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## Evaluation of the effect of hydroalcoholic extracts of *Cassia occidentalis* in carrageenan induced paw edema in Rats

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### ABSTRACT:

Evaluation of the effect of hydroalcoholic extracts of *Cassia occidentalis* in paw edema induced by Carrageenan in Rats. The effect of oral administration of hydroalcoholic extract of *Cassia occidentalis* seeds on carrageenan induced inflammation has been studied and is compared with the effect of oral administration of Diclofenac sodium as standard on rats. A carrageenan resulted in paw edema. Supplementation with hydroalcoholic extract of *Cassia occidentalis* seeds significantly decreased paw edema. The results indicate that the seed of *Cassia occidentalis* is endowed with protected in paw edema.

**KEY WORDS:** *Cassia occidentalis*, carrageenan, diclofenac sodium, paw edema, analysis of variance

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### 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. <sup>[1]</sup>

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. <sup>[2]</sup> The health burden of asthma is increasing globally at an alarming rate, providing a strong impetus for the development of new therapeutics.

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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.<sup>[3]</sup> 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.<sup>[4]</sup> 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.<sup>[5]</sup>

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.<sup>[6]</sup>

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.<sup>[7]</sup>

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

## 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.

#### Paw edema induced by Carrageenan in rats<sup>[8]</sup>

Albino wistar rats of any sex weighing 250-300 g were divided into four groups of six animals each and the treatment given to animals of each group as follows.

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

Group II: Standard (Diclofenac sodium, 20 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.)

One hour after administration of the various agents, oedema was induced by injection of carrageenan (0.1 ml, 1%, w/v in saline) into the sub-plantar tissue of the right hind paw. The linear paw circumference was then measured using the cotton thread method. Measurements of paw circumference were done

immediately before injection of the various agents and at 1 h interval for 5 h.

#### 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 carrageenan by subplantar route to healthy adult rats resulted in paw edema. In control animals, the subplantar injection of carrageenan produced a local edema that increased progressively to reach maximal intensity at 5 h after the injection of the phlogistic agent (5.42 ± 0.03). However, supplementation with hydroalcoholic extract of *Cassia occidentalis* seeds significantly ( $P < 0.001$ ) decreased paw edema. Animals were treated with Diclofenac sodium (20 mg/kg, i.p.), as a standard drug; HECS (30 mg/kg and 60 mg/kg, p.o.) as a test drug. The group of animals pretreated with hydroalcoholic extract of *Cassia occidentalis* seeds showed significant reduction in paw edema (3.45 ± 0.02 and 3.33 ± 0.06) at the dose 30 mg/kg, 60 mg/kg b.w. when challenged with carrageenan. The reduction in paw edema of test drug (HECS) was comparable to standard control (Diclofenac sodium) (20 mg/kg b.w.). (Table 1, Figure 1)

#### DISCUSSION

Airway inflammation has been demonstrated in all forms of asthma. Even in mild asthma, there is an inflammatory response involving infiltration, particularly with activated eosinophils and lymphocytes, with neutrophils and mast cells. The degree of bronchial hyperresponsiveness and airway obstruction is closely linked to the extent of inflammation.<sup>[9]</sup> Anti-inflammatory drugs suppress the inflammatory response by inhibiting infiltration and activation of inflammatory cells as well as their synthesis, or release of mediators and the effects of inflammatory mediators. Carrageenan rat paw edema is a suitable test for evaluating anti-inflammatory drugs which has been frequently used to assess the anti-edematous effect of natural products.<sup>[10]</sup>

In the present study, carrageenan induced inflammation is useful in detecting orally active anti-inflammatory agents. Oedema formation due to carrageenan in the rat paw is a biphasic event. The initial phase is attributed to the release of histamine and serotonin. The edema produced at the peak (3 h) is thought to be due to the release of kinin-like substances, especially bradykinin. The second phase is sensitive to most clinically effective anti-inflammatory drugs.<sup>[11-12]</sup> It is well established fact that non-steroidal anti-inflammatory drugs (NSAIDs) exert their anti-inflammatory activity by inhibition of prostaglandin biosynthesis.<sup>[13]</sup> The anti-edematogenic mechanism of action of *Cassia occidentalis* seeds may also be related to prostaglandin synthesis inhibition. Inflammation pain results from the release of hyperalgesic mediators - prostaglandins and catecholamines - which are supposed to act by regulating the sensitivity of pain receptors.<sup>[14-15]</sup>

In conclusion, the presented data indicate that administration of the hydroalcoholic extract of *Cassia occidentalis* seeds to rats with carrageenan induced paw edema, reduced the paw edema, supporting folk information regarding antiasthmatic activity of the plant. The mechanism underlying this effect is still unknown, but is apparently related to reduction in paw edema. These effects could conclude that *Cassia occidentalis* has an antiasthmatic property.

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**Table 1 Effect of hydroalcoholic extract of *Cassia occidentalis* on Carrageenan-induced rat paw edema in rats**

Group	Dose (mg/kg)	Time (hour) and Paw size (cm) (Mean±SEM)					
		0h	1h	2h	3h	4h	5h
Disease Control	0.2 ml	2.7	3.3	3.9	4.5	5.2	5.4
		3 ±	8 ±	7 ±	8 ±	3 ±	2 ±
		0.0	0.0	0.0	0.0	0.0	0.0
Standard control	20	3	5	4	5	3	3
		2.5	2.9	3.2	3.2	3.4	3.3
		8 ±	3 ±	2 ±	0 ±	5 ±	8 ±
HECS	30	0.0	0.0	0.0	0.0	0.0	0.0
		3	4	5	6	4	5
		2.5	2.9	3.2	3.4	3.4	3.4
HECS	60	3 ±	5 ±	7 ±	2 ±	7 ±	5 ±
		0.0	0.0	0.0	0.0	0.0	0.0
		7	6	6	6	4	2
HECS	60	2.4	2.7	3.1	3.1	3.3	3.3
		3 ±	8 ±	0 ±	8 ±	2 ±	3 ±
		0.0	0.0	0.0	0.0	0.0	0.0
		7	7	6	5	5	6

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

**Figure 1: Effect of hydroalcoholic extract of *Cassia occidentalis* on Carrageenan-induced rat paw edema in rats**

