groups (n=6)	Duration of catalepsy (sec) at Mean $\pm$ SEM						
	15 Min	30 min	60 min	90 min	120 min	150 min	180 min
I	36.66 $\pm$ 1.76	139.16 $\pm$ 3.63	142.5 $\pm$ 2.18	176.83 $\pm$ 2.83	188.83 $\pm$ 2.90	145.33 $\pm$ 2.52	91 $\pm$ 5.13
II	37.66 $\pm$ 2.48	139.33 $\pm$ 4.11	131.66 $\pm$ 3.27	168.33 $\pm$ 2.31	182.66 $\pm$ 2.21	157.33 $\pm$ 5.20	89.16 $\pm$ 2.91
III	34.56 $\pm$ 2.27	130.83 $\pm$ 3.74	141.66 $\pm$ 2.56	166.5 $\pm$ 2.82	187.66 $\pm$ 2.49	137.5 $\pm$ 2.27	83 $\pm$ 2.12
IV	33.83 $\pm$ 1.01	125.66 $\pm$ 3.51	145.16 $\pm$ 1.51	163.5 $\pm$ 3.89	188.5 $\pm$ 1.40	129.5 $\pm$ 2.39	86.33 $\pm$ 2.33
V	29.33 $\pm$ 1.08	127.83 $\pm$ 3.25	135.33 $\pm$ 2.24	161.83 $\pm$ 7.41	183.16 $\pm$ 3.12	131.16 $\pm$ 5.95	79.16 $\pm$ 2.04

Values are mean  $\pm$  SEM; n = 6 in each group, One way ANOVA followed by Dunnett's test.

### Discussion

Histamine plays an important role in the symptomatology of allergic reactions. Drugs which have the capacity to control the histamine release and its further effects can be called as antihistaminic or antiallergic drugs<sup>19</sup>. Uvnas (1969) studied the mast cell degranulation and in correlation with the release of histamine after administration of mast cell degranulating agent (compound 48/80)<sup>20</sup>. Lakadwala et al., (1980) have shown that clonidine releases histamine from mast cells in a similar manner to a selective liberator like compound 48/80<sup>9</sup>.

Preliminary phytochemical analysis revealed the presence of alkaloids, tannins, steroids, triterpenoid, saponins and flavonoids. Plants containing flavonoids have been reported to possess antihistaminic, antiallergic and mast cell degranulation properties<sup>21, 22</sup>.

In clonidine induced catalepsy, hydroalcoholic extract reduces histamine content significantly. It may be due to the inhibitory effect on release of calcium from an intra cellular store of mast cells<sup>23, 24</sup> and stabilization of the mast cell membrane<sup>21</sup>. The observation of this study indicated that the hydroalcoholic extract of *Ageratum conyzoides* Linn inhibited clonidine induced catalepsy and not inhibited haloperidol induced catalepsy. Thus the hydroalcoholic extract of *Ageratum conyzoides* Linn leaves has antihistaminic activity. However it does not possess antidopaminergic activity. All the above findings lend credence to the beneficial use of hydroalcoholic extract in the treatment of asthma and related conditions. However further studies with other experimental models is in progress.

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