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STUDY OF HERBAL FORMULATION CONSISTING OF VARIOUS INDIGENOUS PLANTS FOR THEIR ANTI-ASTHMATIC ACTIVITY IN EXPERIMENTAL ANIMALS

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Received: 09-10-2010; Revised: 15-11-2010; Accepted: 27-11-2010

ABSTRACT
Asthma is defined as a disease characterized by wide variation over short period of time in resistance to flow in intrapulmonary airways. Presently synthetic drugs used in asthma are known to show various side-effects and cases of resistance cases are also common. Ayurveda being a time tested system offers safe and effective remedy against asthma. Four drugs selected are *Passiflora incarnate***,** *Passiflora incarnate, Adhatoda vasica, Ocimum sanctum*. All these drugs are mentioned in Ayurveda at a dose of 500mg they produce effective results. They are tested on various models and are selected on their pharmacological basis as they target specific etiological factors in asthma. Testing was done for their animal activities (preclinical trials). Seven different methods of pharmacological antiasthmatic testing protocols were used for this purpose.

KEYWORDS- antiasthma, Ayurveda, *Passiflora incarnate, Passiflora incarnate, Adhatoda vasica, Ocimum sanctum*

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INTRODUCTION

Asthma is defined as a disease characterized by wide variation over short period of time in resistance to flow in intrapulmonary airways. Increased resistance to airflow is due to environmental factors especially inhaled substances in concentrations that do not affect the majority of persons. The strongest risk factors for developing asthma include a family history of asthma especially in childhood allergic sensitization.[1] Currently synthetic drugs such as bronchodilators, methylxanthines, Mast cell stabilizers, Leukotriene inhibitors, steroids are used for treatment. Although synthetic drugs give instant relief from symptoms of asthma, but they cause lot of undesirable effects (like those of steroids). Moreover, their efficacy goes on decreasing with their continuous use. Herbal drugs like on the other hand, provide prolonged effects and have less side effects[2].

Earlier many work has been done on herbal drugs regarding their potential as anti-asthmatic, such as olea-europea[3],Tylophora, boswelia, glycyrrhiza, coleus,Ginkgo, Fagopyrum to name a few. In the present study drugs selected are *Passiflora incarnate, Picrorrhiza kurroa, Adhatoda vasica, Ocimum sanctum*. All these drugs have been individually proven for their efficacy in asthma but are combined for the first time. *Passiflora incarnate* consist of flavonoids which are antioxidanys and are also effective in relieving various symptoms of asthma. *Adhatoda vasica* is expectorant, *Picrorrhiza kurroa* Preclinically, it has been found that this drug prevents allergen and platelet activating factor induced bronchial obstruction and ocimum is immunostimulant.

All the above drugs are evaluated in this study by using seven different asthmatic models which proves a wide dimension of this anti-asthmatic formulation. These parameters are

Effect of test drug on histamine induced contraction of smooth muscles, Effect of test drug on histamine release from mast cell, Effect of test drug on catalepsy, Effect of test drug on Antigen-antibody (AG-AB) reaction, Bronchodilator model

MATERIALS AND METHODS

Procurement of plant

Leaves of Passiflora incarnate, Picorrhiza kurroa, Adhatoda vasica, Ocimum sanctum were purchased from local nursery. All the plants/parts were identified and authenticated at Department of Botany, University of Pune by Prof. Dr. S.S.Deokule.

Preparation of extract

All four medicinal plants were procured and subjected to aqueous extraction through maceration at room temperature. All the extracts were then filtered through filter paper, dried and stored.

Animals and animal methods

For acute oral toxicity and LD_{50} determination OECD guideline 425 (Organization for economic co-operation and development) was followed. Administration of different doses of preparation did not have any toxic effects. The animals were alive, healthy and active during the observation period of 14 days.

Pharmacological evaluation of the herbal plants using various in-vitro and in-vivo screening methods

1. Effect of test drug on histamine induced contraction of smooth muscles
    - A. Isolated goat tracheal chain prepartion
    - B. Isolated guinea pig ileum preparation
    - C. Bronchial hyperactivity in guinea pig
2. Effect of test drug on histamine release from mast cell
    - A. Clonidine induced catalepsy in mice
3. Effect of test drug on catalepsy
    - A. Haloperidol induced catalepsy in mice.
4. Effect of test drug on Antigen-antibody (AG-AB) reaction
    - A. Passive paw anaphylaxis in rats.
5. Bronchodilator model

Isolated goat tracheal chain preparation

In most animal species such as rodents, dog and human trachea, histamine causes bronchoconstriction where as, in cat and sheep it shows the bronchorelaxation. The guinea pig tracheal chain is a classical preparation but it is not very sensitive for many agonists.[4]

It is reported that there is preponderance of H_1 excitatory and a scanty population of H_2 inhibitory receptors on isolated goat tracheal preparation. The agonists like ACh, histamine, 5-Hydroxytryptamine and bradykinin causes dose dependent contraction of goat trachea. The both goat tracheal chain and strip preparation are suitable for screening spasmogenic activity on respiratory smooth muscle. The goat tracheal chain is easier to handle and prepare, it is also much more sensitive than guinea-pig tracheal chain.[5,12]

Isolated guinea pig ileum preparation

Histamine is an autocoid having profound physiological effect in the body. Besides the triple response caused by it, histamine has spasmogenic response on intestinal smooth muscle. By acting on H_1 receptors it causes the contraction of intestinal smooth muscle. This model was used to screen the effect of all 4 combinations on histamine-induced contraction on intestinal smooth muscle.[6,7]

Histamine induced Bronchoconstriction in Guinea pigs

Histamine causes very strong smooth muscle contraction, profound hypotension, and capillary dilation in cardiovascular system. In each species there is a characteristic predominant response to histamine that is the cause of death. The experimental bronchial asthma can be induced in guinea pigs by exposing them to 0.2 % histamine aerosol. Histamine when inhaled has been shown to induce bronchoconstriction by direct H_1-receptor activation. Histamine when inhaled shows hypoxia and leads to convulsion in guinea pigs.This model was used to screen the effect of all 4 combinations on histamine aerosol induced bronchoconstriction by measuring the time required for appearance of preconvulsive dyspnea caused by the histamine [preconvulsive time (PCT)], which was recorded for each animal. [8]

Passive Paw Anaphylaxis in rat

Allergic asthma is a chronic inflammatory process occurring due to exposure of allergen resulting in the activation of T-lymphocytes with subsequent release of inflammatory mediators. Immuno-modulating agents are useful in the treatment of asthma by inhibiting the antigen-antibody (AG: AB) reaction and there by inhibiting the release of inflammatory mediators.[9]

Clonidine-induced catalepsy in mice

Catalepsy is a condition in which the animal maintains imposed posture for a long time before regaining the normal posture. Catalepsy is a sign of extra pyramidal effect of drugs that inhibits dopaminergic transmission or increase/release histamine (inhibitory neurotransmitter) in brain. Clonidine, a α_2 adrenoreceptor agonist, induces dose dependent catalepsy in mice, which is inhibited by histamine H_1 receptor antagonist but not by H_2 receptor antagonist.

The clonidine releases histamine from these mast cells and thus catalepsy produced by clonidine is mediated by histamine via H_1 receptors.[10,11]

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Table 1: Effect of dose of combination (500mg) **on Histamine induced contraction of isolated goat tracheal chain preparation**

Sr. No.	- Log Molar concentration of Histamine	Control Group % Maximum contraction	Test Group % Maximum contraction
1	0.1	25.524 ± 2.123	17.25 ± 1.208**
2	0.2	53.124 ± 3.520	32.812 ± 1.532**
3	0.4	70.426 ± 1.910	46.464 ± 2.53**
4	0.8	90.724 ± 1.420	56.313 ± 3.00**
5	1.6	93.725 ± 2.623	66.742 ± 4.56**

n=6, Values in Mean ± SEM.
Control = D.R.C. of Histamine in absence of Combination
Test = D.R.C. of Histamine in presence of Combination I
Statistical analysis done by using Student t-test
**p < 0.001, significantly different from control group

Table 2: Effect of aqueous extract of Combination I (500g)on Histamine induced contraction of isolated guinea pig ileum preparation

Sr.No.	- Log Molar concentration of Histamine	Control (% Maximum contraction)	Test (% Maximum contraction)
1	0.1	18.397 ± 0.931	13.00 ± 1.106**
2	0.2	32.00 ± 0.533	20.00 ± 1.63**
3	0.4	61.21 ± 2.520	42.50 ± 2.180**
4	0.8	90.79 ± 2.87	54.33 ± 2.96**
5	1.6	91.38 ± 2.88	65.66 ± 1.39**

Table 3: Effect of combination I against histamine induced bronchoconstriction in guinea pigs

Group	Latent period of convulsion (sec.) (Mean ± SEM)			
	Before	1 hr	4 hr	24 hr
Group I(500g)	39.6 ± 2.13	54.2 ± 1.74**	64.4 ± 2.06**	46.4 ± 1.07

Table 4: Effect of combination against histamine induced bronchoconstriction in guinea pigs

% Protection		
1 hr.	**4 hr.**	**24 hr.**
26.9	38.51	14.65

Table 5: Effect of combination on Clonidine induced catalepsy in mice

Duration of catalepsy (sec) Mean ± SEM at						
15 Min	**30 Min**	**60 Min**	**90 Min**	**120 Min**	**150 Min**	**180 Min**
21.8 ± 0.12*	98.6 ± 0.65**	132.6 ± 1.76**	189.4 ± 1.71**	211.7 ± 3.17**	198.8 ± 3.42**	107.8 ± 5.75**

Table 6: Effect of combination on Passive Paw Anaphylaxis

Paw Edema volume in ml (Mean ± SEM) at			
1 hr	**2 hr**	**3 hr**	**4 hr**
0.56 ± 0.04*	0.41 ± 0.042**	0.38 ± 0.044**	0.32 ± 0.047**

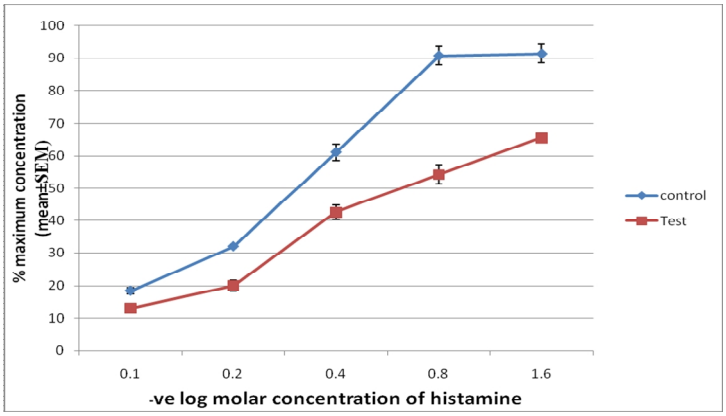

Figure 1: Effect of aqueous extract of combination I on Histamine induced contraction of isolated goat tracheal chain preparation

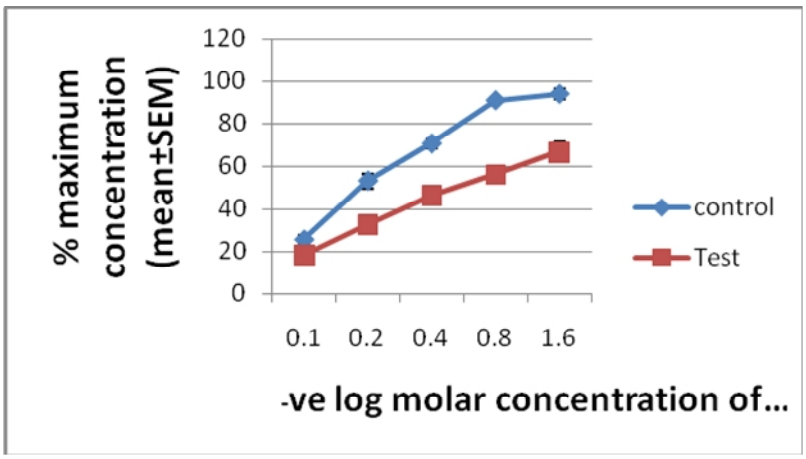

Figure 2: Effect of aqueous extract of Combination I on histamine induced contraction of isolated guinea pig ileum preparation

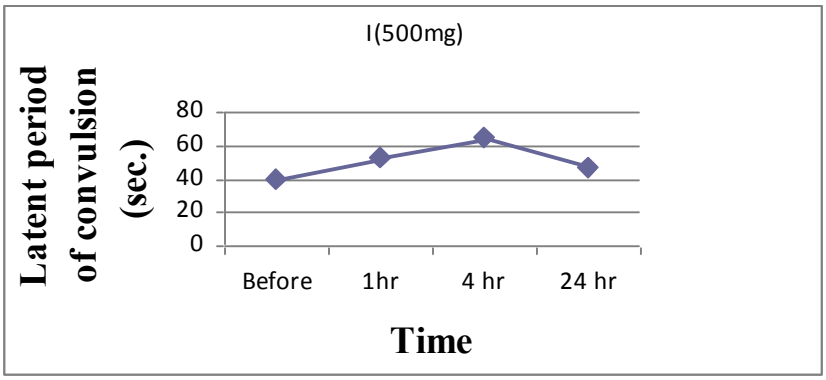

Figure 3: Effect of combination I against histamine induced bronchoconstriction in guinea pigs

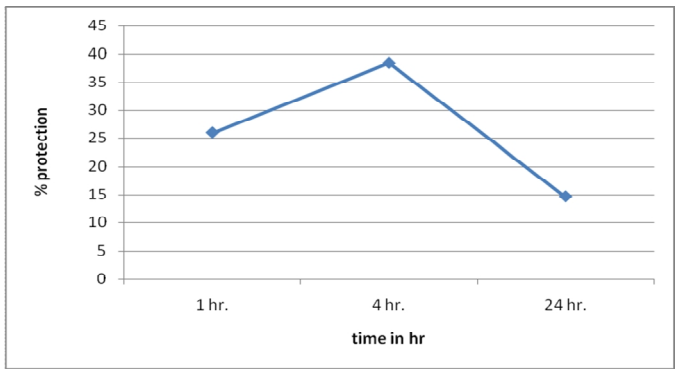

Figure 4: Effect of combination against histamine induced bronchoconstriction in guinea pigs

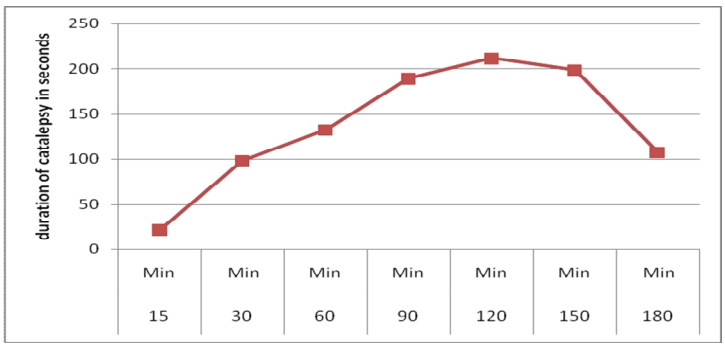

Figure 5: Effect of combination on Clonidine induced catalepsy in mice

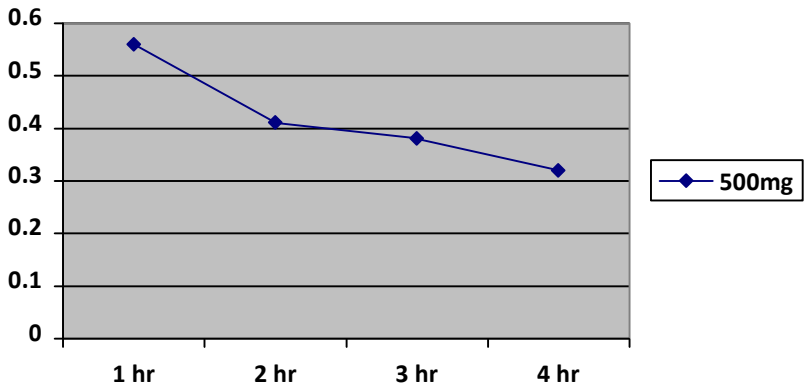

Figure 6: Effect of combination on Passive Paw Anaphylaxis

Source of support: Nil, Conflict of interest: None Declared