Evaluation of Anti-inflammatory activity of Coccinia indica leaves extracts

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ABSTRACT

The effects of Coccinia indica leaves 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 Coccinia indica leaves extracts 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<0.0 & **p<0.01) compared with the control in both of these models. The dry powdered leaves 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.

Key words: Coccinia indica, Anti-inflammatory activity, Carrageenan-induced oedema, Dextran induced oedema, Leaves extract.

INTRODUCTION

Inflammation is physiological process in response to tissue damage resulting from microbial pathogen infection, chemical irritation, and/or wounding [1]. The relation between inflammation & atherosclerosis, diabetes, cancer, arthritis and Alzheimer’s disease has been well substantiated [2-5]. The functioning of the immune systems finally balanced by the activities of proinflammatory and anti-inflammatory mediators or cytokinins. The mediators of the inflammation such as cytokines, prostaglandins, and free radicals have direct or indirect effect on pathophysiology of disease. Chronic inflammation develops from unresolved symptomatic acute inflammation with or without any clinical manifestations. This may activate macrophages and lymphocytes which release inflammatory mediators and result in excessive formation of reactive oxygen and nitrogen species that damage DNA and cell membranes. Inflammatory cell releases prostaglandins with concomitant increases in expression of key enzyme cyclooxygenase which in turn can activate several transcription factors including NF-κB [6]. Inflammation activates a variety of inflammatory cells, which induce and activate oxidant generating enzymes like NADPH oxidase, xanthine oxidase, myeloperoxidase etc., which produce superoxide anion and other reactive nitrogen species like nitric oxide through activation of inducible nitric oxide synthases’ (iNOS) [7].

Free radicals play major role in persistence of inflammation. During the process of inflammation, phagocytes secrete chemically reactive oxidants, radicals and electrophilic compounds that bring about the elimination of infectious agents[8, 9]. These inflammatory mediators can damage the surrounding host tissue. [10, 11]. Many drugs of plant origin having antioxidant activity have been reported to have anti-inflammatory activity [12, 13].

The plant Coccinia indica also called as tindora,Tondli in Marathi, Kundari (Oriya),ghloda,kundri,kundra (Malayalam),Kovakkat Tamil,dondakaya(telugu),Coccinia indica is a dioecious perennial herbaceous vine. Stems mostly glabrous, produced annually from a tuberous rootstock; tendrils simple, axillary. Leaves alternate, simple, blade broadly ovate, 5-lobed, 5-9 x 4-9cm, acute and mucronate at the apex, cordate with a broad sinus at the base; surfaces glabrous or scaly, with 3-8 glands near the base; margins denticulate; petiole 1-5cm long. Inflorescence usually of solitary, axillary flowers. Calyx of 5 subulate, recurved lobes 2-5mm long on the hypanthium; peduncle 1-5cm long. Corolla campanulate, white, 3-4.5cm long, deeply divided into 5 ovate lobes. Stamens 3, present as staminodes in female flowers. Ovary inferior, Fruit a smooth, bright red, ovoid to ellipsoid berry 2.5-6cm long. The leaves are used as a poultice in treating skin eruptions. The plant is used as a laxative. It is used in the treatment of oedemas.

Collection of plant materials and preparation of extracts

The leaves of Coccinia indica were collected in the month of August from the local market of Etawah, Uttar Pradesh state, India, and authenticated by M.S.Mondal, Additional Director of Botanical, Survey of India, Government of India, and Hawarah-711103. A voucher specimen was submitted at Institute’s herbarium department for future reference (AI 205). Shade dried leaves 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. 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.

Materials and Methods

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Drugs and chemicals

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

Phytochemical screening

The dry powdered leaves and various extracts were found to contain alkaloids, glycosides, saponins, tannins and carbohydrates etc. By standard methods of analysis [19, 20].

Animals

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

Control group: Dextran + 2% Tween 80 (10 mg/kg b.w) Standard group: Dextran + Diclofenac Sodium (10mg/kg b.w)

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

Acute Toxicity Studies

The acute toxicity study was carried out in adult female albino rats by the ‘up and down’ Staircase method (21). The animals were fasted overnight and next day extracts of Coccinia indica leaves 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 [22].

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 [23]. 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 & injects 0.1 ml underneath the planter region.

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The extracts were subjected to phytochemical screening for the presence of type of.

Table 1. Anti-inflammatory activity of extracts of Coccinia indica leaves on Dextran-induced paw edema in rats.

<table>
<thead>
<tr>
<th>Group / Treatment</th>
<th>Dose (mg/kg, p.o)</th>
<th>Mean paw edema (ml) ± S.E.M</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 / Control</td>
<td>0.2 ml</td>
<td>1.05 ± 0.06</td>
<td>90.04*</td>
</tr>
<tr>
<td>Group 2/ Diclofenac sodium</td>
<td>10</td>
<td>0.20 ± 0.03</td>
<td>79.04</td>
</tr>
<tr>
<td>Test 1 / Ethanol</td>
<td>250</td>
<td>0.47 ± 0.07</td>
<td>55.24</td>
</tr>
<tr>
<td>Test 2 / Ethanol</td>
<td>500</td>
<td>0.32 ± 0.02</td>
<td>69.52*</td>
</tr>
<tr>
<td>Test 3 / Aqueous</td>
<td>250</td>
<td>0.55 ± 0.09</td>
<td>47.76</td>
</tr>
<tr>
<td>Test 4 / Aqueous</td>
<td>500</td>
<td>0.44 ± 0.04</td>
<td>58.09</td>
</tr>
</tbody>
</table>

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

Table 2. Effect of ethanol extract of Coccinia indica leaves on Dextran-induced paw edema in Rats.

<table>
<thead>
<tr>
<th>Group / Treatment</th>
<th>Dose (mg/kg, p.o)</th>
<th>Mean paw edema (ml) ± S.E.M</th>
<th>% of inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1 / Control</td>
<td>0.2 ml</td>
<td>0.97 ± 0.06</td>
<td>-</td>
</tr>
<tr>
<td>Group 2/Diclofenac sodium</td>
<td>10</td>
<td>0.19 ± 0.02</td>
<td>86.68*</td>
</tr>
<tr>
<td>Test 2 / Ethanol</td>
<td>500</td>
<td>0.24 ± 0.07</td>
<td>75.25*</td>
</tr>
</tbody>
</table>

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

CONCLUSION

It can be concluded that the extracts of Coccinia indica leaves have 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, flavonoids, saponins, & other phytoconstituents such as ephedra, lupin, betasitosterol, cephaladine-A, cephaladine-B, stigma 7-en -3-one, taxaraxone taxaralex. The results of anti-inflammatory activity revealed that all the extracts exhibited dose dependent anti-inflammatory activity. At the dose of 500mg/kg the ethanol and aqueous extracts have shown maximum inhibition of the edema (69.52% and 58.09% respectively) as compared to the control. The detailed results are shown in Table 1. All the extracts were tested for anti-inflammatory activity against carrageenan induced edema model. Among them only ethanol extract (500mg/kg b.w) has shown maximum activity compared to the control group using carrageenan induced oedema model, therefore only this extract showed better activity profile compared to the aqueous extract hence it can be said to possess majority of the activity. The present study demonstrates the efficacy of Coccinia indica leaves 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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