EVALUATION OF BRONCHODILATOR AND ANTI-ANAPHYLACTIC ACTIVITY OF HYDROETHANOLIC EXTRACT OF POLYHERBAL COMPOUND- BHARANGYADI

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ABSTRACT

In the present investigation, we studied the effect of hydroethanolic extract of an Ayurvedic drug, Bharangyadi on various experimental models of bronchial asthma. The polyherbal compound Bharangyadi contains Bharangi (Clerodendrum serratum), Sati (Hedychium spicatum) & Pushkarmula (Inula racemosa) as ingredient herbs. Significant (P < 0. 05) increase in preconvulsion time was observed on pre-treatment with Bharangyadi extract when the guinea pigs were exposed to histamine aerosol. This bronchodilating effect of Bharangyadi compound was comparable to Mepyramine malate. Alcoholic extract of Bharangyadi produced significant dose dependent protection by egg albumin induced anaphylaxis. Antimicrobial activity of the polyherbal compound was tested against various respiratory pathogens like Escherichia coli (E. coli), Staphylococcus aureus (S. aureus) and Pseudomonas aeruginosa (P. aeruginosa). Our data suggest that antiasthmatic activity of Bharangyadi compound may be due to its bronchodilator, anti-allergic and antimicrobial activity. The study evidence that drug has potent bronchodilator and anti-anaphylactic properties.

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INTRODUCTION:

The human respiratory tract is universally exposed to air pollution and rapidly changing atmospheric conditions. The care for the respiratory tract should be stressed more often now-a-days, especially in view of a dramatic increase in the incidence of life-threatening diseases like asthma [1]. Asthma is one of the most prevalent chronic health conditions among children and adults. According to World Health Organisation (WHO) estimated 300 million people suffer from Asthma, 255, 000 people died of Asthma in 2005 (WHO 2004) and over 80% of Asthma deaths are reported from low and lower-middle income countries [2]. Asthma creates a substantial burden on individuals and families as it is more often under-diagnosed and under-treated [3]. There is a noticeable increase in health care burden from asthma in several areas of the world. The Global Strategy for Asthma Management and Prevention Guidelines define Asthma as ‘a chronic inflammatory disorder of the airways associated with increased airway hyper-responsiveness, recurrent episodes of wheezing, breathlessness, chest tightness, and coughing, particularly at night/early morning’. Despite the availability of a wide range of anti-asthmatic drugs, the relief offered by them is mainly symptomatic and short lived. Moreover their side effects are also quite disturbing. Hence a continuous search is needed to identify effective and safe remedies to treat bronchial asthma. Ayurveda is traditional system of Indian medicine using mainly herbal products for curing diseases. In Ayurvedic system of medicine mainly polyherbal compounds are used for the treatment of Asthma. Clerodendrum serratum, Hedychium spicatum and Inula racemosa, are extensively used in Ayurvedic system of medicine for the treatment of Bronchial Asthma. Bharangi (Clerodendrum serratum) is found to have anti-inflammatory [4,5], antihistaminic [6-8], antiallergic, antioxidant and hepatoprotective properties [9]. In Ayurvedic system of medicine, it is mainly used in respiratory tract diseases. Sati (Hedichium spicatum) is found to possess hypotensive, hypoglycaemic, anti-inflammatory, vasodilator, antispasmodic, tranquillizer, anti-bacterial, anti-fungal, CNS-depressant, hypothermic, spasmolytic & analgesic effects[10-12]. Pushkarmoola (Inula racemosa) has been found prove beneficial for cardiovascular system, angina and dyspnoea [13, 14]. Bharangyadi is a mixture of Clerodendrum serratum, Hedychium spicatum and Inula racemosa. In clinical practice this polyherbal drug proves to be very efficacious in the management of bronchial asthma thus the present study plan to search the anti-asthmatic potential of an indigenous polyherbal drug-Bharangyadi by evaluating the anti-anaphylactic and bronchodilator activity on experimental models.

MATERIALS AND METHODS:

Plant Material:

The plants Clerodendrum serratum, Hedychium spicatum, & Inula racemosa were collected from local market of Varanasi. The identification of the drugs was done by Prof.A.K. Singh, Department of Dravyaguna, S.S.U., Varanasi (Identification number DG/ AKS / 604).

Extraction of the Plant Material and Sample Preparation:

Hydroalcoholic Extraction (Distilled water: Ethanol = 2:1) of drug was carried out by Hot percolation method through Soxhlet apparatus. Thereafter extract was dried using rotary evaporator and dried extract was put to the process of standardization. The percentage yield was noted.

Drugs and Solvents

1. Ova albumin (Sigma Chemical Co. U.S.A.) – Intraperitoneal injection of 0.5ml and 10% w/v solution.
2. Histamine dihydrochloride (Sigma Chemical Co. U.S.A.) dissolved in distilled water to make desired concentration.
3. Mepyramine malate (Sigma).
Animals:
1. **Rats:** Colony bred descendants of Charles–Foster strain procured from Animal Research Branch of the Institute of Medical Sciences, Banaras Hindu University, and Varanasi. The animals were of either sex weighing 100±20g.
2. **Guinea pig:** Local bred supplied by M/s Zoological Emporium, Institute of Medical Sciences, Banaras Hindu University, Varanasi of either sex, weighing 300-450g.

Experimental/Methodology

**Egg Albumin Induced Anaphylaxis in Guinea Pigs**

Guinea pigs were sensitized by two intraperitoneal injections of 0.5 ml and 10% w/v solution of egg albumin at a 48-h interval. After sensitization, the animals were divided into two groups. Animals of group I received 0.5% CMC and served as control group. Animals of Group II received ethanolic extract of Bharangyadi compound (500mg/Kg,p.o, once daily) for 14 days. On day 14, two hours after treatment, the animals were challenged with 0.5 ml of 2% w/v solution of egg albumin into the saphenous vein. Guinea pigs were observed for the onset of symptoms such as dyspnoea and cyanosis, duration of persistence of symptoms (min.) and mortality. The severity score with respect to symptoms was recorded using the method of Gupta et al. as: increased respiratory rate, dyspnoea for 10 min, dyspnoea and cyanosis for 10 min and collapse. Guinea pigs remaining alive after the antigen challenge were counted to record the percentage of mortality due to anaphylactic shock by using the following equation:

\[
\text{Mortality rate} \%(\%) = \frac{\text{Number of guinea pigs collapsed}}{\text{Total number of experimental animals}} \times 100
\]

**Bronchodilator Effect**

**Studies on Histamine Induced Bronchospasm in Guinea Pigs**

Guinea pigs of either sex weighing 350 - 500 g were selected and randomly divided into four groups each containing four animals. The drugs were administered orally in 0.5% sodium carboxymethyl cellulose (CMC). The single dose treatments were given one and half an hour before the study. The following schedule of treatment was administered:

- Group I: 0.5% CMC (control)
- Group II: Ketotifen (1 mg/kg) (anti-histaminic drug as standard drug )
- Group III: Alcoholic extract of Bharangyadi compound(100 mg/kg)
- Group IV: Alcoholic extract of Bharangyadi compound (200 mg/kg)

One and half hour later the animals were exposed to 0.2% histamine aerosol (in histamine chamber through glass nebulizer) and time for pre convulsion state (PCD) was noted for each animal [15]. The end point for PCD was determined from the time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions. As soon as PCD commenced, the animals were removed from chamber and placed in fresh air to recover. This time for PCD was taken as day 0 value. After 15 days of wash out period, the animal of group III& IV were again given same schedule of drug and expose to histamine aerosol and time for PCD was noted. The % increase in time of PCD was calculated using the following formula.

\[
\text{Percentage increase in time of PCD} = 1 - \frac{T_1}{T_2} \times 100
\]

Where: T1= time for PCD onset on day 0, T2 = time for PCD onset on day 15.
RESULTS AND DISCUSSION

As shown in Table No. 1, an intravenous challenge of egg albumin resulted in a fatal anaphylactic shock in 70% of the animals in control group characterized by symptoms of dyspnoea, asphyxia and collapse. Pre-treatment with ethanolic extract of Bharangyadi compounds (500mg/Kg, p.o), significantly protected the sensitized guinea pigs against anaphylactic shock as the onset of symptoms were delayed, less severe and none of the animals collapsed.

| Table 1: Effect of Bharangyadi Compounds on egg albumin induced anaphylaxis in rats |
|---------------------------------|---------------------------------|-----------------|-----------------|-----------------|
| **Group**                        | **Pre convulsion time (min)**   | **Duration (min)** | **Severity (score)** | **Mortality (%)** |
| I (Control)                      | 1.246 ± 0.056                   | 23.66 ± 0.345    | 9.4 ± 0.306       | 70%             |
| II Bharangyadi compound 500mg/Kg, p.o. | 2.35 ± 0.217*                    | 8.56 ± 0.32*     | 3.51 ± 1.15*      | 0%              |

Statistical analysis for symptoms by paired t-test; values are mean ± SEM; n = 5 in each group; significantly different from control group *P < 0.001.

| Table 2: Effect of polyherbal formulations on histamine-aerosol in guinea pigs |
|---------------------------------|-------------------------------|----------------|----------------|----------------|
| **Treatment**                   | **Dose (mg/kg)**              | **Onset of**   | **Protection** | **% Increase in** |
|                                 |                               | convolution in sec. | (%)           | preconvulsion time |
| Control                         | saline, 1.0 ml/kg             | 91.45 ± 0.093    | 0             | 0              |
| Mepyramine                      | 10mg/Kg                       | 1028.0 ± 4.553   | 90***         | 33.93 ± 3.12   |
| Bharangyadi Compound            | 100                           | 780 ± 0.396      | 78**          | 25.05 ± 2.16***|
| Bharangyadi Compound            | 200                           | 820 ± 0.674      | 82***         | 34.23 ± 2.78***|

n=5 in each group; **P < 0.01, ***P < 0.001 vs. control; (χ2 test with Yate’s correction).
Figure 1: Showing graphical presentation of % protection in Bronchospasm on exposure of Histamine

In present study, significant increase in preconvulsion time was observed due to pretreatment with polyherbal compound *Bharangyadi*, when the guinea pigs were exposed to histamine aerosol. The bronchodilating effect of polyherbal compound was comparable to the standard drug. *Bharangyadi* compound produced 78% and 82% protection with 25.05± 2.16% and 34.23± 2.78 % elongation in preconvulsion time (Table 2), (Figure 1). Bronchial asthma is characterized by increased airway reactivity to spasmogens. An initial event in asthma appears to be the release of inflammatory mediators (e.g. Histamine, Tryptase, Leukotrienes and prostaglandins). Some of these mediators directly cause acute bronchoconstriction, airway hyperresponsiveness and bronchial airway inflammation. Spasmolytic drugs like beta adrenergic agonists, xanthine derivatives and anticholinergics relax the airway smooth muscles and are used as quick relief medications in acute asthmatic attacks.

Histamine when inhaled has been shown to induce bronchoconstriction by direct H1-receptor activation and also by a naturally mediated bronchoconstrictor effect via vagal reflexes. In the present study, ethanolic extract of *Bharangyadi* (100, 200mg/kg) significantly protected the Guinea pigs against histamine-induced bronchospasm. The guinea pigs exposed to histamine aerosol showed signs of progressive dyspnoea leading to convulsions.

Anaphylaxis caused by egg albumin induced a rapid and huge increase of plasma catecholamines especially adrenaline. Anaphylaxis induced by egg albumin is true type with type-1 hypersensitivity reaction induced by IgE mediated immune response mainly against ovaalbumin and ovamucoid. Anti-anaphylactic activity of drug suggest that it might prohibit the antigen antibody (IgE bound to the surface of mast cell) interaction or stabilize the mast cell and prevent the massive liberation of various inflammatory mediators such as histamine etc. Thus probably it act at three stages in disruption of pathogenesis as 1. Desensitization by preventing antigen –antibody interaction 2. Stabilizing mast cell membrane 3. Mediator antagonism. This result is in accordance with earlier studies were prolonged treatment with the Saponin from Clerodendrum serratum is reported to have antihistaminic and anti-allergic activities [16, 17].
The present findings reveal protection against egg albumin induced anaphylactic shock characterized by decrease in intensity and delay in the development of symptoms of dyspnoea, asphyxia and collapse. In line with this notion, anti-anaphylactic effect of Bharangyadi may be due to inhibition of phenomenon of sensitization or non-availability of antibodies on the mast cell surface.

CONCLUSION

The result of this study suggested that the drug has anti-histaminic and desensitization properties by virtue of which it act as anti-asthmatic in addition to other possible mode of action that should be investigate in future. The effect could be more pronounced if sufficient dose of the fractionated isolate was used. In general the effects observed by the extract in this study may account, at least in part, for the reported beneficial anti-asthmatic action of Bharangyadi compound.

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