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Full Length Research Paper

Antihistaminic evaluation of formulated polyherbal cough syrup

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Allergic rhinitis is one of the most common allergic disorders throughout the world. The conventional therapies are effective in alleviating symptoms but the efficacy is limited and not persistent. Many of the indigenous herbal preparations used by the tribes are little known to the public due to lack of scientific evaluation. The aqueous ethanol extracts of various traditional herbs like *Adhatoda vasica, Acorus calamus, Glyzyrrhiza glabra, Ocimum sanctum, Tylophora asthmatica, Piper longum* and *Solanum xanthocarpum* was evaluated for its antihistaminic activity by the inhibition of histamine induced contractions on the guinea pig ileum. The results showed that the formulated cough syrup inhibited histamine induced contractions of guinea pig ileum at 2.5 to 25 µg/ml concentrations in a dose dependent manner ($p < 0.01$, $p < 0.05$) and also significantly ($p < 0.05$) inhibited degranulation of mast cells. All the results were well comparable with the standard benadryl cough syrup (diphenhydramine).

Key words: Antihistaminic syrup, formulation, asthma, siddha, ayurveda.

INTRODUCTION

The current market is flooded with enormous medicated products for the treatment of asthma as it is one of the most common diseases prevailing in the world population. These drugs effectively control the incidence of sign and symptoms of asthma but none promise to provide a complete relief. Among them antileukotrienes are the newest classes of antihistaminic drugs available. Moreover, they are also associated with a number of side effects like kidney, liver failure, increased hunger, compromised immune system and high blood pressure (Ghosh et al., 2003). Therefore the world is in search for novel herbal remedies which can serve mankind from dawn of civilization. Ancient system of Indian medicine (Ayurveda) has recommended indigenous plants as a potential source for novel drugs that can be used in the treatment if asthma and allergic disorders. World Health Organization (WHO) has called the attention of many countries to the ever increasing interest of the public in the use of herbal medicines and encourages countries to identify and exploit those aspects of traditional medicine that provide safe and effective remedies (Akah et al., 1997).

Allergic rhinitis is a collection of symptoms, predominantly in the nose and eyes, caused by airborne particles of dust, dander or plant pollens in the people who are allergic to those substances. Allergic rhinitis involves inflammation of the mucous membranes of the nose, eyes, eustachian tubes, middle ear, sinuses and pharynx. In this study, a formulation was prepared from the aqueous ethanol extract of *Adhatoda vasica* (Jayanth, 1999), *Acorus calamus* (Yende et al., 2009), *Glyzyrrhiza glabra* (Nicole et al., 2009), *Ocimum sanctum* (Pratibha et al., 2005), *Tylophora asthmatica* (Betsy et al., 2007), *Piper longum* (Seung et al., 2008), *Solanum Xanthocarpum* (Gupta, 1994) and screened for antihistaminic properties. These seven herbs used were commonly used in Indian traditional system like Siddha and Ayurveda for the treatment of allergic rhinitis. An attempt to prepare a single formulation were undertaken in the present study, after reviewing various literatures for each of these promising plants with afore mentioned activities.

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Table 1. Formulation of antihistaminic cough syrup.

Extracts of plants	Each 10 mL of syrup (mg)
A. vasica	100
A. calamus	100
G. glabra	100
O. sanctum	100
T. asthmatica	100
P. longum	50
S. xanthocarpum	50
Syrup base	q.s.

MATERIALS AND METHODS

Collection of plant materials

The leaves of *A. vasica, O. sanctum, T. asthmatic* and the rhizomes of *A. calamus* were collected from medicinal plant garden, Shankaralingam Bhuvaneshwari College of Pharmacy, Sivakasi, Tamilnadu, India. The fruits of *P. longum, S. xanthocarpum* and the roots of *G. glabra* were purchased from the local market, Sivakasi, Tamilnadu, India. All the plant materials were authenticated by Dr. P. Jayaraman, Director, Plant Anatomy Research Center, Chennai, Tamil Nadu, India. Voucher specimens were deposited in the herbarium, SB College of Pharmacy, Sivakasi, India.

Preparation of extracts

The collected plant materials (2 kg) were dried under shade, size reduced into coarse powder and macerated separately with 4 L of water-ethanol mixture (1:1). After 7 days of maceration, all the extract was filtered out and concentrated under vacuum using rotary vacuum evaporator (Sunilson et al., 2008). The residue obtained was kept in a dessicator for the present study.

Preparation of herbal syrup

The simple syrup (66.67% w/v) was prepared as per Indian pharmacopoeia. One gram of each extracts of *A. vasica, A. calamus, G. glabra*, *O. sanctum* and *T. asthmatica* and 0.5 g of each extracts of *P. longum* and *S. xanthocarpum* were dissolved in simple syrup I.P. and the volume was made up to 100 mL (Table 1).

Evaluation of formulated cough syrup

Physical parameters like specific gravity, pH, and refractive index were analyzed as per the standard procedure mentioned in Indian Pharmacopoeia. The colour and odour were also recorded in Table 2.

Pharmacological screening

Animals

Twenty four healthy adult albino rats (100 - 150 g) and five guinea pigs (300 - 400 g) of either sex were selected from the animal house of S.B. College of Pharmacy, Sivakasi, Tamilnadu. They were kept in the departmental animal house under the conditions of light (14 h light/10 hdark) at 27 ± 2°C and relative humidity 44 - 56%, for 1 week before and during the experiments. They were fed with pellet diet (Hindustan Ltd., Bangalore, India) and water was allowed to have *ad libitum*. All animals are handled according to the approval and current guidelines of Institutional Animal Ethical Committee (IAEC NO:SBCP/F.9(J)/06a/2006) and the ethical guidelines for investigations of experimental pain in conscious animals (Zimmerman, 1983).

Table 2. Evaluation of physical parameters.

Parameter	Formulated syrup
Specific gravity	1.2927
pH	4.6
Refractive index	1.546
Colour	Brownish black
Odour	Characteristic

Antihistaminic activity

Overnight fasted guinea pigs were killed by a blow on the head and exsanguinated. The abdomen was cut open, and a good length of the ileum was placed on a Petri dish containing Tyrode solution at 37°C. A 2.5 cm long piece of the distal part of the ileum was used for the study. Experiments were performed in organ baths containing 40 mL Tyrode solution at 37°C and bubbled with air. The concentrations of the ileal strips to histamine were recorded on smoked kymograph paper with frontal writing lever having a 10-fold magnification and 1.0 g tension. The preparation was allowed to equilibrate for 30 min during which the Tyrode solution was changed at intervals of 10 min. The responses of the histamine, standard drug (benadryl) and the herbal cough syrup were studied at 2.5 - 25 µg/mL dose levels. Their effect on contractions induced by histamine (0.5 µg/mL) was also studied. The percentage inhibition of the extracts on contractions induced by histamine was calculated.

Mast cell stabilizing activity

Twenty four rats were divided into four groups of six animals each. Group I received vehicle only (saline 2 mL) and served as control. Group II (sensitized control group, received only saline 2 mL), group III (herbal cough syrup 5 mL/kg b.wt. orally once daily for 14 days) and group IV (benadryl 5 mL/kg body weight orally once daily for 14 days) were sensitized by injecting 0.5 mL of horse serum subcutaneously along with 0.5 mL triple antigen containing 20,000 million *Bordetella pertusis* organisms. On day 14, 2 hour post-treatment the rats were sacrificed and the intestinal mesentry were excised and placed in Ringer solution (NaCl 154, KCl 5.6, $CaCl_2$ 2.2, $NaHCO_3$ 6.0, glucose 5.55 mmol/L of distilled water) at 37°C. The mesenteric pieces were challenged with 5% horse serum for 10 min after which the mast cells were stained with 1% toludine blue and examined microscopically for the number of intact and degranulated mast cells (Grover, 1990).

Statistical analysis

All the data are expressed as mean ± SEM. The values obtained for the above parameters were compared with control group using one way ANOVA followed by Student's test (Kulkarini, 1999). The values of $p < 0.01$ and $p < 0.001$ were considered to indicate a significant difference between the groups.

Table 3. Effects of the herbal formulation and standard on histamine (0.5 µg/mL) induced contractions on guinea pig ileum.

S/No.	Treatment	Mean concentrations (mm)		% inhibition of histamine responses	
	Dose of drugs (µg/mL)	Standard syrup (Diphenhydramine HCl)	Herbal antihistaminic syrup	Standard syrup (Diphenhydramine HCl)	Herbal antihistaminic syrup
1.	-	42 ± 1.48	39 ± 1.72	-	-
2.	2.5	34 ± 1.02	30 ± 1.26	19.04	23.07*
3.	5.0	26 ± 1.21	15 ± 0.78	38.09*	61.53**
4.	10	20 ± 0.60	10 ± 0.46	52.38**	74.35**
5.	20	11 ± 0.53	02 ± 0.21	73.80**	94.87**
6.	25	05 ± 0.002	00 ± 0.00	88.09**	100.00**
7.	-	31 ± 1.30	28 ± 1.18	-	-

Values are Mean ± S.E.M, *P < 0.05, **p < 0.01 (compared the values with control group).

Table 4. Effect of herbal formulation on mast cell stabilizing in sensitized rats.

Groups	Mast cells %	
	Intact	Disrupted
Control (saline 2 mL)	79.26 ± 3.02	16.68 ± 1.86
Sensitized control	9.31 ± 0.97	86.13 ± 2.44
Herbal formulation (5 mL/kg)	61.74 ± 3.94*	34.62 ± 1.30*
Benadryl (2 mL/kg)	65.25 ± 2.26*	30.72 ± 2.09*

Values are Mean ± S.E.M, *p < 0.05 (compared the values with control group), n = 6.

RESULTS

The physical parameters like colour, odour, specific gravity, hydrogen ion concentration and refractive index for the formulated herbal cough syrup were recorded in Table 2. Histamine at 0.5 µg/mL produced contractile responses on guinea pig ileum. Herbal formulation in the concentration range of 2.5 - 25 µg/mL revealed significant ($p < 0.01$, $p < 0.05$) antihistaminic activity in a dose-dependent manner, showing a 100% inhibition of histamine response at maximum dose. The results were tabulated in Table 3. The herbal drug formulation at 5 mL/kg b.wt. was found to significantly ($p < 0.05$) inhibit degranulation of mast cell in pretreated sensitized animals and when challenged with horse serum, the effect of herbal formulation was also comparable with the standard drug (Table 4).

DISCUSSION

Although, a number of synthetic preparations have proved to be effective for managing asthma symptoms, a curative therapy for asthma is lacking. Asthma is a chronic inflammatory airways disease characterized by bronchial hyper responsiveness to various stimuli and increased numbers of both inflammatory cells and inflammatory mediators (Yang et al., 2007). The major goals in the management of asthmatic attack include relaxation of the bronchial tree thereby relieving airway obstruction and hyper responsiveness and inhibition of the release of inflammatory mediators in asthma such as histamine, 5-HT and leukotrienes (Akah et al., 1997). In the present study, evaluation of antihistaminic formulation prolonged the latent period of convulsion following exposure to histamine. This may be suggestive of an antihistaminic activity of the formulation due to direct bronchodilator effect or due to its H_1-blocking effect or may be spasmolytic activity. Basophils, mast cells and their preformed de novo synthesized mediators are essential contributors in the pathogenesis of allergic disorders. These molecules are potent, vasoactive and bronchoconstrictor agents and they modulate local immune responses and inflammatory cell infiltration (Schroedor et al., 1994). Antigen challenge in sensitized animals resulted in mast cells degranulation, which is an important characteristic of anaphylaxis. In the present study our herbal formulation exhibited pronounced protection against the mast cell degranulation following antigen challenge in sensitized animals. Mast cell stabilizing activity of the formulated cough syrup may be attributed to the presence of plant phyto constituents, which are known for their mast cell stabilizing potential against antigen-antibody reaction and/or due to the

suppression of IgE antibody production, which is responsible for degranulation of mast cells (Palit et al., 1983). Hence the resultant antihistaminic effect may be caused by the suppression of antibody production and stabilization of the mast cell membrane, inhibition of antigen-induced histamine release or non-availability of antibodies on the mast cell surface (Kale et al., 2006). The cough suppressant activity elicited by the formulated herbal syrup may also be attributed to the presence of some phyto constituents such as vasicinone and vasicinol (Jayant, 1999), Glycyrrhizin and Piperine (Joy et al., 1998) in the extracts of *A. vasica*, *G. glabra* and *P. rlongum* respectively. All the above findings support the traditional claims in Ayurveda and Siddha for use of this formulation in the treatment of asthma and bronchitis by virtue of its bronchodilating and antiallergic activity.

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