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Research Article

Evaluation of anti-inflammatory potential of *Cassia obtusifolia* Linn. leaves in Wistar rats

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ABSTRACT

The present study was aimed to screen *Cassia obtusifolia* leaves for anti-inflammatory potential using carrageenan-induced paw edema and cotton pellet-induced granuloma models in Wistar rats. In this study, 90% methanol extract at two dose levels (250 and 500 mg/kg, p.o.) and n-Butanol fraction of methanol extract (50 and 100 mg/kg, p.o.) were studied in carrageenan-induced paw edema model. The methanol extract (500 mg/kg) and n-Butanol fraction of methanol extract (50 and 100 mg/kg) showed significant ($p<0.05$) anti-inflammatory potential in this model, Moreover, the anti-inflammatory effect of n-Butanol fraction of methanol extract at the dose of 100 mg/kg was comparable to diclofenac sodium (20 mg/kg, p.o). Therefore, n-Butanol fraction of methanol extract (50 and 100 mg/kg, p.o.) have been further evaluated in cotton pellet induced granuloma model. Preliminary phytochemical screening of the n-Butanol fraction of methanol extract revealed the presence of glycosides, steroids, flavonoids and triterpenoids. The present study hence proved the anti-inflammatory potential of methanol extract of *Cassia obtusifolia* leaves. On further fractionation, the n-Butanol fraction has potentiated the therapeutic efficacy.

INTRODUCTION

Inflammation is a patho-physiological response of mammalian tissues to a variety of noxious agents including infectious organisms, toxic chemical substances, physical injury etc resulting local accumulation of plasma fluid and blood cells [1]. It is a part of host defense, but when the response becomes worse it may be fatal. The non-steroidal anti-inflammatory drugs (NSAIDs) are considered important because of their wide therapeutic potential, but their prolonged use may cause severe adverse side effects [2]. Medicinal plant-based drugs owe the advantage of being simple, effective and exhibit broad spectrum activity. Medicinal plant products when compared to their synthetic counterparts minimize the adverse effects [3]. As a result, a search for other alternatives seems necessary and beneficial.

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Cassia obtusifolia Linn. (CO), belongs to family Leguminosae, grows as a weed during rainy season throughout India and other tropical regions of the world like America, Asia and Africa. It is commonly known as 'Sicklepod'. The plant has been traditionally used for the treatment of dizziness, dysentery and eye inflammation etc. [4]. The leaves are used traditionally as a remedy for gout, sciatica, joints pain, stomach-ache and head-ache [5]. The leaf decoction is used as febrifuge and for treatment of gingivitis, urinary tract infections, diarrhea, fever and cough [6]. Chemical review of the plant leaves revealed the presence of phytoconstituents like anthraquinones, phytosterols, triterpenoids and flavonoids [7]. No scientific evidence is available pertaining anti-inflammatory activity of the leaves so far. Therefore, the present study was designed to investigate the anti-inflammatory effect of CO leaves.

MATERIALS AND METHODS

Plant material

The leaves of CO were collected in the month of September from the local area of Dist. Ludhiana

(Mullapur), Punjab, and was authenticated by Dr. H. B. Singh at National Institute of sciences Communication and Information Resources (NISCAIR), New Delhi (Specimen voucher no. NISCAIR/RHMD/CONSULT/2009-10/1275/7, dated 23/09/2009). A voucher specimen has been kept in the pharmacognosy department of the institute for future reference.

Preparation of extracts and fractionation

The air-dried coarsely powdered leaves of CO (665 g) were defatted with petroleum ether (40-60°C), the marc was extracted with 90% methanol using soxhlet extraction (48 h). The methanol extract (ME) obtained was dried and concentrated under vacuum. ME was further washed with petroleum ether to remove colour pigments and then dissolved in water (150 ml), residue and other insoluble matter were removed by filtration. The filtrate was partitioned successively with ethyl acetate and n-Butanol in a separatory funnel and concentrated under vacuum to obtain an ethyl acetate fraction (5.59 % w/w) and n-Butanol fraction (NBF) (9.05 % w/w) respectively.

Experimental animals

Wistar albino rats (180-220 g) of either sex were used for this study. The studies were performed in accordance with the CPCSEA guidelines. The experimental protocol was subjected for the permission from animal ethical committee and cleared by the same (Reg. No.816/04/C/ CPCSEA). Animals were kept in polypropylene cages (3 rats in each cage) and standard environmental conditions were maintained. The animals were acclimatized with laboratory conditions for one week before the experiment. The rats were fed with commercially available normal chow diet (Aashirwad Industries Ltd., Punjab), and water ad libitum.

Acute toxicity studies

The ME of the leaves of CO was studied for its safety profile using organization of economic co-operation and development (OECD) 423 guidelines, 1987 using Wistar rats of either sex (n = 3) [9].

Anti-inflammatory activity

1. Carrageenan-induced rat paw edema

In this model, paw edema was induced by carrageenan injection (0.1 ml, 0.9 % w/v, normal saline) into sub-planter region of the left hind paw [10]. Wistar rats were divided into six groups each comprising of six animals. Grouping was done as follows:

Group I – Carrageenan control (received, vehicle 1% w/v CMC, p.o.)
Group II – Standard treated (diclofenac sodium 20 mg/kg, p.o.)
Group III – ME treated (250 mg/kg, p.o.)
Group IV – ME treated (500 mg/kg, p.o.)
Group V – NBF of ME treated (50 mg/kg, p.o.)
Group VI – NBF of ME treated (100 mg/kg, p.o.)

All the test drugs were administered one hour prior to carrageenan injection. The paw volume was measured plethysmographically at different time intervals of 1 h, 2 h, 3 h and 4 h after carrageenan injection. The % inhibition of paw volume was measured using formula:

$$\% \text{ Inhibition} = (Vc - Vt) / Vc \times 100$$

Where Vc is paw volume in control group and Vt is the paw volume after drug treatment.

2. Cotton pellet granuloma model

In this model the animals were anaesthetized with thiopental sodium (40 mg/kg, i.p). An incision was made in the lumber region and sterile cotton pellets (50±1 mg/kg) were inserted on either side in the scapular region. The animals were divided into four groups as follows:

Group I – Control (Normal saline 0.9% w/v)
Group II – Served as Standard (DS 15 mg/kg, p.o.)
Group III – NBF of ME (50 mg/kg, p.o.)
Group IV – NBF of ME (100 mg/kg, p.o.)

Test drugs were administered orally, daily for 9 consecutive days at regular intervals of 24 h, starting from the day of pellet implantation. On 10th day, animals were sacrificed; cotton pellets were removed surgically; cleaned from extraneous tissue; and dried at 60°C till constant weight achieved [11]. The increment in dry weight of pellets over 50 mg was taken as an index of granuloma formation, which indicates the effectiveness of the drug against inflammation.

Statistical analysis

The results are expressed as mean±SD. Statistical analysis was performed using analysis of variance (ANOVA) followed by multiple comparisons tests. The results were regarded as significant at $p<0.05$. All statistical analysis was performed using Graph Pad Prism software (5.04 version).

RESULTS

Acute toxicity study

The ME of leaves CO showed no mortality as well as behavioral changes in the animals at the dose of 2000 mg/kg.

Carrageenan-induced paw edema model

Sub plantar injection of carrageenan (1% w/v, 0.1 ml) caused significant increase in paw volume as compared to its normal basal value in successive 1 h to 4 h. Pretreatment with ME at two dose levels of 250 and 500 mg/kg, p.o. showed significant ($p<0.05$) % inhibition of paw volume (37.14%, 41.41%, 43.90%, 53.65%, 56.52%, 60.86%, 49.96% and 54.42%, respectively). Moreover, NBF of ME at the doses of 50 and 100 mg/kg, p.o. showed significant ($p<0.05$) % inhibition of paw volume (34.28%, 42.85%, 50.24%, 58.53%, 63.04%, 73.91%, 58.00% and 67.74%, respectively as compared to vehicle treated control rats) in dose-dependent manner. The NBF of ME (50 mg/kg and 100 mg/kg, p.o.) showed results at par to diclofenac sodium (20 mg/kg, p.o.) (Fig. 1).

Cotton pellet-induced granuloma model

For this study, NBF of ME was selected at the two dose levels (50 and 100 mg/kg, p.o.). The NBF had shown maximum anti-inflammatory effect on carrageenan model. Hence, selected for its effect on (sub-chronic) cotton pellet-induced granuloma model. In this model, inter-scapular implantation of sterile cotton pellets caused significant granuloma tissue formation as indicated by elevated weight of cotton pellet. The NBF of ME (50 and 100 mg/kg, p.o.) and diclofenac sodium (15 mg/kg, p.o.) treated animals showed significant ($p<0.05$) decrease in granuloma tissue formation as compared to control group (Table 1).

DISCUSSION

The present study demonstrated the evidences for the anti-inflammatory effects of ME and it's NBF of leaves of CO in experimental animals. The acute toxicity study indicated that ME was found to be safe up to 2000 mg/kg as evidenced by no mortality as well as behavioral change. The carrageenan-induced paw edema is a well known method for investigating or evaluating new drug therapies for acute inflammatory pathological condition [12]. Carrageenan is reported to cause paw edema which is a biphasic event. The initial phase is attributed with the release of histamine and serotonin causing vasodilatation and increased capillary permeability; the second phase is due to release of bradykinin, prostaglandins, protease and lysosomal enzymes regulating the process of adhesion molecules [13], cell migration, activation and degranulation [14-16]. The study also showed the significant paw edema formation after sub plantar administration of carrageenan, characterizing the cellular events of acute inflammation. ME (250 and 500 mg/kg, p.o.) and NBF of ME (50 and 100 mg/kg, p.o.), respectively showed marked protective effect in dose-dependent manner in cellular events during edema formation and in all the stages of acute inflammation. The NBF (100 mg/kg) showed maximum inhibition of edema.

The cotton pellet granuloma model is indicative of proliferative phase of inflammation involving macrophages, neutrophils, fibroblast cells and collagen formation, which are basic source of granuloma formation [17]. Results revealed that NBF significantly ($p<0.05$) inhibit the granuloma formation in rats. Multiplications of small blood vessels as well as proliferation of fibroblasts are the characteristics features at the repair phase of inflammation. Such proliferating cells penetrate the exudates, producing a highly vascularized reddened mass known as granulation tissue [18]. Reduction in weight of cotton pellets in comparision to vehicle treated group indicates the reduction in the proliferative activity of NBF of ME confirming its potency over chronic inflammatory conditions.

However, preliminary phytochemical investigations of NBF of ME of leaves of CO revealed the presence of steroids, triterpenoids, flavonoids and glycosides which are known to be responsible for the anti-inflammatory activity in other plants [19-21]. It is therefore, possible that anti-inflammatory activity of NBF may be attributed to its flavonoids, triterpenoids and steroidal contents. The results presented in this study should be taken as a basis for further investigation for determination of the exact mode of action of individual constituents of the NBF of ME of CO leaves.

CONCLUSION

The NBF of ME of leaves of CO possesses significant anti-inflammatory effect in both acute and sub-chronic models in Wistar rats at doses 50 and 100 mg/kg, p.o. The results support the traditional use of the leaves of this plant in the treatment of inflammatory conditions and also suggested the presence of steroids and triterpenoids may be biologically active principles, responsible for anti-inflammatory activities of the NBF of the ME of CO leaves.

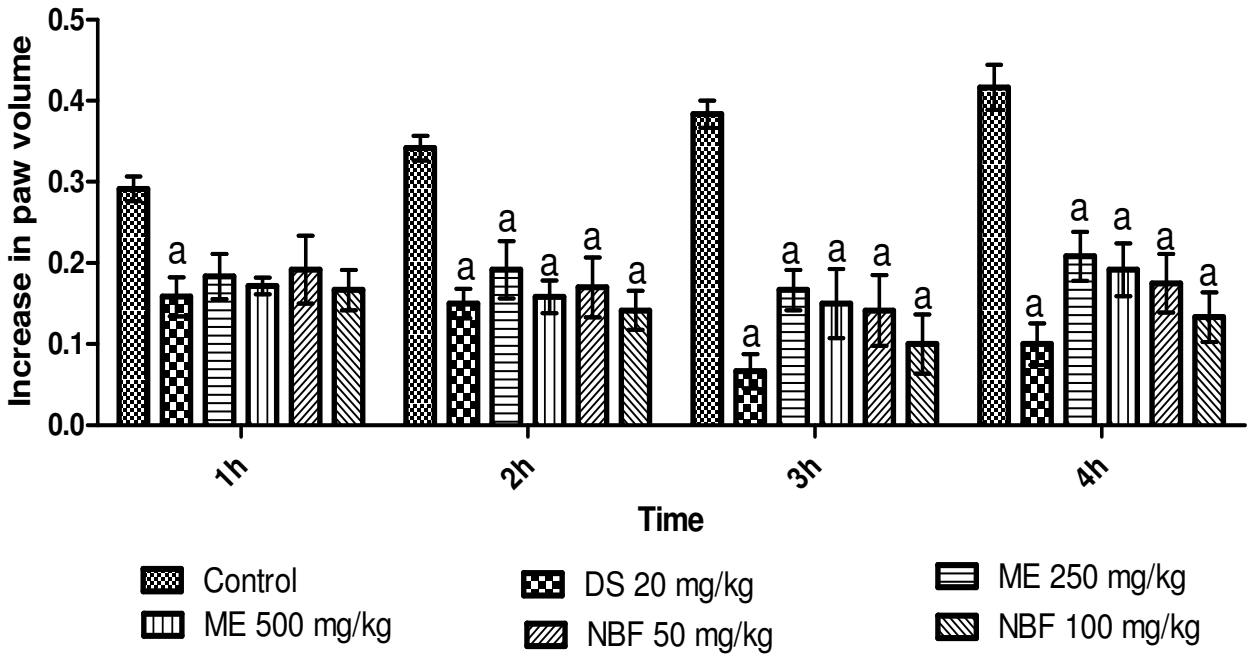

Figure 1: Effect of the test drugs on carrageenan-induced paw edema model in Wistar rats. [a] $p<0.05$ Vs. carrageenan control. [DS = diclofenac sodium; ME = methanol extract; NBF = n-Butanol fraction]

Table 1: Effect of test drug on cotton pellet-induced granuloma model in Wistar rats

Groups	Granuloma weight (Proliferative Phase)	% Granuloma weight
Control	240.41	100
DS 15 mg/kg	56.70	23.58 ± 4.12[a]
NBF 50 mg/kg	149.42	62.15 ± 4.69[a]
NBF 100 mg/kg	121.51	50.54 ± 5.53[a]

Results expressed as mean±SD (n=6) using one way ANOVA followed by Turkey multiple comparison test. a= $p<0.05$; [DS = diclofenac sodium; NBF = n-Butanol fraction]

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