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Effect of ethanolic extract of some anti-asthmatic herbs on clonidine and haloperidol-induced catalepsy in mice

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SUMMARY

The ethanolic extract of some medicinal plants having anti-asthmatic activity such as *Solanum xanthocarpum, Curcuma longa, Glycyrrhiza glabra, Piper longum, A. vasica, A. lebbeck,* and *Tinospora cordifolia* was evaluated for antihistaminic and anti-cataleptic activity. The aqueous solution of ethanolic extract of *S. xanthocarpum* and *G. glabra* potentiated histamine-induced tracheal chain contractions. Whereas, *C. longa, P. longum,* and *T. cordifolia, and A. lebbeck* were without any significant effect on histamine. Only *A. vasica* inhibited histamine-induced tracheal chain contraction. *G. glabra per se* produced contraction of the tracheal chain, which was blocked by pretreatment with atropine. Single dose of *S. xanthocarpum* potentiated clonidine-induced catalepsy but on repeated doses (once in a day for 3 days) inhibited catalepsy. Pretreatment with ethanolic extract of *C. longa, P. longum, T. cordifolia* inhibited catalepsy whereas *G. glabra* and *A. lebbeck* significantly potentiated clonidine-induced catalepsy. None of the extracts inhibited haloperidol-induced catalepsy. Thus the extracts having antihistaminic activity or mast cell stabilizing activity inhibited clonidineinduced catalepsy.

Key words: Catalepsy, clonidine, haloperidol, anti-asthmatic

INTRODUCTION

Catalepsy is a condition in which the animal maintains imposed posture for long time before regaining the normal 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 (Jadhav *et al.*, 1983). They also showed that pretreatment with L-histidine, a precursor of histamine potentiated clonidineinduced catalepsy in dose dependent manner. Muley *et al.*, (1979) showed that intracerebroventricular injection of histamine in conscious mice induced catalepsy, which was inhibited by H_1 receptor antagonist but not by H_2 receptor antagonist. It is known that clonidine releases histamine from mast cells (Lakdawala *et al.*, 1980). Schwartz (1977) 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 receptor blocker, prazosin (Weiner, 1980). Neuroleptic agents also induce catalepsy, but by different mechanism. Neuroleptics inhibit dopamine D_2 receptors in the substantia nigra (Sanberg, 1980; Ossowska *et al.*, 1990).

Therefore it was our objective to study the effect of some of these herbs on clonidine-induced catalepsy. Since catalepsy is a common extrapyramidal side effect of neuroleptic agents and the effect of these herbs on haloperidol-induced catalepsy is not known, we also studied their effect on haloperidol-induced catalepsy in mice. The aerial parts of *Solanum xanthocarpum,* rhizomes of *Curcuma longa,* roots of *Glycyrrhiza glabra,* fruits of *Piper longum,* leaves of *Adhatoda vasica* and *Albizzia lebbeck,* and stems of *Tinospora cordifolia* were used in this study. The anti-histaminic activity of the

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ethanolic extract of these plants was assessed using goat tracheal chain as described earlier by Nag Chaudhari and Lahiri (1974), and Kulshreshtha *et al.*, (1983). The extracts were then tested for their effect on clonidine or haloperidol-induced catalepsy using Bar test (Ferre *et al.*, 1990).

MATERIALS AND METHODS

Animals

Goat trachea was obtained from slaughterhouse. It was immersed in Kreb's solution maintained at 37±1°C. Male albino mice (Swiss strain) weighing 22-25 g were housed under standard laboratory conditions, in groups of five each. The animals had free access to food and water. The ethical committee of the institute approved the protocol of the study.

Drugs

Clonidine (Unichem, INDIA), histamine (Sigma, USA), haloperidol (Searle, India), rhizomes of *Curcuma longa*, roots of *Glycyrrhiza glabra*, fruits of *Piper longum*, stems of *Tinospora cordifolia* purchased from commercial source were identified and authenticated by Dr. S. C. Pal of the Pharmacognosy department. Leaves of *A. lebbeck*, *A. vasica*, and aerial parts of *Solanum xanthocarpum* were collected from the medicinal plant garden of the college.

Assessment of anti-histaminic activity

Goat trachea was cut into individual rings and tied together in series to form a chain. It was suspended in bath containing Kreb's solution (concentration in mM/liter: NaCl, 118; KCl, 4.7; $CaCl_2$, 2.5; $MgSO_4$, 1.2; $NaHCO_3$, 25.0; KH_2PO_4, 1.2; Glucose, 11.1) maintained at 37±1°C, a stream of 5% CO_2 in oxygen was bubbled through the organ tube. One end was tied to an aerator tube and other attached to isotonic frontal writing lever to smoked drum. Tissue was allowed to equilibrate for 45 min. under a load of 400 mg. (Nag Chaudhari and Lahiri, 1974). A dose response curve for histamine was taken in variant molar concentrations. After obtaining a dose response curve of histamine on trachea, the aqueous solution of extract (n=4), except, extracts of *P. longum* and G. glabra, was added to the reservoir and same doses of histamine were repeated. The ethanolic extracts of *P. longum* and *G. glabra* were dissolved in PEG 400 and water. PEG 400 used alone was without any contractile effect. Graph of maximum percentage of contractile response on ordinate and negative logarithm of molar concentration of histamine on abscissa was plotted to record dose response curve of histamine, in absence and presence of aqueous solutions of ethanolic extracts of plants.

Assessment of anti-cataleptic activity

Bar test (Ferre *et al.*, 1990) was used to study the effect of extracts on clonidineinduced catalepsy. Clonidine (1 mg/kg subcutaneously) was injected to mice (n=5) pretreated with vehicle (10 ml/kg i.p.), ethanolic extract of *Solanum xanthocarpum*, *Glycyrrhiza glabra*, *Adhatoda vasica*, *Tinospora cordifolia*, *Albizzia lebbeck* (100 mg/kg each), and *Piper longum* or *Curcuma longa* (50 mg/kg i.p. each). The doses of ethanolic extracts were selected on the basis of preliminary studies (data not shown). 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.

In another set of experiments, one group of mice received two doses of the ethanolic extract of *S. xanthocarpum* (100 mg/kg, 1 h apart), the second group received the *S. xanthocarpum* extract (100 mg/kg i.p.) once daily for 3 days. All 6 of 20 the groups received clonidine 30 min after the last dose and the duration of catalepsy was measured at 15, 30, 60, 90, 120, 150 and 180 min.

Effect on haloperidol-induced catalepsy

The same Bar test was used using haloperidol. Haloperidol (1mg/kg i.p) was injected to mice (n = 5) pretreated with vehicle (10 ml/kg i.p.), ethanolic extract of *S. xanthocarpum* (100 mg/kg i.p.) or *C. longa* (50 mg/kg i.p.). The duration of catalepsy was measured at 15, 30, 60, 90, 120, 150 and 180 min.

Statistical analysis

The data is presented as mean±SEM. The data was analyzed by one-way ANOVA and the Bartlett's test. Prism Graph pad 3 was used for statistical analysis. $P<0.05$ was considered significant.

RESULTS

Assessment of antihistaminic activity

In a graph of maximum percentage of contractile response vs negative logarithm of molar concentration of histamine, indicated a dose dependent contraction of goat tracheal chain. The aqueous solution of ethanolic extract of *S. xanthocarpum* and *G. glabra* potentiated histamine-induced tracheal chain contractions. Whereas *C. longa, P. longum,* and *T. cordifolia, and A. lebbeck* were without any significant effect on histamine. Only *A. vasica* inhibited histamineinduced tracheal chain contraction. *G. glabra per se* produced contraction of the tracheal chain, which was blocked by pretreatment with atropine. The observations are given in Figure 1 - 3.

Clonidine-induced catalepsy

Clonidine produced catalepsy in mice, which remained for 3 hours. Maximum catalepsy was recorded 60 min after clonidine. Single dose of

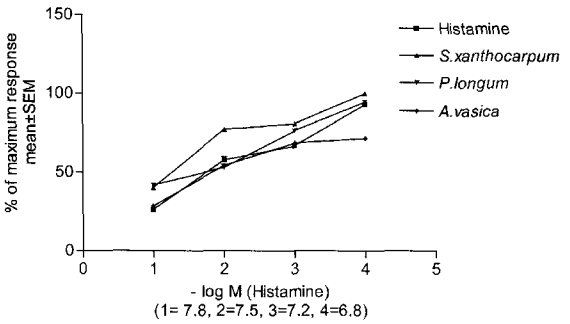

Fig. 1. Effect of ethanolic extract of *S. xanthocarpum, P. longum* and *A. vasica* on histamine-induced contractions in isolated goat tracheal chain preparation.

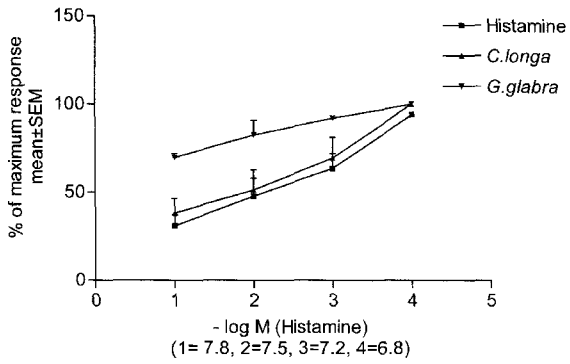

Fig. 2. Effect of ethanolic extract of C. *longa* and *G. glabra* on histamine-induced contractions in isolated goat tracheal chain preparation.

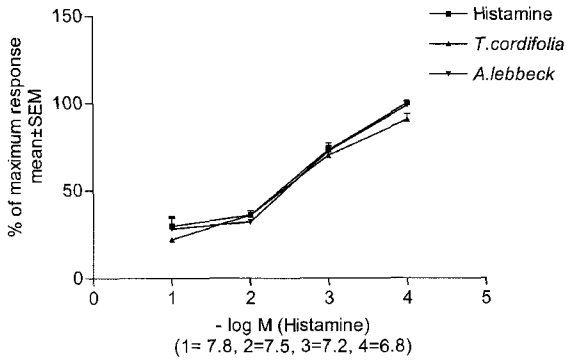

Fig. 3. Effect of ethanolic extract of *T. cordifolia and A. lebbeck* on histamine-induced contractions in isolated goat tracheal chain preparation.

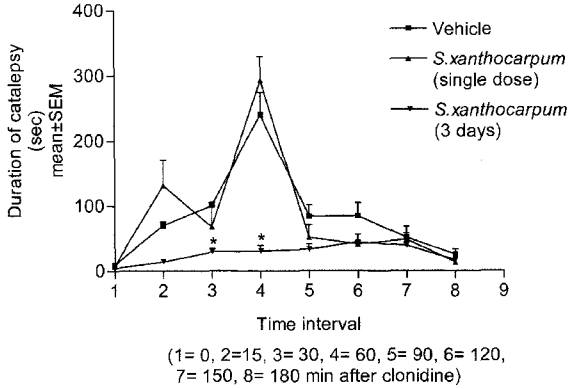

Fig. 4. Effect of ethanolic extract of *S. xanthocarpum* on cilonidine-induced catelepsy in mice.
*P<0.05 compared to vehicle treated group (Bartlett's test)

S. xanthocarpum potentiated catalepsy but on repeated doses (once in a day for 3 days) inhibited catalepsy (P<0.0003, Bartletts test; Fig. 4). Pretreatment with ethanolic extract of *C. longa, P. longum, T. cordifolia* inhibited catalepsy ($F_{3,28}$=3.54; P=0.027; Fig. 5). *G. glabra* and *A. lebbeck* significantly potentiated clonidine-induced catalepsy ($F_{3,28}$= 3.492; P=0.028; Fig. 6).

Haloperidol-induced catalepsy

None of the extracts inhibited haloperidol-induced catalepsy (Data is not shown).

DISCUSSION

Several drugs are known to induce catalepsy in animals. The neuroleptic agents induce catalepsy by inhibiting dopamine D_2 receptors in the substantia nigra (Sanberg, 1980). Chopra and Dandiya (1975)

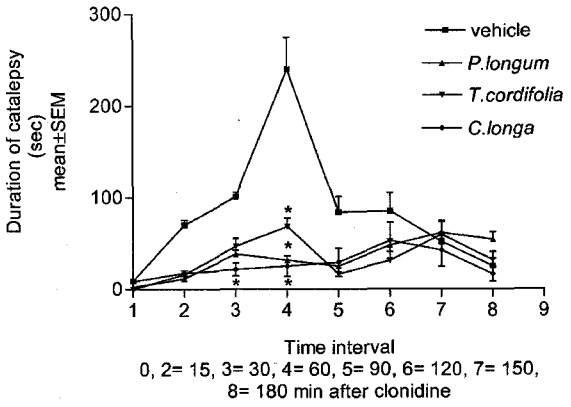

Fig. 5. Effect of ehanolic extract of P. *longum, C. longa* and *T. cordifolia* on clonidine-induced catalepsy in mice. *P<0.05 compared with vehicle treated group (ANOVA, Bartlett's test)

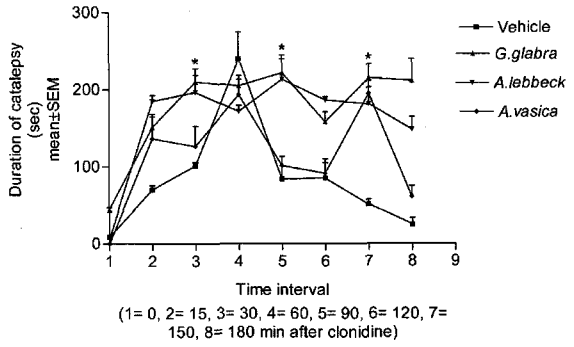

Fig. 6. Effect of ethanolic extract of *G. glabra, A. lebbeck* and *A. vasica* on clonidineinduced catalepsy in mice. *P<0.05 compared to vehicle treated group (ANOVA)

have studied the relative role of acetylcholine and histamine in perphenazine-induced catalepsy and suggested that anticholinergic activity of antidepressants might be due to an increase in dopamine content in the brain or their ability to inhibit release of acetylcholine.

They also showed that different stages of catalepsy appear to be directly correlated with brain histamine content. Jadhav *et al.*, (1983) noticed that clonidine, unlike haloperidol, failed to antagonize apomorphine-induced cage climbing behaviour occurring as a result of direct stimulation of post-synaptic striatal dopamine receptors.

Uvnas (1969) studied the mast cell degranulation and its correlation with the release of histamine after administration of compound 48/80, the mast cell degranulating agent. Both clonidine and compound 48/80 act through the dynamic expulsion of granules without causing any damage to the cell wall (Stanworth 1973), Lakdawala *et al.* (1980) have shown that clonidine releases histamine from mast cells in a similar manner to a selective liberator like compound 48/80. It is known that disodium cromoglycate a standard mast cell stabilizer prevents degranulation of the mast cells by raising the cyclic adenosine monophosphtae (Geetha *et al*, 1981).

The observation of this study indicated that the herbs inhibiting mast cell granulation or having antihistamine activity inhibited clonidine-induced catalepsy and none of the extracts inhibited haloperidol-induced catalepsy. The plants used in this study are being used in the treatment of asthma and the herbs relieve asthma by different ways. *T. cordifolia* has immunomodulatory, mast cell stabilizing and spasmolytic activity (Nadakarni, 1954; Nayampalli *et al*, 1986). *A. vasica* has bronchodilator and mucolytic activity (Atal, 1980). It is reported that *S. xanthocarpum* depletes lung histamine (Gupta,1970) whereas *G. glabra* has anti-inflammatory, expectorant activity (Nadakarni, 1954; Pandey *et al*, 2003). *P. longum* has mast cell stabilizing and bioavailability enhancing property (Dahanukar *et al*, 1983). *A. lebbeck* has anti-allergic property (Das, 1988), whereas *C. longa* exhibits mast cell stabilizing, anti-histaminic and anti-inflammatory activity (Sharma, 2000; Yano *et al*, 2000).

From the present study we concluded that the cataleptic effect of clonidine in the mouse is mediated by histamine release from mast cells, and the clonidineinduced catalepsy was inhibited by *S. xanthocarpum, C. longa, T. cordifolia* and *P. longum.* The effect of these plants on clonidine-induced catalepsy is probably due to their mast cell stabilizing property and the plants do not have activity on dopaminergic transmission.

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