

## Anti-Inflammatory and Anti Cataleptic Effect of Herbal Formulation Consisting of Various Indigenous Plants in Experimental Animals

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### ABSTRACT

We had investigated the effect of Antiasthmatic Herbal Formulation (AHF) on Clonidine induced catalepsy in mice and Passive paw anaphylaxis in rats. The protective effect of AHF containing an extracts of *Curcuma longa*, *Zingiber officinale* and *Alpinia galanga* were studied at the doses of 62.5, 125, 250 mg/kg, showed significant inhibition (\*p<0.05, \*\*p<0.01) of clonidine induced catalepsy in the animals. And significantly reduced (\*p<0.05, \*\* p<0.01) the paw volume at 0.5, 1, 2, 3, and 4 hr time interval and also showed significant percentage inhibition. The findings of the current study revealed that AHF exhibits significant dose dependant anti-inflammatory and anticataleptic activity in in-vivo animal model suggesting its potential in prophylaxis and treatment of asthma.

**Keywords:** anticataleptic, passive paw anaphylaxis, herbal formulation.

### INTRODUCTION

Asthma is a chronic inflammatory and allergic lung disease. It is caused by variety of physical, chemical and pharmacological stimuli which increases the secretion of leucocytes, eosinophils and neutrophils<sup>1-3</sup>. Worldwidely the incidences of respiratory disorders has been increases and reachind epidemic proportions. The prevalence of asthma around the world is approximately 200 million with mortality around 0.2 million per year. The estimated asthma burden in India is more than 15 million. Hospital based study on 20,000 children under the age of 18 years from 1979, 1984, 1994 and 1999 in the city of Bangalore showed a prevalence of 9, 10.5, 18.5, 24.5 and 29.5% respectively<sup>4</sup>. The existing remedies for asthma are known to possess serious side effects such as hypersensitivity, severe hepatic impairment and cirrhosis on prolonged use. Therefore, there is a need to explore new antiasthmatic plant based drugs or formulations with lesser side effects<sup>5</sup>. *Curcuma longa*, *Zingiber officinale* and *Alpinia galangal* are important Indian medicinal plants with anti imflammatory and antiallergic, anticancer and antimicrobial properties. Curcuma majorly contain curcumin, demethoxycurcumin, and bisdemethoxycurcumin while ginger contain the gingerol, shogaol paradols etc. Moreover, alpinia galangal contains the essential oil, galangin, alpinin zerumbinettannins,  $\beta$ -sitosterol etc<sup>6-12</sup>.

The above mentioned plants are well known and scientifically reported antiasthmatic, antiallergic activity.our previously developed AHF was reported for the bronchodilaty activity<sup>8,13-15</sup>.

In the present study, we have evaluated the same formulation for antiasthmatic properties using clonidine induced catalepsy and passive paw edema.

### MATERIALS AND METHODS

#### Plant material

The ethyl acetate and methanolic extracts of *Curcuma longa* and *Alpinia galanga* were purchased from Amsar Pvt.Ltd. Indore India while rhizomes of *Zingiber officinale* were collected from local market of Nagpur, India. The plant specimen was authenticated by Dr. N. M. Dongarwar, Department of Botany, RTMNU Nagpur, India. (Voucher specimen no. 9491).

#### Extraction

The dried rhizomes of *Zingiber officinale* was ground to a fine powder which was then soaked in distilled water for 24 h at room temperature. The resulting solution was filtered and evaporated to remove excess of water (yield 10%)<sup>7-8</sup>.

#### Animals

All the procedures were carried out in strict accordance with the guidelines prescribed by the Committee for the purpose of Control and Supervision on Experimentation on Animals (CPCSEA No- DYP/PSR/IAEC/P-14) and were approved by the Institutional Animal Ethics Committee in year 2010. Adult albino rats of Wistar strains weighing between 150-200 g and albino swiss mice weighing between 25-30 g were used. The above animals, rats and mice of either sex were purchased from National Toxicology Centre, Pune and Serum Institute, Pune respectively. Animals had a free access to standard pellet diet and water. They were housed in a group of five under

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standard laboratory of conditions of temperature ( $25 \pm 2^\circ\text{C}$ ) and 12/12 hr light/dark cycle. The distribution of each animal in each group along with the sequence of trials and the treatment allotted to each group were randomized throughout the group of experiment. Separate groups of fresh animals were used for each experiment.

#### *Acute toxicity studies*

Toxicity studies were conducted as per internationally accepted protocol drawn under OECD guidelines 425 in swiss albino mice at a dose level of formulation up to 5000 mg/kg<sup>16</sup>.

#### *Clonidine induced catalepsy in mice*

Bar test was used to study the effect of AHF on Clonidine induced catalepsy. Mice were divided into six groups, five animals in each group. Animals belonging to group I served as control and were administered the vehicle (10 ml/kg, p.o.). Animals belonging to group II received standard drug Chlorpheniramine maleate (10 mg/kg, i.p.). Animals belonging to groups III received MKTD in dose of 248.5 mg/kg and IV, V and VI received three doses 62.5, 125 and 250 mg/kg p.o. respectively of AHF. 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. All the groups received Clonidine (1mg/kg, s.c.), 1 hr after the test drug administration and the duration of catalepsy were measured at 15, 30, 60, 90, 120, 150 and 180 min<sup>16</sup>.

#### *Passive paw anaphylaxis in rats*

Anti serum to egg albumin was raised in rats using aluminum hydroxide gel as an adjuvant. Animals were given three doses of 100 mcg of egg albumin (s.c.) absorbed on 12 mg of aluminum hydroxide gel prepared in 0.5 ml of saline on 1<sup>st</sup>, 3<sup>rd</sup>, 5<sup>th</sup> day. On 10<sup>th</sup> day of sensitization, the blood was collected from the retro orbital plexus. The collected blood was allowed to clot and the serum was separated by centrifugation at 1500 rpm. Animals were divided into five groups each containing 5 animals. Animals belonging to group I served as control and were administered only the vehicle (10 ml/kg, p.o.). Animals belonging to group II were administered Dexamethasone (0.5 mg/kg, i.p.). Animals belonging to groups III received MKTD in dose of 40.5 mg/kg and IV, V and VI received AHF in dose 62.5, 125 and 250 mg/kg, p.o. respectively. Animals were passively sensitized with 0.1 ml of the undiluted serum into the left hind paw. The contra lateral paw received an equal volume of saline. MKTD 40.5 mg/kg and AHF in dose 62.5, 125 and 250 mg/kg, p.o. respectively were administered 24 hr after sensitization. 1 hr. after test drug administration, the animals was challenged in the left hind paw with 10  $\mu\text{g}$  of egg albumin in 0.1 ml of saline and the paw inflammation was measured by using a Plethysmometer (UGO Basile, 7140)<sup>17,18</sup>.

#### *Statistical Analysis*

All observations were presented as Mean  $\pm$  SEM (Standard error of mean). The data was analyzed by Student's t-test or one- way ANOVA followed by Dunnet's test.  $P < 0.05$  was considered as significant.

## RESULTS

In the present study, the effect of AHF was studied on various antiasthmatic models viz. clonidine induce catalepsy in mice and passive paw anaphylaxis.

#### *Effect of AHF on Clonidine induced catalepsy in mice*

Clonidine (1 mg/kg, s.c.) produced catalepsy in mice, which remained for 2 hr. The vehicle treated group showed maximum duration of catalepsy ( $81 \pm 1.64$  sec.) at 150 minute after the administration clonidine. There was significant inhibition ( $*p < 0.05$ ,  $**p < 0.01$ ) of clonidine induced catalepsy in the animals pretreated with MKTD and AHF 62.5mg/kg, 125mg/kg, 250 mg/kg. Chlorpheniramine maleate (10mg/kg, i.p.) significantly inhibited ( $**p < 0.01$ ) clonidine induced catalepsy in mice at 150 minute after the administration clonidine (Table 1).

#### *Effect of AHF on Passive Paw Anaphylaxis in Rats*

Antiserum to egg albumin was injected 24 hr before administration of the test drugs or standard. Egg albumin was injected after the administration of AHF, MKTD and Dexamethasone. In the vehicle or distilled water treated group, egg albumin increased the paw volume in the sensitized animals, which was measurable up to the time period of 4 h. Pretreatment with AHF 62.5mg/kg, p.o significantly reduced ( $*p < 0.05$ ,  $** p < 0.01$ ) the paw volume at 0.5, 1, 2, 3, and 4 hr time interval and the percentage inhibition was 7.3%, 21.84%, 12.4%, 17.4% and 12% respectively. AHF 125 p.o. significantly reduced ( $**p < 0.01$ ) the paw volume at 0.5, 1, 2, 3 and 4hr time interval and the percentage inhibition was 20.39%, 34.32%, 15.78 %, and 28 % respectively. AHF 250mg/kg p.o. significantly reduced ( $**p < 0.01$ ) the paw volume at 0.5, 1, 2, 3 and 4 hrs time interval and the percentage inhibition was 22.4%, 46.8%, 37.9%, 36.3% and 41.4% respectively. MKTD significantly reduced ( $*p < 0.05$ ) the paw volume at 0.5, 1, 2, 3 and 4 hrs time interval and the percentage inhibition was 6.66%, 16.89%, 14.4%, 11.4% and 11.4% respectively. Dexamethasone (0.5 mg/kg, i.p.) significantly reduced ( $**p < 0.01$ ) the paw volume at 0.5, 1, 2, 3 and 4 hrs time intervals and the percentage inhibition was 40.78%, 51.4%, 40.05%, 40.02% and 47.05% respectively (Table 2)

## DISCUSSION

Allergy and anaphylaxis are the most responsible factor for disease like asthma, rhinitis, bronchitis, cold, cough, pain, inflammation etc<sup>2</sup>.

Clonidine, a  $\alpha_2$  adrenergic agonist induced dose dependant catalepsy in mice, which is inhibited by histamine ( $H_1$ ) receptor antagonists but not by  $H_2$  receptor antagonist. It is known that clonidine releases histamine from mast cells. Brain histamine does play a definite role in the production of the extra pyramidal motor it has been suggested that the cataleptic effect of clonidine in the mouse be mediated by histamine (via  $H_1$  receptor) which is release from brain mast cells in response to stimulation of  $\alpha_2$  adrenergic receptors by clonidine. AHF inhibited clonidine induced catalepsy in mice and which may be due to potential to antagonize  $H_1$  receptor<sup>2</sup>.

In the late phase, especially in the development of allergic asthma, eosinophiles play role as an inflammatory cell. Eosinophile secretes mediators such as eosinophile

Table 1: Effect of AHF on Clonidine induced catalepsy in mice.

Group	Treatment	Duration of catalepsy (Sec), Mean $\pm$ SEM						
		15min.	30min	60min	90min	120min	150min	180min
I	Control (10 ml/kg p.o.)	25.4 $\pm$ 1.36	41.2 $\pm$ 1.82	48 $\pm$ 1.70	53.6 $\pm$ 4.15	80.4 $\pm$ 1.7	81 $\pm$ 1.64	63.8 $\pm$ 1.7
	Std					7		1
II	(Chlorpheniramine maleate (10 mg/kg, i.p.)	15.4 $\pm$ 1.50**	16.2 $\pm$ 1.92*	25.2 $\pm$ 1.3	32.2 $\pm$ 1.46	33.6 $\pm$ 1.7	59.2 $\pm$ 1.7	20.2 $\pm$ 3.0
	MKTD		*	9**	**	2**	7**	0**
III	(248.5 mg/kg p.o.)	24.8 $\pm$ 1.49	30.4 $\pm$ 2.82*	35.2 $\pm$ 1.3	34.2 $\pm$ 1.93	68.2 $\pm$ 3.7	69.8 $\pm$ 2.2	62.4 $\pm$ 2.3
	MKTD		*	5*	**	6	8**	7**
IV	AHF (62.5 mg/kg, p.o.)	20.6 $\pm$ 1.07	36.4 $\pm$ 1.60	38.4 $\pm$ 3.2	48.2 $\pm$ 3.12	59.8 $\pm$ 9.4	72.6 $\pm$ 2.5	56 $\pm$ 1.78
V	AHF(125 mg/kg, p.o.)	21.2 $\pm$ 2.43	35.6 $\pm$ 2.70	35 $\pm$ 5.30*	47.4 $\pm$ 5.30	49.4 $\pm$ 4.3	60 $\pm$ 1.48*	48.4 $\pm$ 1.8
VI	AHF(250 mg/kg, p.o.)	18 $\pm$ 2.23*	24.2 $\pm$ 1.28*	30.4 $\pm$ 0.9	37.6 $\pm$ 1.36	44.6 $\pm$ 1.6	45.2 $\pm$ 2.0	39.2 $\pm$ 2.4
			*	2**	**	3**	1**	3**

Table 2: Effect of AHF on Passive Paw Anaphylaxis in Rats.

Groups	Treatment	Paw Edema Volume (ml) Mean $\pm$ SEM				
		0.5 hr	1hr	2hr	3hr	4hr
1	Control (10 ml/kg p.o.)	0.466 $\pm$ 0.006	0.545 $\pm$ 0.02	0.412 $\pm$ 0.003	0.386 $\pm$ 0.003	0.372 $\pm$ 0.002
	Std					
2	((Dexamethasone 0.5 mg/kg, i.p.)	0.276 $\pm$ 0.041**	0.265 $\pm$ 0.026**	0.247 $\pm$ 0.003**	0.231 $\pm$ 0.003**	0.197 $\pm$ 0.004**
	MKTD					
3	(40.5 mg/kg, p.o.)	0.345 $\pm$ 0.005	0.453 $\pm$ 0.02	0.353 $\pm$ 0.036**	0.342 $\pm$ 0.018	0.335 $\pm$ 0.022**
	AHF (62.5 mg/kg, p.o.)	0.432 $\pm$ 0.0003	0.426 $\pm$ 0.050*	0.361 $\pm$ 0.0003**	0.319 $\pm$ 0.02**	0.33 $\pm$ 0.002*
4	AHF (125 mg/kg, p.o.)	0.374 $\pm$ 0.003**	0.358 $\pm$ 0.019**	0.347 $\pm$ 0.010**	0.280 $\pm$ 0.002**	0.268 $\pm$ 0.002**
	AHF (250 mg/kg, p.o.)	0.362 $\pm$ 0.002**	0.29 $\pm$ 0.017**	0.256 $\pm$ 0.008**	0.246 $\pm$ 0.002**	0.218 $\pm$ 0.002**
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cationic protein (ECP) eosinophile derived neurotoxin (EDNT), granulocyte macrophage colony stimulating factor (GM-CSF), tumor necrosis factor(TNF) AND prostaglandin (PG) which results in epithelial shedding, bronchoconstriction and promotion of inflammation in respiratory tract. Eosinophilia is associated with respiratory disorder, often allergic in nature together with pulmonary infiltrates that are detectable on chest films. Immunomodulating agents are useful in the treatment of allergy by virtue of inhibiting the antigen-antibody (AG:AB) reaction thereby inhibiting release of inflammatory mediators. The beneficial effect of ahf could be due to either inhibition AG:AB (hypersensitivity reaction-1) i.e. producing antiallergic and anti-inflammatory properties<sup>2</sup>.

In conclusion, the present study confirmed that the AHF exhibits significant dose dependant antiasthmatic activity in in-vivo animal model suggesting its potential in prophylaxis and treatment of asthma.

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