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Evaluation of Anti-Inflammatory Activity of Methanol Extract of *Coccinia Indica* (Cucurbitaceae) Fruit

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ABSTRACT

The study was designed to investigate the anti-inflammatory activity of methanol extract of fruit of *Coccinia indica* (cucurbitaceae) (MECI) was evaluated against several models of inflammation in rats. Inflammation was induced by subplanter injection into right hind paws of rats by injecting 1%w/v of carrageenan, histamine and dextran solution individually in each groups and antiinflammatory action of methanolic extract of the fruits of *Coccinia indica* (MECI) (100, 150 and 200 mg/kg, p.o.) was evaluated and compared with standard drug indomethacine (20mg/kg,p.o). The rats were also implanted with cotton pellet and granuloma formation was compared of methanolic extract of the fruits of *Coccinia indica* (MECI) against diclofenac sodium. (10 mg/kg, p.o.) The extract showed 44.66%, 26.81% and 32.74% inhibition at the dose level of 200 mg/kg, p.o. for carageenan, histamine and dextran induced paw oedema respectively; when compared to that of control group. The effect was comparable with that of the standard drug indomethacin (20 mg/kg, p.o.). The extract also has effectively and significantly reduced the cotton pellet induced granuloma in rats. The percentage inhibition was 59.05% at the dose level of 200 mg/kg, p.o. From the present research work it was concluded that methanol extract of fruit of *Coccinia indica* (cucurbitaceae) (MECI) has shown significant activity which may be due to some short of phytochemicals such as tannins, saponins, reducing sugars and triterpenoids. and compared with Dexamethasone as standard drug.

Keywords: *Coccinia indica*, carrageenan, histamine, dextran, cotton-pellet.

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INTRODUCTION

The fruit of plant *Coccinia indica* Wight and Arn. (Cucurbitaceae) is used in folklore medicine for the treatment of various ailments such as fever, leprosy, bronchitis, asthma, jaundice, blood diseases and inflammation. The fruit is also used as aphrodisiac, allays thrist and in biliousness.[1] It is reported to possess antioxidant[2], hypoglycaemic[3,4,5,6,7,8,9,10] and anti-diabetic[11,12,13,14,15,16,17,18,19] activity. Chitooligosaccharide – specific lectin have been isolated from *Coccinia indica*.[20]

As the use of the plant for anti-inflammatory activity is not supported by experimental data, so an attempt was made to evaluate the anti-inflammatory activity of methanolic extract of the fruits of *Coccinia indica*(MECI).

Figure 1: coccinia indica fruit, flower and leaves

MATERIAL AND METHODS

Plant material

Fruits of Coccinia indica were collected in month of February, 2011 from Midnapore, West Bengal, India and were authenticated by Department of Botony,Rajasthan University,Jaipur,India with authentification number RUBL 21149. A voucher specimen was deposited in our departmental herbarium for further reference. Freshly collected fruits were washed with distilled water and dried at room temperature. The dried fruit were pulverized to coarse powder. The powder was extracted with methanol in Soxhlet extractor. The extract (MECI) was evaporated under reduced pressure by a rotator vacuum evaporator until all the solvent had been removed to give an extract sample with a yield of 9.38% w/w. The extract was stored in a refrigerator. A weighed amount of the extract was suspended in 2% v/v aqueous Tween 80 solution for the present investigation.

phytochemical screening of extract

Preliminary qualitative analysis of methanol extract showed the presence of tannins, saponins,

reducing sugars and triterpenoids.

Animals

Wistar albino male rats (200-250g) were placed in wire netted cages in a controlled room temperature 22±1 ^{0}c and relative humidity of 60-70%. They were kept under standard conditions of 12/12 h light and dark cycle. The animals were maintained with standard pellet diet and water ad libitum. The animals were deprived of food for 24 h before experimentation but allowed free access to tap water. All studies were carried out using 6 rats in each group. The institute of Animal Ethical Committee approved the animal studies. (reg. no.1356/ac/10/CPCSEA/9/july/10)

Carrageenan induced rat paw oedema

Oedema was induced by sub plantar injection of 0.1 ml of 1% w/v freshly prepared suspension of carrageenan (S.D Fine Chemicals, India) in the right hind paw to each animal of five groups. The fruit extract was suspended in 2% v/v aqueous Tween 80 solution. The test groups received MECI (100, 150 and 200 mg/kg, p.o.), the standard group received indomethacin (20 mg/kg p.o.) and the control group received the vehicle only (2% v/v aqueous Tween 80 solution, 10 ml/kg, p.o.). All the doses were given orally 30 min prior to the injection of carrageenan. The volume of injected and contra-lateral paws was measured immediately before and at the third hour after induction of inflammation using Plethysmometer.[21]

The anti-inflammatory activity was evaluated based on the ratio of changes in paw diameter in treated and untreated group as per the formula given below:

Inhibition (%) = [control (V_t-V_0) – (V_t-V_0) treated] / [(V_t -V_0) control] x 100, where V_t is the paw volume 3 h after carrageenan injection and V_0 is the paw volume before carrageenan injection.

Histamine induced rat paw oedema

0.05 ml of 1%w/v freshly prepared solution of histamine (Sigma Aldrich, USA) was given by subplantar injection into right hind paws of the rats to induce oedema[22] in the second model. The standard groups received indomethacin (20 mg/kg, p.o.) and the control groups received the vehicle only. The test group received the extract MECI (100, 150 and 200 mg/kg, p.o.); the same procedure as that described in the carrageenan model as followed.

Dextran induced rat paw oedema

In this model, oedema was induced by sub plantar injection of 0.05 ml of freshly prepared 1% w/v solution of dextran (Sigma Aldrich, USA) into the right hind paw of the rats.[21] The same procedure as that described in the carrageenan model was followed.

Cotton pellet induced granuloma

The rats were anaesthetized and 10 mg of sterile cotton pellets were inserted into the axilla of each rat. The test groups received the extract MECI (100,150 and 200 mg/kg, p.o.), the standard group received diclofenac sodium (10 mg/kg, p.o.) and the control group received the vehicle only (2 % v/v aqueous Tween 80 solution, 10ml/kg, p.o.), for 7 consecutive days from the day of cotton pellet implantation. The animals were anesthetized again on day 8 and the cotton pellets were surgically removed, freed from extraneous tissue; incubated at 37^{o}C for 24 h and dried at 60^{o}C to constant weight. The increment in the dry weight of the pellets was taken as a measure of granuloma formation.[23]

Statistical analysis

All results are expressed as the mean ± SEM. The results were analysed for statically significance by one way ANOVA followed by Dunnett's't' test. P values < 0.05 were considered as significant.

RESULTS AND DISCUSSION

The results for anti-inflammatory activity of methanol extract of *Coccinia indica* fruit (MECI) against acute pedal oedema are presented in table 1-3. MECI showed significant anti-inflammatory activity and the results are comparable to that of indomethacin. The extract (200 mg/kg, p.o.) showed maximum inhibition (Carrageenan 44.6%, Histamine 26.81%, and Dextran 32.74%). In the dose of 200 mg/kg, p.o. the extract MECI caused a 59.05% reduction in granuloma weight (Table 3) against chronic inflammation by cotton pellet induced granuloma

Table 1: Effects of methanolic extract of *C.indica* on carrageenan- induced rat paw oedema

Treatment	Dose mg/kg	Paw volume[a] (ml)		Inhibition (%)
		Before carrageenan	3 h after carrageenan	
Control (2% Tween 80)	-	2.70±0.02	3.73±0.01	-
MECI	100	2.72±0.01	3.36±0.01[b]	37.86
MECI	150	2.73±0.02	3.34±0.03[b]	40.77
MECI	200	2.70±0.02	3.27±0.01[b]	44.66
Indomethacin	20	2.74±0.01	3.30±0.02[b]	45.63

[a] values are expressed as mean ± SEM (n=6).
[b]values are statistically significant at p < 0.01 vs. Control, one way ANOVA followed by Dunnett's 't' test.

The present study establishes the anti-inflammatory activity of the methanol extract of the fruits of C. *Indica* in the model used. It is evident that carrageenan induced oedema is commonly used as an experimental animal model of acute inflammation. It is believed to be biphasic, the first phase mediated by release of histamine, serotonin and kinin and then by prostaglandin in the later phase.[24] The extract exhibited a significant anti-inflammatory activity in the cotton pellet

test. This reflected its high efficacy to reduce an increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharide, which are natural proliferative events of granuloma tissue formation.[25] It seems that either with or without drug , the gain in weight of the pellets was a linear function of time. Actually, this linearity continued for eight day and then levelled off. Seven days was chosen as a convenient duration of the experiments and one day's incubation at 37^0C temperature.[22] The effect of the extract in the inflammatory process induced by stimulus injection indicates that they act by affecting a time delayed system in a similar fashion to diclofenac sodium.

Table 2: Effect of methanolic extract of C. *Indica* on histamine-induces rat paw oedema

Treatment	Dose (mg/kg)	Paw volume [a](ml)		Inhibition (%)
		Before histamine	3h after histamine	
Control	-	2.66±0.01	3.63±0.03	-
MECI	100	2.68±0.02	3.55±0.02[b]	10.31
MECI	150	2.67±0.04	3.42±0.01[b]	22.68
MECI	200	2.69±0.01	3.40±0.01[b]	26.81
Indomethacin	20	2.68±0.02	3.38±0.02[b]	27.83

[a]values are expressed as mean± SEM (n=6)
[b]values are statistically significant $p < 0.01$ vs. Control, one way ANOVA followed by Dunnett's 't' test.

Table 3: Effects of methanolic extract of C.*indica* on dextran-induced rat paw oedema

Treatment	Dose (mg/kg)	Paw volume[a] (ml)		Inhibition (%)
		Before dextran	3h after dextran	
Control(2% Tween 80)	-	2.68±0.04	3.81±0.02	-
MECI	100	2.70±0.02	3.67±0.03[b]	14.16
MECI	150	2.68±0.03	3.55±0.02[b]	23.01
MECI	200	2.72±0.01	3.48±0.02[b]	32.74
Indomethacin	20	2.74±0.02	3.32±0.01[b]	48.67

[a]values are expressed as mean ± SEM (n=6).
[b]values are statistically significant at $p < 0.01$ vs. Control, one way ANOVA followed by Dunnett's 't' test.

CONCLUSION:

We can conclude the usefulness of fruits of C.*indic*a in treating inflammatory diseases.[1] From the present research work it was concluded that the methanolic extract of *coccinia indica* fruit has shown significant anti-inflammatory activity which ponins may be due to some short of Phytochemicals such as tannins, Triterpenes and compared with dexamethasone as standard drug. More detailed phytochemical and pharmacological studies are necessary to identify the active principles and mechanism of action.

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