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Evaluation of the Effect of Hydroalcoholic Extracts of Cassia Occidentalis Seeds in Clonidine Induced Mast Cell Degradation in Rats

D. M. Patel¹, Urvisha V. Bangoriya², Dr. H. M. Tank³, Ms. P. H. Tank⁴

1. Sal Institute of Pharmacy, Ahmedabad, Gujarat, India.

2. Drug Inspector, Food and Drug Administration, Gujarat, India.

3. Matushri V. B. Manvar College of Pharmacy, Dumiyani, Rajkot, Gujarat, India.

4. Department of Pharmacy, Saurashtra University, Rajkot, Gujarat, India.

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*For Correspondence:

Dr. Dhaval M. Patel,

Sal Institute of Pharmacy, Ahmedabad,
Gujarat, India.

(www.jpsbr.org)

ABSTRACT:

Evaluation of the effect of hydroalcoholic extracts of Cassia occidentalis seeds in mast cell degradation induced by clonidine in Rats. The effect of oral administration of hydroalcoholic extract of Cassia occidentalis seeds 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 seeds significantly increased the percentage protection against mast cell degradation. The results indicate that the seed of Cassia occidentalis is endowed with protected in mast cell degradation.

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

INTRODUCTION:

Acute respiratory infection, tuberculosis and chronic obstructive pulmonary disease rank third, fourth and fifth respectively as per the global health situation. Respiratory diseases are second to cancer as the causes of death and disability to adults.^[1]

Asthma is a disease characterized by recurrent 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.

Asthma is the commonest disease in children in economically developed countries and it is also usual in adults and it is increasing in prevalence and severity.

Around 275 million people around the globe suffer from asthma and this number is rising worldwide, deaths from this condition have reached 18 million annually. The number of deaths from asthma also has increased in the United States of America. The world health organization says about five thousand Americans die from asthma attacks each year. In India, rough estimates indicate a prevalence of between 10-15% in 5-11 years old children.^[2] The health burden of asthma is increasing globally at an alarming rate, providing a strong impetus for the development of new therapeutics.

Bronchial asthma is a complex disease with several clinically well-defined pathogenic components, including recurrent reversible airway obstruction, chronic airway inflammation and development of airway hyperresponsiveness.^[3] Airway inflammation is the primary problem in asthma. An initial event in asthma appears to be the release of inflammatory mediators (e.g.,

histamine, tryptase, leukotrienes and prostaglandins) triggered by exposure to allergens, irritants, cold air or exercise. 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.^[4] The gross pathology of asthmatic airway display lung hyperinflation, smooth muscle hypertrophy, lamina reticularis thickening, mucosal edema, epithelial cell sloughing, cilia cell disruption, and mucus gland hypersecretion. Regardless of the triggers of asthma, the repeated cycles of inflammation in the lung with injury to the pulmonary tissues followed by repair may produce long term structural changes (“remodeling”) of the airways.^[5]

The world health organization (WHO) has recognized herbal medicine as an essential building block for primary health care of vast countries like India and China. India is perhaps the largest producer of medicinal herbs and is rightly called the “botanical garden of the world”. There are very few medicinal herbs of commercial importance, which are not collected or cultivated in this country. 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. Since independence in 1947, India has made tremendous progress in agro technology, process technology, standardization, quality control, research and development. Large numbers of herbs are being used for the treatment and prophylaxis of asthma since ancient time, however systemic studies that identify all possible mechanism of each and every herbal antiasthmatic drug are lacking.

Various allopathic drugs like corticosteroids, anticholinergics, mast cell stabilizers leukotriene antagonists, B2 receptor agonist etc., are in use for the treatment for 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.^[6]

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. India is perhaps the largest producer of medicinal herbs and is rightly called the “botanical garden of the world”. 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. Since independence in 1947, India has made tremendous progress in agro technology, process technology, standardization, quality control, research and development. So many herbal drugs are used in treatment of bronchospasm. Mostly used herbal drugs are *Curcuma longa*, *C. gigantia*, *Indigofera tinctoria*, *Asystasia gangetica*, *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 of *Cassia occidentalis* 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.^[7]

In light of this, the objective of the study is to evaluate the effect of hydroalcoholic extracts of *Cassia occidentalis* seeds in mast cell degradation.

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 ethanols: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 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 (SJT-58/2012) 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.

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* seed (30 mg/kg, p.o.)

Group IV: Hydroalcoholic extract of *C. occidentalis* seed (60 mg/kg, p.o.)

On 7th day rats were anesthetized with ether, 10 ml of normal saline injected into peritoneal cavity. After gentle abdominal massage, 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° 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* seeds significantly (*P* < 0.001) decreased mast cell degranulation. Treatment with Sodium cromoglycate (50 mg/kg, i.p.), as a standard drug; HECS (30 mg/kg and 60 mg/kg, p.o.) given 7 days. The group of animals pretreated with hydroalcoholic extract of *Cassia occidentalis* seeds showed significant protection in degranulation of mast cells (54.50 ± 2.01 and 30.00 ± 0.19) at the dose 30 mg/kg, 60 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 (HECS) was comparable to standard control (Sodium cromoglycate) (50 mg/kg b.w.). (Table 1, Figure 1)

Table 1: Effect of hydroalcoholic extract of *Cassia occidentalis* seeds 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***
HECS	30	45.50 ± 1.03	54.50 ± 2.01	54.50 ± 2.01***
HECS	60	70.00 ± 0.98	30.00 ± 0.19	30.00 ± 0.19***

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

DISCUSSION

In the present study, the hydroalcoholic extract of *Cassia occidentalis* Linn. seeds was found to inhibit the degranulation of mast cells induced by an immunological and a non-immunological stimulus. It is known that the

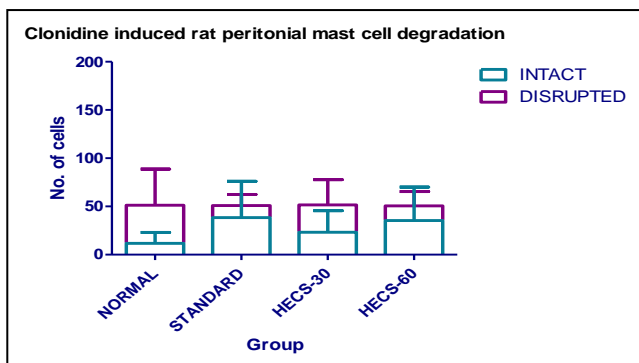


Figure 1: Effect of hydroalcoholic extract of *Cassia occidentalis* seeds on Clonidine induced mast cell degranulation in rats

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 seeds 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* seeds to rats with clonidine induced mast cell degranulation, 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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