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Evaluation of the effect of hydroalcoholic extracts of Cassia occidentalis in clonidine induced mast cell degradation in Rats

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ABSTRACT:

Evaluation of the effect of hydroalcoholic extracts of Cassia occidentalis in mast cell degradation induced by clonidine in Rats. The effect of oral administration of hydroalcoholic extract of Cassia occidentalis leaves on clonidine induced mast cell degradation has been studied and is compared with the effect of oral administration of Sodium cromoglycate as standard on rats. A clonidine resulted in mast cell degradation. Supplementation with hydroalcoholic extract of Cassia occidentalis leaves significantly increased the percentage protection against mast cell degradation. The results indicate that the leaf of Cassia occidentalis is endowed with protected in mast cell degradation.

KEY WORDS: Cassia occidentalis, clonidine, sodium cromoglycate, mast cell degradation, analysis of variance

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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)[1] 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. [2-4]

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. Leaves are 15 to 20 cm long, arranged alternately along the stem, pitiolate, stipulate, peripinnate; Petiole with a distinct spherical gland that is 3-5 mm at its asymmetric base, 3 to 5 pairs of leaflets short stalked, ovate oblong to ovate lanceolate, acute or acuminate, glabrous, 4.5 to 10.5 cm length and 3 to 4.5 cm breadth. Leaves are green in colour, foetid in odour and slightly bitter in taste. They have slippery and papery texture, entire margin, pubescent surface and reticulate venations.

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

Preparation of extract:

Leaves of *Cassia occidentalis* were collected and properly cleaned and dried under shade to remove excess of moisture. The dried plant material was then subjected to size reduction to coarse powder and passed the powder from sieve no. 40. About 500 gm of air dried powder of leaves of *Cassia occidentalis* were extracted in soxhlet with 20:80 ethanols:water hydroalcoholic mixture in soxhlet apparatus by continuous hot extraction. After each extraction, the solvent was recovered using distillation assembly, and the both extracts were concentrated. The extracts were preserved in air tied container for experiment.

Animal selection:

Healthy adult Albino Wistar rats 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.

Mast cell degradation induced by Clonidine in rats[8]

Healthy adult Albino Wistar rats were grouped into 7 groups. Each group contained six rats. Duration of treatment of standard and test drugs was seven days.

Group I: Disease control (0.5% w/w Sodium CMC, 0.2 ml/kg, p.o. as vehicle)

Group II: Standard received Sodium cromoglycolate (50 mg/kg, i.p.)

Group III: Hydroalcoholic extract of *C. occidentalis* leaf (100 mg/kg, p.o.)

Group IV: Hydroalcoholic extract of *C. occidentalis* leaf (200 mg/kg, p.o.)

On 7th day rats were anesthetized with ether, 10 ml of normal saline injected into peritoneal cavity. After gentle abdominal message, the peritoneal fluid were collected in centrifuge tubes placed over ice and 2,000 rpm for 5 min. Supernatant solution was discarded and the cells were washed twice with saline and resuspended in 1 ml of saline. To this 0.5 µg/ml of clonidine solution was added and incubated at 37^{o} C in water bath for 10 minutes. After 10 min., it was stained with 1% clonidine solution and examined microscopically for the number of intact and degranulated mast cells. A total of 100 cells were counted from different visual areas and percentage protection against clonidine induced mast cell degranulation was calculated.

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, staining with clonidine to healthy adult rats resulted in mast cell degradation. % protection in mast cell degradation was higher in disease control group. However, supplementation with hydroalcoholic extract of *Cassia occidentalis* leaves significantly ($P < 0.001$) decreased mast cell degranulation. Treatment with Sodium cromoglycate (50 mg/kg, i.p.), as a standard drug; HECL (100 mg/kg and 200 mg/kg, p.o.) given 7 days. The group of animals pretreated with hydroalcoholic extract of *Cassia occidentalis* leaves showed significant protection in degranulation of mast cells (32.36 ± 1.98 and 39.34 ± 1.8) at the dose 100 mg/kg, 200 mg/kg b.w. when challenged with clonidine. These significantly decreased the percentage protection in rats. The protection in mast cell degranulation of test drug (HECL) was comparable to standard control (Sodium cromoglycate) (50 mg/kg b.w.). (Table 1, Figure 1)

DISCUSSION

In the present study, the hydroalcoholic extract of *Cassia occidentalis* Linn. leaves was found to inhibit the degranulation of mast cells induced by an immunological and a non-immunological stimulus. It is known that the physiological stimulus for the release of histamine from mast cells is provided by a combination of antigen with specific antibody fixed on the cell surface. This combination is believed to transiently increase the permeability of membrane to calcium ions showing an absolute requirement for calcium ions for the secretory process to occur.[9] Anaphylactic and clonidine induced secretion from mast cells share a common requirement as far as the presence of calcium ions is concerned. However, clonidine can utilize intracellular calcium stores to initiate the release process, even in the absence of calcium in the extracellular medium. [10] On the other hand, anaphylactic release requires the presence of calcium in the extracellular medium which moves onto the cell via calcium gates in the membranes.[11,12] A significant protection of rat peritoneal mast cells from disruption by antigen and clonidine by extract *Cassia occidentalis* Linn. points towards its ability to interfere the release and/or synthesis of mediators of inflammation, indicating its mast cell stabilizing activity. Hence it may be assumed that the cytoprotective effect induced by extract of *Cassia occidentalis* Linn. on mast cell surface could be due to its ability to alter the influx of calcium ions.

In the present study, the hydroalcoholic extract of leaves of *Cassia occidentalis* Linn. were found to inhibit the degranulation of mast cells induced by an immunological and a non-immunological stimulus. It is known that the physiological stimulus for the release of histamine from mast cells is provided by a combination of antigen with specific antibody fixed on the cell surface. This combination is believed to transiently increase the

permeability of membrane to calcium ions showing an absolute requirement for calcium ions for the secretory process to occur.[9]

In conclusion, the presented data indicate that administration of the hydroalcoholic extract of *Cassia occidentalis* leaves to rats with clonidine induced mast cell degradation, reduced and prevented the degranulation, supporting folk information regarding antiasthmatic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to protection of degranulation. These effects could conclude that *Cassia occidentalis* has an antiasthmatic property.

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TABELS AND FIGURES

Table 1: Effect of hydroalcoholic extract of Cassia occidentalis on Clonidine induced mast cell degranulation in rats

Groups	Dose (mg/kg p.o.)	Mast cells %		% protection
		Intact	Disrupted	
Disease Control	0.2 ml	23.00 ± 0.57	77.00 ±1.97	77.00 ±1.97
Standard control	50	76.00 ± 0.88	24.00 ± 0.98	24.00 ± 0.98
HECL	100	60.66 ± 1.02	39.34 ± 1.80	39.34 ± 1.80
HECL	200	67.64 ± 1.08	32.36 ± 1.98	32.36 ± 1.98

All values represented as Mean ± S.E.M. of six animals. *** indicates significance at the level of p< 0.001.

Figure 1: Effect of hydroalcoholic extract of Cassia occidentalis on Clonidine induced mast cell degranulation in rats