EVALUATION OF THE EFFECT OF HYDROALCOHOLIC EXTRACTS OF CASSIA OCCIDENTALIS IN HISTAMINE INDUCED BRONCHOSPASM ON GUINEA PIGS

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ABSTRACT
Evaluation of the effect of hydroalcoholic extracts of Cassia occidentalis in bronchospasm induced by histamine hydrochloride on Guinea pigs. Exposure to an aerosol of 0.1% Histamine hydrochloride in closed chamber was given to induce bronchospasm on Guinea pigs. The effect of oral administration of hydroalcoholic extract of Cassia occidentalis seeds on histamine induced bronchospasm has been studied and is compared with the effect of oral administration of Ketotifen as standard on Guinea pigs. An administration of an aerosol of Histamine hydrochloride resulted in bronchospasm as well as decreased pre-convulsion time. Supplementation with hydroalcoholic extract of Cassia occidentalis seeds significantly increased the pre-convulsion time. The results indicate that the seed of Cassia occidentalis is endowed with bronchospasmolytic activity.

KEYWORDS: Cassia occidentalis, histamine, ketotifen, pre-convulsion time, analysis of variance.

INTRODUCTION
Asthma is a complex syndrome with many clinical phenotypes in both children and adults. Its major characteristics include a variable degree of air flow obstruction, bronchial hyperresponsiveness, and airway inflammation. For many patients, the disease has its roots in infancy, and both genetic factors (atopy)¹¹ and environmental factors (viruses, allergens and occupational exposure) contribute to its inception and evolution. Asthma is a common and costly health condition. More than the 30 million people in the united state have asthma. More women than men suffer from asthma and have a much higher death rate. Every year, asthma is responsible for about 5,00,000 hospitalization and 5,000 death only in USA. Around 8% people of the Swiss population suffer from asthma. In Western Europe as a whole, asthma has doubled in ten years. There are about 3 million asthmatics in Japan of whom 70% have severe and 30% have moderate asthma.¹²-⁴
An initial event in asthma seen to be the release of inflammatory mediators (e.g., histamine, leukotrienes and prostaglandins) triggered by exposure to allergens, irritants, cold air or exercise. The mediators are released from bronchial mast cell, alveolar macrophages, T-lymphocytes and epithelial cells. Some mediators directly cause acute bronchoconstriction, termed the “early-phase asthmatic response”. The inflammatory mediators also direct the activation of eosinophils and neutrophils, and their migration to the airway, where they cause injury. This is called “late-phase asthmatic response” results in epithelial damage, airway edema, mucus hypersecretion and hyperresponsiveness of bronchial smooth muscle.\[5\]

Microscopically, asthma is characterized by presence of increased number of eosinophils, lymphocytes, and plasma cells in the bronchial tissues, bronchial secretion, and mucus. The cross linkage of two IgE antibodies molecules by allergen causes mast cells to degranulate; releasing histamine, leukotrienes, and other mediators that perpetuate the airway inflammation. IL-5 activates the recruitment and activation of eosinophils. The activated mast cells and eosinophils also generate their cytokines that help to perpetuate the inflammation. The repeated cycles of inflammation in the lung with injury to the pulmonary tissues followed by repair may induce long term structural changes of the airways.\[6\]

Asthma is a disease characterized by redcurrant of reversible airway obstruction with attack of wheeze, shortness of breath and often nocturnal cough. Essential features of asthma are airway inflammation which causes bronchial hyper responsiveness which in turn results in recurrent reversible airway obstruction. There are various causative factors for asthma like allergens, drugs induced asthma, cold air, irritant chemicals etc.

Various allopathic drugs like corticosteroids, anticholinergics, mast cell stabilizers leukotriene antagonists, B2 receptor agonist etc., are in use for the treatment for asthma. In the most extents, these drugs have been helpful in the symptomatic relief, treatment and prophylaxis of asthma. But the involvement of debilitating side effects is major drawback of these drugs. For example, long-term treatment with corticosteroids leads osteoporosis, skeletal muscle myopathy, obesity etc.\[7\]

As a result of problems in asthma, there is high prevalence of usage of alternative traditional of medicines for the treatment of asthma. Ayurveda offers a unique insight into comprehensive approach to asthma management through proper care of respiratory tract. Medicinal herbs have been in use for thousands of years, in one form or another, under the indigenous system of medicine like Ayurveda, Siddha, and Unani. Mostly used herbal drugs are Curcuma longa, Indigofera tinctoria, Asystasia gangetica, C. gigantia, Bryonia laciniosa etc. Options available
today have many limitations including risk of adverse effects, where as herbal world offers many options with safety, efficacy & availability with economical aspects also. *Cassia occidentalis*, commonly known as “Kasundri”, is one of the leading drugs used as alternative system of treatments. As per traditional method, it is known for its expectorant activity. Seeds are 40 or more in each pod which are ovoid, compressed at one end and rounded at the other, 6 mm long, 4 mm broad, hard, smooth, shining, dark olive green or pale brown in color.

In light of this, the objective of the study is to evaluate the effect of hydroalcoholic extracts of *Cassia occidentalis* in bronchospasm.

**MATERIALS AND METHODOLOGY**

**Collection and identification of plant material:**

Collection of seed part of *Cassia occidentalis* was done from the wild sources nearby Saurashtra University campus and Kalawad road area of city Rajkot during Mid October to December, 2009 and identification and authentication were done by local botanist and by national authority. (Specimen No: SSIPER/Herb/01)

**Preparation of extract:**

The seeds of the plant *Cassia occidentalis* Linn are collected and dried under roof. The dried seeds are roasted in hot air oven at 110°C. Roasted seeds were powdered and allowed to pass from sieve no. 40. This powder was then placed in the soxhlet apparatus for extraction process. About 500 gm of air dried powder of seeds of *Cassia occidentalis* were extracted in soxhlet with 20:80 ethanol:water hydroalcoholic mixture in soxhlet apparatus by continuous hot extraction. After extraction, the solvent was recovered using distillation assembly, and the extract was concentrated. The extract was preserved in air tied container for experiment.

**Animal selection:**

Healthy, New Zealand guinea pigs of either sex were used for this study. They were housed at ambient temperature (22±1°C), relative humidity (55±5%) and 12h/12h light dark cycle. Animals had free access to standard pellet diet and water given *ad libitum*. The protocol of the experiment was approved by the Institutional Animal Ethical Committee (IAEC) as per the guidance of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Social Justice and Empowerment, Government of India.

**Bronchospasm induced by Histamine in guinea pigs**

Healthy New Zealand guinea pigs of either sex weighing range of 550-700 g were used and divided into four groups. Each groups containing six animals.

Group I: Disease Control (vehicle)
Group II: Standard (Ketotifen, 1 mg/kg, p.o.)
Group III: Hydroalcoholic extract of *C. occidentalis* seed (30 mg/kg, p.o.)
Group IV: Hydroalcoholic extract of *C. occidentalis* seed (60 mg/kg, p.o.)

All test and standard drug were dispersed in 0.5% Sodium CMC, 0.2 ml/kg, p.o. as vehicle. The animals were kept in a closed chamber (30×30×15 cm) and exposed to an aerosol of 0.1% Histamine hydrochloride and pre-convulsion time (PCT) (The time of aerosol exposure to the onset of dyspnoea leading to the appearance of convulsions) was noted. As soon as symptoms like convulsion had appeared in animals, animals were removed from the chamber and placed in fresh air to recover. PCT was taken as basal value. Later, all treatments were given orally to all respective groups once daily for 7 days. After 7 days, two hours after the respective drug treatment, animals were exposed to histamine hydrochloride aerosol and PCT was measured by nebulizer pump (Aero space nebulizer). The effect of drug was calculated by the following formula.

\[
% \text{ Increase in PCT} = [1 - T1/T2] \times 100,
\]

Where, 
T1= PCT on day 0,
T2= PCT on day 7.

**Statistical analysis:**
Results were expressed as mean ± SEM. Differences among data were determined using one-way ANOVA followed by Student–Newman–Keul’s test (Graphpad Prism software for Windows, Version 4.10.1998). Differences between the data were considered significant at \( P < 0.05. \)

**RESULTS**
In the present study, administration of 0.1% histamine to healthy guinea pigs resulted in bronchospasm and seen in the form of Pre-Convulsion Dyspnoea (PCD). % increase in Pre-convulsion time was lower in disease control group. However, supplementation with hydroalcoholic extract of *Cassia occidentalis* seeds significantly \( (P < 0.001) \) delayed onset of pre-convulsion dyspnoea. Treatment with Ketotifen (1 mg/kg, p.o.), as a standard drug; HECS (30 mg/kg and 60 mg/kg, p.o.) given 7 days before aerosol exposure delayed onset of pre-convulsion dyspnoea (PCD) \((73.48 \pm 0.53 \%, \ 40.52 \pm 0.47 \% \ \text{and} \ 62.94 \pm 0.52 \% \) respectively in guinea pigs. These significantly and dose dependently increased the onset of convolution time in guinea pigs. The bronchodilating effect of test drug was compared to standard control (ketotifen) (1 mg/kg). (Table 1, Figure 1)
Table 1: Effect of hydroalcoholic extract of *Cassia occidentalis* on Histamine induced bronchospasm in guinea pigs

<table>
<thead>
<tr>
<th>Groups</th>
<th>Dose (mg/kg p.o.)</th>
<th>% Increase in preconvulsion time</th>
</tr>
</thead>
<tbody>
<tr>
<td>Disease Control</td>
<td>0.2 ml</td>
<td>2.71 ± 0.20</td>
</tr>
<tr>
<td>Standard control</td>
<td>1</td>
<td>73.48 ± 0.53***</td>
</tr>
<tr>
<td>HECS</td>
<td>30</td>
<td>40.52 ± 0.47***</td>
</tr>
<tr>
<td>HECS</td>
<td>60</td>
<td>62.94 ± 0.52***</td>
</tr>
</tbody>
</table>

All values represented as Mean ± S.E.M. of six animals.

*** indicates significance at the level of *p* < 0.001.

**DISCUSSION**

Since bronchodilators, mediator release inhibitors and anti-inflammatory drugs are the different classes of drugs used conventionally in the treatment of bronchial asthma; various animal models and experimental protocols were used in the present study to evaluate anti-asthmatic activity of seeds of *Cassia occidentalis* Linn. Bronchial asthma is characterized by increased airway reactivity to spasmogens.\(^9\) 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.

Guinea pig ileum was used for screening of antihistaminic activity. Stimulation of H1 receptors produces graded dose related contraction of isolated guinea pig ileum.\(^{[10]}\)

In present study, significant increase in preconvulsion time was observed due to pretreatment with seeds of \textit{Cassia occidentalis} Linn., when the guinea pigs were exposed to either histamine aerosol. This bronchodilating effect of seeds at high dose was comparable to ketotifen. Spasmylytic effect of \textit{Cassia occidentalis} Linn. seed was also evaluated by observing the effect of hydroalcoholic extract of seeds (30 and 60 mg/kg) on histamine induced ileum contractions to seek for scientific evidence for beneficial use of seeds in spasm produced by any means. The results showed antagonistic effects of the seeds against the contraction induced by the standard spasmogens. The maximum effects of histamine induced contractions were inhibited in the presence of the seeds extract at 60 mg/kg.

In conclusion, the presented data indicate that administration of the hydroalcoholic extract of \textit{Cassia occidentalis} seeds to Guinea pigs with histamine induced bronchospasm, reduced and prevented the spasm of bronchi, supporting folk information regarding antiasthmatic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to dilatation of bronchi. These effects could conclude that \textit{Cassia occidentalis} has an antiasthmatic property.

\textbf{REFERENCES}


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