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ANTI ALLERGIC AND ANTIASTHMATIC EFFECT OF HERBAL FORMULATION ON MURINE MODEL OF ASTHMA

P. R. Dongre [*1], C. A. Doifode [1], S.B. Taywade [1] and S. S. Bhujbal [2]

Shri Sacchidanand Shikshan Santha's [1], College of Pharmacy Koradi, Nagpur - 441111, Maharashtra, India.
Dr. D.Y. Patil Institute of Pharmaceutical Sciences and Research Pimpri [2], Pune - 411018, Maharashtra, India.

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Correspondence to Author:
Priti R. Dongre

Assistant Professor,
Department of Pharmacognosy,
College of Pharmacy Koradi, Nagpur - 441111, Maharashtra, India.

E-mail: pritidongrengp@rediffmail.com

ABSTRACT: *Curcuma longa, Zingiber officinale* and *Alpinia galanga* are traditionally and scientifically reported for the treatment of respiratory disorders such as asthma, bronchial congestion and allergic reactions in the respiratory tract. As part of a continuing effort aimed at the development of efficacious antiallergic and antiasthmatic herbal formulation for the treatment of asthma and related respiratory diseases. We have formulated antiasthmatic herbal capsules (AHF) using *Curcuma longa, Zingiber officinale* and *Alpinia galanga* extracts. The protective effect of the antiasthmatic herbal formulation (AHF) containing extracts of *Curcuma longa, Zingiber officinale,* and *Alpinia galanga* were studied on Milk-induced Leukocytosis and Eosinophilia in mice and also compared with the available marketed formulation. Subcutaneous injection of milk in a dose of 4ml/kg produced significant increase $p<0.05$, $p<0.01$ in leukocytes count after 24 h of its administration. Mice pretreated with MKTD at a dose of 248.5 mg/kg, p.o. exhibited inhibition $p<0.01$ and AHF at the dose of 62.5, 125, 250 mg/kg, p.o. exhibited inhibition $p<0.01$ of milk-induced leukocytosis. Subcutaneous injection of milk at a dose of 4 ml/kg produced a significant (###$p<0.01$) increase in the leucocytes count after 24 hr of its administration. In the groups of mice pretreated with MKTD and AHF 62.5, 125, 250 mg/kg, p.o., there was significant (*$p<0.05$, **$p<0.01$) inhibition of milk-induced Eosinophils. The findings revealed that the antiallergic and antiasthmatic activity of AHF might be due to marked inhibition of leukocytes and eosinophils.

INTRODUCTION: Asthma is a chronic inflammatory lung disease characterized by airway hyper-responsiveness (AHR) to allergens, airway edema, and increased mucus secretion along with an increase in infiltration of leukocytes, eosinophils into the airways [1, 2]. Pathogenesis of asthma majorly involves airway inflammation coupled with AHR to a variety of physical and pharmacological stimuli [3].

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The incidence of respiratory diseases increases globally and is reaching epidemic proportions. The prevalence of asthma around the world is approximately 200 million with mortality around 0.2 million per year. The estimated burden of asthma 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 [4]. The already existing remedies for asthma are known to possess detrimental side effects like 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.

Thus, continuous efforts have been progressed in the development of herbal formulation as well as traditional knowledge-based drugs for the treatment of human diseases such as asthma and respiratory disorders [5].

Evaluation of the effects of these formulations on organs and systems has also been contributed to developing the scientific support for their therapeutic applications [2, 6].

Curcuma longa, Zingiber officinale and *Alpinia galanga* traditionally and scientifically reported for antiasthmatic, anti-allergic and other respiratory related diseases [7, 8, 9, 10, 11, 12]. AHF (antiasthmatic herbal formulation) a herbal formulation containing mainly the ethyl acetate, aqueous and methanolic extracts of *Curcuma longa, Zingiber officinale* and *Alpinia galanga* respectively.

The dry rhizomes of *Curcuma longa* contain curcumin, a main bioactive component, demethoxycurcumin, and bisdemethoxycurcumin. The traditional use of turmeric or natural curcuminoids in folk medicines are multiple, and some are based on their antioxidant, anti-inflammatory and antiallergic properties which have been confirmed by various experimental studies. Curcumin is also found to be a potent blocker of nuclear transcription factor (NF)-Kb, which is linked to a variety of diseases including allergy and asthma. *Zingiber officinale* has been found to exert anti-inflammatory activity and is reported to be a potent inhibitor of inflammatory mediators such as prostaglandins and leukotrienes.[5] *Alpinia galanga* has been found to exert antiplatelet activity [12] and antiallergic activity [13].

In the present study, the effect of AHF a herbal formulation was studied on various aspects of asthma using various animal models like milk induced eosinophils and leukocytosis.

MATERIALS AND METHODS:

Plant Material: The ethyl acetate and methanolic extracts of *Curcuma longa* and *Alpinia galanga* were procured from Amsar Pvt. Ltd. Indore. The 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, RTM Nagpur University, 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%)[7].

Animals: All the procedures were carried out in strict accordance with the guidelines prescribed by the Committee for Control and Supervision of Experimentation on Animals (CPCSEA No-DYPIPSR/IAEC/P-14) and were approved by the Institutional Animal Ethics Committee in the 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 free access to standard pellet diet and water. They were housed in a group of five under standard laboratory of conditions of temperature (25 ± 2 ºC) 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 an 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 [16].

Statistical Analysis: All observations were presented as Mean ± SEM (Standard error of the mean). The data were analyzed by Student's t-test or one- way ANOVA followed by Dunnet's test. $P<0.05$ was considered significant. Milk-induced Leukocytosis and Eosinophilia in mice.

Mice were divided into six groups (n=5). Animals belonging to Group-I received distilled water 10 ml/kg, p.o. An animal belonging to Group- II, III, IV, V, and VI received boiled and cooled milk in a dose of 4 ml/kg (subcutaneously). An animal belonging to Group- III received marketed formulation Damana capsules (248.5 mg/kg, p.o.) and IV, V and VI received AHF 62.5, 125, 250 mg/kg, p.o. respectively 1 h before milk injection. Blood samples were collected from each mouse by

retro-orbital plexus, under light ether anesthesia. Total leukocyte count was recorded in each group before and after 24 h milk injection. Differences in total leukocyte and eosinophils count before and after 24 h drug administration were calculated [17].

RESULTS: In the present study, the effect of AHF was studied on various antiasthmatic models *viz.* milk induced leukocytosis and eosinophilia. Subcutaneous injection of milk in a dose of 4ml/kg, produced significant increase $p<0.05$, $p<0.01$ in leukocytes count after 24 h of its administration. Mice pretreated with MKTD at the dose of 248.5 mg/kg, p.o. exhibited inhibition $p<0.01$ and AHF at a dose of 62.5, 125, 250 mg/kg, p.o. exhibited inhibition $p<0.01$ of milk-induced leukocytosis **Table 1**.

TABLE 1: EFFECT OF AHF ON MILK INDUCED LEUKOCYTES IN MICE

Group	Treatment	Difference in no. of Leukocytes (per cu mm) Mean ± SEM
I	Control (10 ml/kg p.o.)	80 ± 12.247
II	Intox (4 ml/kg, sc)	4540 ± 507.54$^{###}$
III	MKTD (248.5 mg/kg, p.o.)	3900 ± 621.18**
IV	AHF (62.5 mg/kg, p.o.)	4340 ± 639.21**
V	AHF (125 mg/kg, p.o.)	3580 ± 451.16**
VI	AHF (250 mg/kg, p.o.)	2980 ± 219.14**

Subcutaneous injection of milk at a dose of 4 ml/kg produced a significant ($^{###}p< 0.01$) increase in the leucocytes count after 24 hr of its administration. In the groups of mice pretreated with MKTD and AHF 62.5, 125, 250 mg/kg, p.o., there was significant (*$p<0.05$, **$p<0.01$) inhibition of milk-induced Eosinophils **Table 2**.

TABLE 2: EFFECT OF AHF ON MILK INDUCED EOSINOPHILS IN MICE

Group	Treatment	The difference in no. of eosinophils (%) Mean ± SEM
I	Control (10 ml/kg p.o.)	2.8 ± 0.37
II	Intox (4 ml/kg)	9 ± 0.70$^{###}$
III	MKTD (248.5 mg/kg, p.o.)	6.4 ± 0.50**
IV	AHF (62.5 mg/kg, p.o.)	7.2 ± 0.37*
V	AHF (125 mg/kg, p.o.)	6 ± 0.54**
VI	AHF (250 mg/kg, p.o.)	5.4 ± 0.50**

DISCUSSION: Herbal formulation used in the treatment of asthma include some antistress herbs that enabled adaptation to stress since excessive stress or nervous debility may aggravate symptoms of asthma. The normalization effect of an adaptogen can be observed in milk induced leukocytosis after parenteral administration of milk [17]. In the present study, the distilled water treated group of mice after parenteral administration of milk showed a significant increase in leukocyte count, whereas the group treated with AHF showed a normal leukocyte count. This indicates the adaptogenic activity of AHF.

Most allergic and non-allergic asthmatics, including those with mild asthma, have bronchial eosinophilia and there is a significant association between eosinophils activation and severity of asthma as well as bronchial hyperresponsiveness.

The total eosinophils count reflects asthmatic activity and is useful in the management of asthma. In the present study parenteral administration of milk (4 ml/kg) showed significantly increased in eosinophil count 24 h after administration of milk, whereas the group treated with AHF significantly inhibited eosinophilia in mice.

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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CONFLICT OF INTEREST STATEMENT: The authors declare that they have no conflict of interest.

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