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Evaluation of Anti-inflammatory activity of Methanolic extract of *Cassia obtusifolia* seeds in Wistar rats

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ABSTRACT

The present study was aimed to screen Cassia obtusifolia (CO) seeds for anti-inflammatory potential using carrageenan-induced paw oedema (Acute model) and cotton pellet induced granuloma (sub-chronic model) models in Wistar rats. In the study, 90% methanolic extract (ME) of the seeds was prepared and studied in carrageenan-induced paw oedema model at three different dose levels: 125, 250 and 500 mg/kg, orally. The ME (500 mg/kg) showed significant ($P<0.05$) anti-inflammatory activity in this model therefore, selected for further evaluation in sub-chronic model. The results of ME (500 mg/kg) were at par with diclofenac sodium (20 mg/kg). The study shows that CO seeds have anti-inflammatory potential and can be studied further to find out the compound responsible for the activity.

Keywords: Carrageenan; *Cassia obtusifolia*; Cotton pellet granuloma; Diclofenac sodium.

INTRODUCTION

Inflammation is an immediate physiological response of a body to various stimuli including pathogens, noxious substances such as chemicals or physical agents etc. It is the processes, essential for immune surveillance, optimal repair, and regeneration after injury [1]. A sustained, excessive or inappropriate inflammation may results in numerous diseases including rheumatoid arthritis, psoriasis and inflammatory bowel disease. Inflammation is also considered as a fundamental contributor to the cancer, diabetes and other cardiovascular system related diseases [2].

Cassia obtusifolia (CO) is one of the medicinally important plants of *Cassia* genus belongs to family Leguminosae. Various species of the genus are well documented to possess anti-inflammatory activities and the genus is also reported to contain anthraquinones and flavonoids,

these constituents are well documented to possess anti-inflammatory activity. The leaves of CO are being used traditionally to get relief from vomiting, stomach-ache and head-ache [3]. The plant seeds are used for the treatment of acute inflammation in eyes. Chemical review of the plant seeds revealed the presence of phytoconstituents like anthraquinones and flavonoids. To the best of our knowledge no report is available pertaining anti-inflammatory activity of the seeds. Hence, present study was aimed to investigate the anti-inflammatory activity of CO seeds.

MATERIALS AND METHODS

Animals

Wistar albino rats of either sex weighing 180-200 g were used. Animals were kept in the animal house, ISF College of Pharmacy, Moga (Pb) India (CPCSEA, Reg. No.816104) in polypropylene cages (3 rats in each cage) and standard environmental conditions were maintained. The animals were acclimatized for one week before the experiment. The rats were fed with commercially available normal chow diet (Aashirwad Industries Ltd., Punjab), and water *ad libitum*. The experimental protocol was subjected to Institutional Animal Ethical Committee for the approval and got approved.

Chemicals

Carragennan (Sigma Chemical Co., USA), diclofenac sodium (Novartis, India) and thiopental sodium (NEON Laboratories, India) were incorporated in the study. All other chemicals and reagents used were of highest commercial grade available.

Plant material

The plant material was collected from Ludhiana region (Punjab), India and was authenticated by Dr H B Singh at National Institute of sciences Communication and Information Resources, New Delhi. The voucher specimen is kept in the Pharmacognosy Department of the Institute for future reference.

Preparation of extract

The seeds were separated from the pods and dried under shade. The dried seeds were pulverized and first defatted with petroleum ether, and the marc was used in order to obtain 90% methanolic extract (ME) using Soxhlet extractor (48 h). The extract was dried and concentrated under vacuum and kept in a desiccators till further use.

Preparation of test solutions

Test solutions were prepared by suspending ME and diclofenac sodium separately in warm solution of 1% CMC.

Carrageenan induced paw edema model [4]

In this model ME was administered orally in animals at three dose levels (125, 250 and 500 mg/kg). The rats were divided into five groups of six rats in each. Grouping was done as follows:

Group I	*Carrageenan control (received, vehicle 1% w/v CMC)*
Group II	*Standard treated (DS 20 mg/kg)*
Group III	*Test I (ME 125 mg/kg)*
Group IV	*Test I (ME 250 mg/kg)*
Group V	*Test I (ME 500 mg/kg)*

All the test drugs were administered 30 min prior to carrageenan injection (0.1 ml of 1% w/v in saline) in sub plantar region of hind paw of each animal. The degree of oedema was measured using a plethysmograph at 30, 60, 90, 120 min, 2, 3, 4 and 24 h after carrageenan injection.

Cotton pellet induced granuloma model [5]
The dose of ME (500 mg/kg) showed most significant effect in carrageenan induced paw oedema model. In this model, inter-scapular implantation of sterile cotton pellets (20 mg/kg) was done in the animals under anaesthesia by injecting thiopental sodium (40 mg/kg, *i.p*).

The animals were divided into three groups viz:
First group was served as a control where animals received only normal saline (1%). Group II animals were given DS (15 mg/kg) and third group animals got the treatment of ME (500 mg/kg). Test drugs were administered for 10 days. On 11^{th} day animals were sacrificed by cervical dislocation, cotton pellets were removed and dried at 60^0 C till the constant weight obtained. The cotton implantation caused significant granuloma tissue formation over the cotton pellet as indicated by elevated cotton weight. The comparison of % weight difference in test drug treated and control animals were calculated, which indicates the effectiveness of the drug against inflammation.

RESULTS

In carrageenan model ME (500 mg/kg) treated animals showed significant inhibition ($P<0.05$) of % paw volume at 30, 60, 90, 120 min and 3, 4 and 24 h were found to be 72.22, 46.11, 38.05, 37.14, 30.15, 42.22 and 59.72, respectively). Moreover, ME at the dose of 250 mg/kg showed the paw volume of 77.77, 61.66, 41.66, 42.85, 37.3, 47.77, 63.88 and 83.33 % respectively as compared to carrageenan control animals).

Animals treated with ME (500mg/kg) showed granuloma tissue formation up to 76.24 whereas standard diclofenac treated group showed 52.49 as compared to control group.

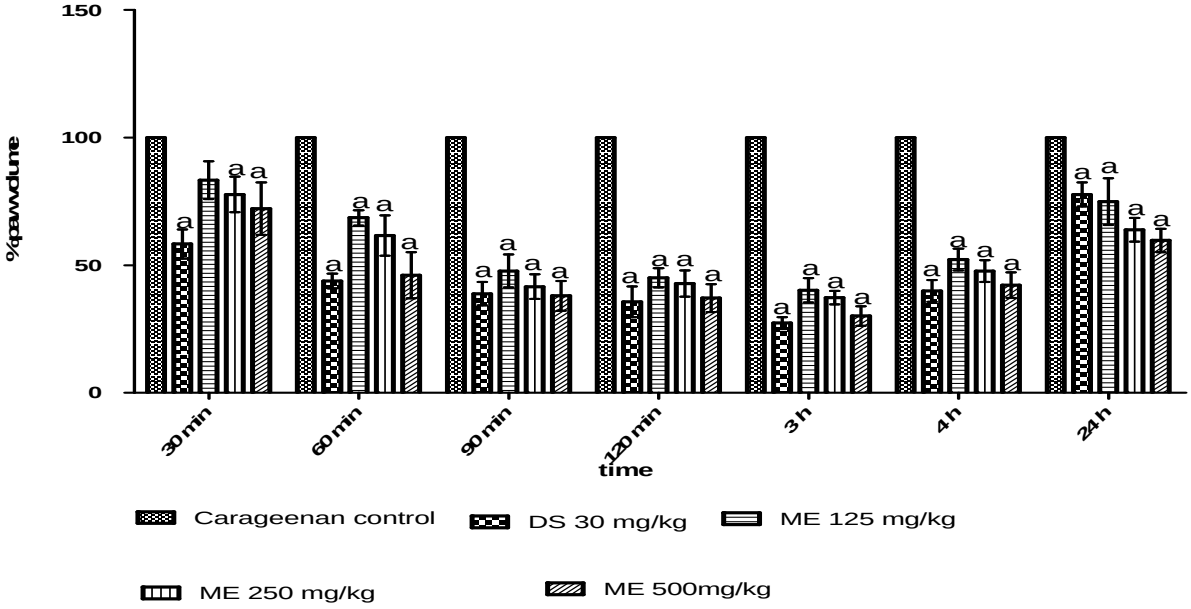

Figure 1. Effect of test drugs on carrageenan induced paw oedema
Results are expressed as Mean ±SEM. a= P<0.05 sta tistically significant (n=6). ME: methanolic extract, DS: diclofenac sodium.

Cotton pellet induced granuloma model

In this model both ME (500 mg/kg) and DS (15 mg/kg) treated animals showed reduction in the granuloma formation. Results are depicted in table 1.

Group	Granuloma weight	% Granuloma weight
Control	104.27	100
Diclofenac Sodium (15 mg/kg)	59.36	$49.35^{a} \pm 3.71$
ME (500 mg/kg)	84.87	$78.39^{a} \pm 1.15$

Results are expressed as Mean ± SEM; a= P<0.05 statistically significant (n=6); ME = Methanolic extract.

DISCUSSION

The study demonstrated the protective effect of CO seeds in both, acute and chronic inflammation using carrageenan induced paw oedema and cotton-pellet induced granuloma models in rats. The carrageenan induced rat paw oedema model is highly sensitive to NSAIDs like DS and has been accepted for evaluating new drug therapies for the inflammation. Carrageenan is well reported to cause paw oedema (a biphasic event) in rats. The initial phase results in the release of histamine and serotonin causing vasodialation and increased permeability of capillaries; whereas the release of bradykinin, prostaglandins, protease and lysosomal enzymes which regulate the process of adhesion of molecules is attributed to the second phase [6]. Subcutaneous injection of carrageenan into the rat paw produces accumulation of plasma and fluid and plasma protein exudation also takes place along with neutrophil extravasations [7]. The early phase of inflammation begins immediately after carrageenan injection and extends up to 6 h whereas the late phase remains up to 24 h [8]. The ME (500 mg/kg) treated animals showed significant prevention of paw oedema from 60 min to 24 h. It indicates the ameliorating effect of ME on carrageenan induced inflammation, which may be due to the suppression of the release of various inflammatory mediators. Cotton pellet granuloma model is indicative of proliferative phase of inflammation involving macrophages, neutrophil, fibroblast cells and collagen formation resulting granuloma formation in the cotton-pellet induced granuloma model [9, 10]. The model is widely used models for chronic inflammation occurred by means of development of proliferated cells existed in the form of granuloma. NSAIDS inhibit the granuloma formulation by preventing granulocyte infiltration, generation of collagen fibres, fibroblast and suppressing muco-polysaccharides [11]. The ME (500 mg/kg) treated group showed decreased granuloma formation which may be due to marked suppression of proliferative phase. The Phytochemical screenings of ME revealed the presence of triterpenoid glycosides and carbohydrates, which have already been reported to possess anti-inflammatory activity [12-15]. Thus, triterpenoid glycosides and carbohydrates present in the plant may be responsible for its anti-inflammatory activity.

CONCLUSION

The present study on ME (250 & 500 mg/kg) of CO seeds showed a significant anti-inflammatory activity in the rats in both acute and chronic model. Thus, it can be said that CO seeds may reduce the risk of inflammation related disease. It further requires fractionation and isolation of the responsible compounds.

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