Effect of PHE against Trypsin and Egg-albumin Induced Experimental Model of Asthma in Mice

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ABSTRACT

The present investigation was carried out to study the effect of PHE in experimental induced bronchial asthma in mice. Trypsin and egg-albumin induced chronic model of asthma was used and various parameters were measured on 35th day. The asthmatic control group showed lower level of pO₂, tidal volume, airflow rate and higher respiratory rate, serum bicarbonate level, eosinophil count in bronchoalveolar lavage (BAL) fluid compared to normal control group. Dexamethasone and PHE treated groups showed higher pO₂ level, tidal volume, airflow rate whereas lower respiratory rate, serum bicarbonate level, eosinophil count in bronchoalveolar lavage fluid compared to asthmatic control group. Histopathological examination of lungs showed more prominent alveolar and muscular layer destruction in asthmatic control group than dexamethasone and PHE treated groups. PHE has beneficial effect in asthma and might be used for the treatment of bronchial asthma.

KEYWORDS: asthma; trypsin; egg albumin; eosinophil; dexamethasone.

Introduction

Bronchial asthma is an inflammatory disorder of the airways characterized by various airway obstruction, airway inflammation and bronchial hyper responsiveness (Djukanovic et al., 1990) and is a global health problem that results from a complex interplay between genetic and environmental factors (Phillip, 2003). Nearly 7–10% of the world population suffers from bronchial asthma. Among several respiratory diseases affecting man, bronchial asthma is the most common disabling syndrome. The currently used drugs for the treatment of this dreadful disease in modern medicine have major limitations owing to low efficacy, associated adverse events and compliance issues (Salib et al., 2003). Therefore, there is a dire need to identify effective and safe remedies to treat bronchial asthma (Govindan et al., 1999).

Herbal medicines are being used by nearly about 80% of the world population, primarily in developing countries for primary health care (Kamboj, 2000). It has been reported that there has been an alarming increase in number of diseases and disorders caused by synthetic drugs prompting a switch over to traditional herbal medicine (Ghule and Patil, 2001). Ayurveda is a traditional Indian Medicinal System practiced for thousands of years and has described several drugs from indigenous plant sources in the treatment of bronchial asthma and allergic disorders (Charaka, 1949). The polyherbal formulations described in Ayurveda have been the basis of treatment of various human diseases. Selection of scientific and systematic approach for the biological evaluation of herbal formulations based on their use in the traditional systems of medicine forms the basis for an ideal approach in the development of new drugs from plants.

In the light of above background, the present study aimed at evaluation of a polyherbal formulation for the possible anti-asthmatic action using experimental animals.

Materials and Methods

Chemicals and Equipments

Trypsin and egg albumin were purchased from Rakesh Chemicals, India. Dexamethasone was obtained from Suvik Pharmaceutical Private Limited, India. Curcuminoids, Vasicine and Solasodine were procured from Avance Phytochemical Ltd, Ahmedabad, India while Piperine was obtained from Amsar Pvt Ltd, Indore, India. Saline (0.9 % m/V NaCl solution) and phosphate buffered saline (pH 7.2) (8 g NaCl, 0.2 g KCl, 1.44 g NaHPO₄·2H₂O and 0.24 g KH₂PO₄ in 1 L of distilled water) was used. All the chemicals were of analytical grade.
Student physiograph from Biodevices, India was used to measure AHR in mice. Arterial O₂ tension (pO₂) was performed with the help of Pulse Oxymeter; Nasan Surgical Devices, India.

**Test material**
The plant materials (leaves of *Adhatoda vasica* Nees., roots of *Clerodendrum serratum* Linn., rhizomes of *Curcuma longa* Linn., fruits of *Solanum xanthocarpum* Schrad & Wendl. and fruits of *Piper longum* Linn.) were obtained from Government Ayurvedic Udhyayan, Gandhinagar, Gujarat, India. They were identified and authenticated by the Department of Pharmacognosy, K.B. Institute of Pharmaceutical and Education Research, Gandhinagar, India. Voucher specimens (PH/508/002, PH/508/003, PH/508/004, PH/508/005, and PH/508/006) were deposited at the Department of Pharmacognosy, K.B. Institute of Pharmaceutical and Education Research, Gandhinagar, India. The individual plants were evaluated with regard to their standard specifications according to the ‘Ayurvedic Pharmacopoeia of India’. Ethanolic extracts were prepared by extracting each plant with ethanol using soxhlet extractor and each extract was standardized to 0.25%/w/w vasicine, 3.4%/w/w piperine, 1.56%/w/w curcumin and 0.04%/w/w solasodine by HPTLC method. PHE was prepared using ethanolic extract of *Adhatoda vasica* Nees. (leaves), *Clerodendrum serratum* Linn. (roots), *Curcuma longa* Linn. (rhizomes), *Solanum xanthocarpum* Schrad & Wendl. (fruits) and *Piper longum* Linn. (fruits) in proportion of 40%, 30%, 10%, 10% and 10%, respectively (Gohil et al., 2011).

**In Vivo Study**

**Animals**
Healthy albino mice of either sex (N=24), weighing 25-30 g; procured from Zydu's Research Centre, India. The animals were housed at 25 ± 1ºC; 50 ± 15% RH for 12 hours light-dark cycles, in polypropylene cages with free access to food and water ad libitum. The animals were divided into four groups of six animals each.

- **Group I – Normal control**
- **Group II – Asthma**
- **Group III – Asthma + PHE**
- **Group IV – Asthma + Dexamethasone**

All animals (except group-I) were exposed to aerosol of trypsin (1 mg mL⁻¹, 1 mL⁻¹ per min.) once daily for 5 min, followed by a rest of 2 h and then exposed to egg albumin aerosol (1% m/V solution, 1 mL⁻¹ per min.) for 3 min. This procedure was repeated for 10 days and later, egg albumin aerosol was discontinued whereas trypsin exposure was continued till the 21st day. On the 21st day after last exposure to trypsin, the animals were examined for parameters mentioned below. Group II animals were exposed to trypsin and egg albumin, but did not receive any drug treatment. They served as asthmatic control animals. Animals of group-III received PHE (200 mg kg⁻¹, p.o) and animals of group-IV received dexamethasone (5 mg kg⁻¹, p.o) from day 22 to day 35. Group I animals did not receive any treatment except saline and served as normal control. On day 35, after 2 h of the last dose of treatment, only egg albumin challenge was given.

On day 1 before any exposure (basal value), on day 21 after trypsin exposure and on day 35 after egg albumin challenge, the following parameters were measured for each animal: pO₂, lung function test (respiratory rate, air flow rate, tidal volume), blood testing (bicarbonate).

On day 35, in addition to the above parameters, the following parameters were also measured: BAL, and histopathology of lung tissue.

**Measurement of pO₂**
The measurement of arterial O₂ tension (pO₂) was performed done with the help of Pulse Oxymeter instrument according to the methods described by Apps et al., 1992 and Fabbri et al., 1997.

**Measurement of serum bicarbonate**
The method used in the present study to measure serum bicarbonate level was slightly modified from that described by Godkar, 1996. About 1-2 mL⁻¹ of blood was collected from each animal under anesthesia after 1 h of the exposure to egg albumin. The serum was separated from blood by minimum exposure to air and stored in a sealed tube till bicarbonate level was estimated. For bicarbonate level measurement, 10 mL⁻¹ of 1 g per dL⁻¹ saline was pipetted out in 100 mL⁻¹ beaker. To this, 0.1 mL⁻¹ of the serum and 2 drops of phenol red indicator were added and mixed well. In the above mixture, NaOH (0.01 N) was added drop wise till the end point was achieved (7.35 pH or color changed from yellow to pink). The volume of NaOH required was noted down and considered as control reading (X mL⁻¹). In another set, 9.0 mL⁻¹ of 1 g per dL⁻¹ saline and 1 mL⁻¹ HCL (0.01 N) were added. The above procedure was repeated and volume of NaOH required was noted (Y mL⁻¹).

Now, Burette Reading R = Y mL⁻¹ – X mL⁻¹ and serum bicarbonate level (mEq L⁻¹) = (1-R) × 100.

**Measurement of respiratory rate, airflow rate and tidal volume**
The measurement of tidal volume, respiratory rate and airflow rate were done with the help of Respiratory volume transducer that were used with strain gauge coupler and student physiograph. The strain gauge coupler was calibrated with the help of standard 0.02 cc volume calibrator (Khandpur, 1996; Guyton and Hall, 2006).
BAL fluid: differential leukocyte count

On the 35th day, after 3 h of egg albumin challenge or just prior to animal death, whichever were earlier, the tracheobronchial tree was lavaged with 1 mL⁻¹ of saline 3 to 4 times. The fluid was collected and centrifuged at 2000 rpm for 5 min. The supernatant was discarded and pellet was re-suspended in 0.5 mL⁻¹ saline. A thin film of 2000 rpm for 5 min. The supernatant was discarded and kept for 15 min or more. It was washed off with tap water and dried. The number of each type of leukocytes was determined under the microscope at 450× magnification (Vogel, 2002).

Histopathology of lungs

On the 35th day, the animals were sacrificed and their lungs were dissected out. The procedure used for histopathological study was fixation of the tissue with formalin, embedding in paraffin blocks, sectioning with microtome (0.7 μ thicknesses) and finally staining by hematoxylin and eosin stain technique (Garg et al., 1996).

Statistical analysis

The experimental results have been expressed as the mean ± SEM (N=6). Statistical significance of difference in parameters amongst groups was determined by one way ANOVA followed by Tukey’s multiple range test. p<0.05 was considered statistically significant.

Results and Discussion

Trypsin and egg albumin induced asthma model is clinically relevant experimental model that replicates most of the features of chronic human asthma. Trypsin induces inflammatory changes and airway remodeling while egg albumin incorporates allergic components of asthma (Schmidlin et al., 2002; Ebeling et al., 2005; Kim et al., 2009). Therefore, this study was basically focused on determining the effect of PHE in trypsin and egg-albumin induced experimental model of asthma.

In asthmatic patients, analysis of blood gases reveals a severe hypoxemia with arterial oxygen (pO2) lower than 60 mmHg, hypocapnia and respiratory alkalosis (Papiris et al., 2002). As severity of airflow obstruction increases, pCO2 first normalizes and subsequently increases. Increased pCO2 level in serum will eventually result in increased bicarbonate levels because carbon dioxide in blood is transported as bicarbonate (Sembulingum and Sembulingum, 2006). In the present study, the sensitized animal when challenged with egg albumin, showed a lower serum pO2 level and higher bicarbonate level similar to that of observed in patients of asthma. Animals treated with PHE and dexamethasone prior to the challenge with antigen significantly improved the condition. Serum oxygen level (pO2) was significantly (p<0.05) higher and serum bicarbonate level remained significantly (p<0.05) lower than the asthmatic control group (Table 1).

Next important parameters for asthma include the respiratory functions like tidal volume, respiratory rate and airflow rate. Tidal volume is the volume of air inspired or expired per breath. In asthma, which is an obstructive disease, there is a difficulty in expiration, and hence the volume of air expired is decreases. In addition, there is shallow and rapid breathing thus decreasing the tidal volume and simultaneously increasing the respiratory rate. The lungs do not provide adequate respiratory exchange due to constricted air flow volume and the levels of oxygen in the blood begin to fall (Venegas et al., 2005). As a consequence of this, air flow rate, which is directly proportional to tidal volume and respiratory rate, also decreases. In the present study, significantly (p<0.05) lower tidal volume and air flow rate were observed in asthmatic control group as compared to normal control group after egg albumin challenge but there was a significant (p<0.05) increase in tidal volume and air flow rate in dexamethasone and PHE-treated animals. In contrast to tidal volume and air flow rate, significantly (p<0.05) lower respiratory rate was observed in dexamethasone and PHE-treated groups as compared to asthmatic control group (Table 2).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Group I</th>
<th>Group II</th>
<th>Group III</th>
<th>Group IV</th>
</tr>
</thead>
<tbody>
<tr>
<td>pO2 level (%)</td>
<td>95.67±1.54</td>
<td>67.00±3.31***</td>
<td>82.00±2.63*</td>
<td>90.67±2.35*</td>
</tr>
<tr>
<td>Serum bicarbonate level (mmol L⁻¹)</td>
<td>26.17±1.52</td>
<td>55.67±1.92***</td>
<td>32.17±2.04*</td>
<td>27.17±1.59*</td>
</tr>
</tbody>
</table>

Values represent mean ± SEM of six animals in each group.
Group I – normal control, group II – asthma, group III – PHE (200 mg kg⁻¹), group IV – dexamethasone (5 mg kg⁻¹).
Significant difference between groups I and group II: *** p<0.001.
Significant difference between groups III-IV and group II: * p<0.05.

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<td>Respiratory rate (breaths per min)</td>
<td>181.33±12.84</td>
<td>81.17±3.15***</td>
<td>165.67±7.57*</td>
<td>175.33±10.41*</td>
</tr>
<tr>
<td>Air-flow rate (mL⁻¹per min)</td>
<td>10.26±0.59</td>
<td>2.19±0.21***</td>
<td>6.98±0.31*</td>
<td>8.72±0.48*</td>
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<tr>
<td>Tidal volume (mL⁻¹)</td>
<td>0.057±0.003</td>
<td>0.027±0.002***</td>
<td>0.043±0.003*</td>
<td>0.050±0.002*</td>
</tr>
</tbody>
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Values represent mean ± SEM of six animals in each group.
Group I – normal control, group II – asthma, group III – PHE (200 mg kg⁻¹), group IV – dexamethasone (5 mg kg⁻¹).
Significant difference between groups I and group II: *** p<0.001.
Significant difference between groups III-IV and group II: * p<0.05.
Asthma is always associated with strong inflammatory component which gives a chronic status to this disease. The presence of peripheral blood eosinophilia and activated eosinophils in the chronic inflammatory infiltrate of the airways is characteristic of both allergic and non-allergic asthma. In asthmatic patients, after transendothelial migration, eosinophils adhere to bronchial epithelium, where they degranulate and release eosinophil cation protein, major basic protein, eosinophil peroxidase and superoxide causing damage to epithelium. This can be observed by a higher eosinophil count (Milos and Snezana, 2002; Wardlaw, 2001). Challenging of animals with trypsin and egg albumin showed significantly ($p<0.001$) higher eosinophil count in asthmatic control group as compared to normal control group on 35th day of study. Also, there was a significant ($p<0.001$) decrease in eosinophil count, in the animals subjected to dexamethasone and PHE treatment (Fig. 1). Various processes involved in bronchial asthma such as inflammatory response can explain various histopathological alterations observed in biopsy of asthmatic patients. In asthma chronic inflammation is responsible for the bronchoconstriction which leads to airway narrowing and decrease in the lumen size of the bronchiole (Kelly and Sorknes, 2005). This can be clearly seen by the histopathological studies of the lung tissue by observing the cross section of bronchi. In the present study, the sections of the lung tissues of animals sensitized with egg albumin depicted marked bronchitis and severe bronchoconstriction [Fig. 2(b)]. Treatment with dexamethasone and PHE prevented the inflammation and bronchoconstriction which leads to normal lumen size and normal cellular structure compared to antigen sensitized animals [Fig. 2(c) & Fig. 2(d)].

PHE is reported to have anti-inflammatory, bronchodilatory, anti-anaphylactic and mast-cell stabilizing activity (Gohil et al., 2011; Gohil and Mehta, 2011) which might be responsible for its anti-asthmatic action.

**Fig. 1.** Effect of PHE and dexamethasone on eosinophil count in BAL fluid.

Values represent mean ± SEM of six animals in each group.
Group I – normal control, group II – asthma, group III – PHE (200 mg kg$^{-1}$), group IV – dexamethasone (5 mg kg$^{-1}$).
Significant difference between groups I and group II: *** $p<0.001$.
Significant difference between groups III-IV and group II: *$p<0.05$. 
Conclusions

It can be concluded that PHE has beneficial effects in trypsin and egg-albumin induced experimental model of bronchial asthma and might be used for the treatment of bronchial asthma. However, further clinical research of PHE will be necessary to support the present investigation.

References

Actinidia arguta, in a murine ovalbumin-induced asthma model. 


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