

## Anti-inflammatory activity of *Cordia dichotoma forst f.* seeds extracts

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**Abstract-** The effects of *Cordia dichotoma forst f.* seeds extracts on different phases of acute inflammation were examined. Investigations were performed using different phlogistic agents-induced paw edema viz., Carrageenan-induced paw oedema and Dextran- induced paw oedema in rats. Various extracts (ethanol and aqueous) of *Cordia dichotoma forst* seeds at a dose of 250 mg/kg and 500 mg/kg orally were tested. Diclofenac sodium at the dose of 10mg/kg was used as standard. Both the extracts showed significant activity (\*p<0.05 & \*\*p<0.01) compared with the control in both of these models. The dry powdered seeds were found to contain alkaloids, glycosides, saponins, tannins and carbohydrates. Thus it is revealed from the screening model used that the ethanol extract and aqueous fraction of this plant possesses acute anti-inflammatory activity.

**Keywords-** *Cordia dichotoma forst*, Anti-inflammatory activity, Carrageenan-induced oedema, Dextran-induced oedema, seeds extracts.

### Introduction

Inflammation, clinically, causes, as shown by Cornelius Celsus of Rome 2000 years ago, rubor (redness), calor (heat), dolor (pain) of the affected region [1] and is a complex biological response of vascular tissues to harmful stimuli including pathogens, irritants or damaged cells [2]. It is defensive mechanism of the body to remove the injurious stimuli as well as initiate the healing process for the tissue. Inflammation, however, if runs unchecked, leads to onset of diseases such as vasomotor rhinorrhoea, rheumatoid arthritis, and atherosclerosis [3]. It is believed that current drugs available such as Opioids and NSAIDs drugs are not useful in all cases of inflammatory disorders, because of their side effects, economy and potency [4,5]. As a result, a search for other alternatives is necessary. The use of plants to treat ailments is as old as antiquity. Records of humans using plants to treat diseases have been recorded as far back as 6000 to 4000 years ago when Ayurvedic physicians started treating tumors with extracts from *Vinca roseus* [6,7]. The plant *Cordia dichotoma forst* commonly known as Indian cherry is a small to medium sized deciduous tree with a short bole and spreading crown, belonging to family *Boraginaceae*. The fruits is globose, yellowish-brown, pink or black and pulpy. It turns black on ripening and pulp gets viscid, the hard stone is 1-4 seeded, seed dispersal is aided by birds and monkeys which feed on the ripe fruits. The plant grows in India, Sri Lanka and other warmer regions. The medicinal attributes of *C.dichotoma* have been known since a long time. The seeds of the plant are anti-inflammatory. The fruits of the plant are used as, astringent, expectorant, anthelmintic, purgative and diuretic [8,9]. The seeds have not been explored for anti-inflammatory activity so far. The present study was therefore aimed at investigating the anti-

inflammatory activity of the seeds extracts with a view to justifying the use of the plant in the treatment of oedemas.

### Materials and Methods

#### Collection of plant materials and preparation of extracts

The seeds of *Cordia dichotoma forst* were collected in the month of August from the local market of Etawah, Uttar Pradesh state, India, and authenticated by Dr.Harish .K. Sharma, Ayurvedic Medical College, Davangere, Karnataka, India. A voucher specimen was submitted at Institute's herbarium department for future reference (AI 101). Shade dried seeds were ground to coarse powder. Powder was first defatted with pet. ether and then extracted with ethanol which is further evaporated to dryness to obtain alcoholic extract. Aqueous extract were obtained by maceration for 24 hrs.

#### Drugs and chemicals

Carrageenan was purchased from Merc Pvt.Ltd, Dextran was purchased from Sigma Pvt.Ltd and Diclofenac Sodium was obtained from Zydus Cadilla Ltd.The solvent and other chemicals of analytical grade were used.

#### Phytochemical screening

Qualitative assay, for the presence of plant phytoconstituents such as carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins were carried out on the powdered seeds following standard procedure [10].

#### Animals

Wistar rats of either sex weighing 160-180 g were procured from institute animal house for experimental study. They were acclimated to laboratory conditions for seven days before commencement of experiments, and were

allowed free access to standard dry pellet diet and water ad libitum. The experimental protocol was approved by the IAEC, for using animals in present study. Animals were fasted overnight with free access to water prior to each experiment. Five animals were used in treated & controlled groups respectively.

### Acute Toxicity Studies

The acute toxicity study was carried out in adult female albino rats by the 'up and down' method [11]. The animals were fasted overnight and next day extracts of the *Cordia dichotoma forst* dissolved in normal saline was administered orally at different dose level. Then the animals were observed continuously for 3 hours for general behavioral, neurological and autonomic profiles and then every 30 minutes for next 3 hour and finally death after 24 hours [12].

### Determination of Anti-Inflammatory Activity Carrageenan induced rat paw oedema model

The rats were divided into six groups containing five rats in each group (one control, one standard & four test groups) acute inflammation was induced according to edema assay [13]. The extracts were suspended in 2.0 % tween 80 & administered orally (250-500 mg/kg/b.w) to rats 1 hour before Carrageenan injection. Diclofenac Sodium (10 mg/kg b.w) is given to standard group. Carrageenan was prepared as 1% w/v solution in 0.9 % w/v NaCl & inject 0.1 ml underneath the planter region.

Control group 1: Carrageenan + 2% Tween 80 (10 ml /kg b.w)

Standard group 2: Carrageenan + Diclofenac Sodium (10 mg/kg b.w)

Test group1: Carrageenan + Ethanol extract (250 mg/kg b.w)

Test group2: Carrageenan + Ethanol extract (500 mg/kg b.w)

Test group3: Carrageenan + Aqueous extract (250mg/kg b.w)

Test group 4: Carrageenan + Aqueous extract (500mg/kg b.w)

The paw volume was measured with Plethysmograph at 0 and 3 hours after Carrageenan injection. The percentage of inhibition of edema was calculated using formula:  
% Inhibition of edema =  $(V_c - V_t / V_c) \times 100$

Where  $V_t$  = Paw volume in test group animals.

$V_c$  = Paw volume in control group animals

### Dextran induced rat paw oedema model

The animals were treated by same procedure as done in Carrageenan induced model but instead of Carrageenan, here 0.1 ml of Dextran (1.0 % w/v in normal saline) was used as the oedemagen. since only ethanol extract (500 mg/kg b.w) has shown maximum activity (69.52%) compared with the control group using

Carrageenan induced oedema model, therefore only this test drug was screened in this model.

Control group: Dextran + 2% Tween 80 (10 ml /kg b.w)

Standard group: Dextran + Diclofenac Sodium (10mg/kg b.w)

Test group1: Dextran + Ethanol extract (500mg/kg b.w)

### Result and Discussion

The extracts were subjected to phytochemical screening for the presences of type of phytoconstituets. The extracts were found to contain carbohydrates, alkaloids, glycosides, flavonoids, tannins and saponins. The results of anti-inflammatory activity revealed that, all the extracts exhibited dose dependent anti-inflammatory activity. At the dose of 500 mg/kg the ethanol and aqueous extracts have shown maximum inhibition of the edema (69.52% and 58.09 % respectively) as compared to control. The detailed results are shown in Table 1, 2. All the extracts were tested for anti-inflammatory activity using carrageenan induced edema models (Table 1). Among them since only ethanol extract (500mg/kg b.w) has shown maximum activity (69.52%) compared with the control group using carrageenan induced oedema model, therefore only this was screened using Dextran - induced oedema model. The present study establishes the anti-inflammatory activity of the ethanol and aqueous extracts of *Cordia dichotoma forst* in a number of experimental models. Carrageenan induced rat paw edema is a suitable experimental animal model for evaluating the anti-edematous effect of natural products [14] and this is believed to be triphasic, the first phase (1hr after carrageenan challenge) involves the release of serotonin and histamine from mast cells, the second phase (2hr) is provided by kinins and the third phase (3hr) is mediated by prostaglandins, the cyclooxygenase products and lipoxygenase products [15]. The metabolites of arachidonic acid formed via the cyclooxygenase and lipoxygenase pathways represent two important classes of inflammatory mediators, prostaglandins (products of the cyclooxygenase pathway) especially prostaglandin E2 is known to cause or enhance the cardinal signs of inflammation, similarly, leukotriene B4 (product of lipoxygenase pathway) is a mediator of leukocyte activation in the inflammatory cascade [16]. From the results, both the extracts of *Cordia dichotoma forst f.seeds* significantly inhibited (\*p < 0.05 & \*\*p<0.01) Carrageenan induced rat paw edema at 250 mg/kg and 500 mg/kg (Table 1). At the 0f 500 mg/kg the activity of the ethanol extracts is comparable than that elicited by Diclofenac sodium. Diclofenac sodium is a cyclooxygenase inhibitor, and can be said to inhibit the cyclooxygenase enzyme but lipoxygenase inhibitors also possess significant

anti-inflammatory activity against carrageenan induced paw edema [17], so inhibition of carrageenan induced paw edema by the crude extract could also be due to its inhibitory activity on the lipoxigenase enzyme. Dextran induced edema is a well known experimental model in which the edema is a consequence of liberation of histamine and serotonin from mast cells [18]. At 500 mg/kg the ethanol extract significantly inhibited Dextran induced rat paw edema (\*\*p<0.01). (Table 2).

### Conclusion

It can be concluded that both the extracts of *Cordia dichotoma forst* has anti-inflammatory activity against carrageenan and dextran (ethanol extracts 500mg/kg) induced paw edema in rats. These activities may be due to their content of tannins, flavanoids, alkaloids, glycosides, saponins and carbohydrates. The ethanol extract showed better activity profile compared to the aqueous extract hence it can be said to possess majority of the activity. This study demonstrates the efficacy of *Cordia dichotoma forst* as an anti-inflammatory agent and also scientifically justifies the use of this plant as an anti-edematous agent in folk medicine, however, further studies are required to determine the constituents responsible for its anti-inflammatory activity and further authenticate its mechanism of action.

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Table 1- Anti-inflammatory activity of *Cordia dichotoma* forst.f.seeds extracts on Carrageenan induced paw edema in rats

Group / Treatment	Dose	Mean paw edema (ml) + S.E.M	% inhibition
	(mg/kg,p.o)	At 3hrs.	
Group 1 / Control	...	1.05 ± 0.06	...
Group 2/Diclofenac Sodium	10	0.220 ± 0.03	79.04**
Test 1 / Ethanol	250	0.47 ± 0.07	55.24*
Test 2 / Ethanol	500	0.32 ± 0.02	69.52**
Test 3 / Aqueous	250	0.55 ± 0.09	47.76*
Test 4 / Aqueous	500	0.44 ± 0.04	58.09*

Results are mean ± S.E.M. (n=5) \*P<0.05 & \*\*p<0.01 compared to control

Table 2- Effect of ethanol extract of *Cordia dichotoma* forst.f.seeds on Dextran-induced paw edema in Rats

Group / Treatment	Dose	Mean paw edema (ml) + S.E.M	% Inhibition
	(mg/kg,p.o)	At 3hrs.	
Group 1 / Control	...	0.97 ± 0.06	.....
Group 2 / Diclofenac Sodium	10	0.19 ± 0.02	86.66**
Test 2/ Ethanol	500	0.24 ± 0.07	75.25**

Results are mean ± S.E.M. (n=5) \*P<0.05 & \*\*p<0.01 compared to control