



EVALUATION OF ANTIINFLAMMATORY ACTIVITY OF MOMORDICA CYMBALARIA AGAINST COTTON PELLET INDUCED GRANULOMA IN WISTAR RATS

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ABSTRACT

Inflammation is a complex biological response of living mammalian tissues to the injury. It is an inbuilt body defense mechanism in order to eliminate or limit the spread of pathogens. There are diverse components to an inflammatory reaction that can contribute to the related symptoms and tissue injury. Edema, granuloma formation and leukocyte infiltration represent such components of inflammation. *Momordica cymbalaria* (Cucurbitaceae) had been used widely for its reported biological activities in traditional system of medicine. The present work is an attempt to investigate anti-inflammatory activity of various fractions of *Momordica cymbalaria* fruit on cotton pellet-induced granuloma in wistar rats. Butanol fraction of *Momordica cymbalaria* (BFM) showed 43.82%, 50.25% and 50.68% reduction in granuloma formation respectively at 50mg/kg, 100mg/kg & 200mg/kg doses. The effects of BFM were significant at every test dose as compared to standard. The BFM showed almost same activity as that of standard at a relatively low dose level (100 mg/kg). The results of the present study demonstrate that the butanol fraction of *Momordica cymbalaria* possess significant anti-inflammatory activity. Further detailed molecular investigation is necessary to delineate the underlying protective mechanism of *Momordica cymbalaria* against inflammation.

KEY WORDS

Inflammation, Pathogens, Granuloma, Cotton-pellet, Leukocyte infiltration.

INTRODUCTION

Inflammation is an inbuilt protective mechanism of the body, evoked by various stimuli such as disease-causing organisms, ecological factors, ischemia, immunological reactions, biological factors and free radicals. Macroscopically the cardinal signs of inflammation are erythema (redness), edema, tenderness, pain and heat. Inflammatory response occurs in three discrete phases each apparently mediated by different mechanism. The first phase is an

acute transient phase in which the initial response to tissue injury is triggered by the release of several autacoids like, serotonin, bradykinin, prostaglandins, leukotrienes and histamine. (Gallin *et al*, 1992). This phase is also characterized by local vasodilatation and increased capillary permeability. The second phase is a delayed sub-acute phase which is characterized by infiltration of leucocytes and phagocytic cells. The response occurs when immunologically competent cells are activated in

response to foreign organisms or antigenic substances liberated during the acute inflammatory response and the final phase is characterized by a chronic proliferative phase in which tissue degeneration and fibrosis occurs. Chronic inflammation involves the release of a number of mediators such as interleukins 1, 2 and 3, Granulocyte-macrophage colony-stimulating factor (GM-CSF), Tumor necrosis factor alpha (TNF- α 2), interferon and Platelet derived growth factor (PDGF). These mediators are involved in progression of various diseases like cardiovascular and neurodegenerative disorders. Presently for the management of pain and inflammatory conditions the drugs used are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids. e.g. hydrocortisone. All of these drugs are highly potent, not suitable for prolonged use and possess many adverse effects. Moreover, synthetic drugs are very expensive to develop and cost of development ranges from 0.5 to 5 million dollars. Several laboratories in India are actively engaged in anti-inflammatory drug research from indigenous plants as these are cheap, easily available and have minimum side effects. Several of the botanical species belonging to the genus *Momordica* are used in folk-lore medicine and among them *Momordica charantia* is used as traditional medicine to cure several ailments such as antidiabetic, abortifacient, anthelmintic, contraceptive, dysmenorrhea, eczema, emmenagogue, antimalarial, galactagogue, gout, jaundice, abdominal pain, kidney (stone), laxative, leprosy, leucorrhea, piles, pneumonia, psoriasis, purgative, rheumatism, fever and scabies (Grover et al., 2004). Besides, *Momordica charantia* other species of the *Momordica* genus are being studied to identify their constituents as well as for anti-inflammatory activities. Hence the present work is an attempt to investigate anti-inflammatory activity of *Momordica cymbalaria* fruit on cotton pellet-induced granuloma in wistar rats.

MATERIALS AND METHODS

Collection, identification and authentication of plants

The plant *Momordica cymbalaria* belongs to family Cucurbitaceae. Fruits of *Momordica cymbalaria* were collected in the month of June from the Alva Pharmacy, Mangalore and authenticated by Dr. MD. Mustafa, Assistant Professor, Department of Botany, Kakatiya University, Warangal. The fruits were dried under shade

then fine powder was prepared with the help of mixer grinder.

Preparation of Extracts

To identify the active principle(s) of *M. cymbalaria* Crude Ethanolic Extract of *Momordica cymbalaria* (CEE) was fractionated successively using different organic solvents into chloroform (CFM) and butanol fractions (BFM). The Crude Ethanolic Extract of *Momordica cymbalaria* (CEE), Chloroform Fraction of *Momordica cymbalaria* (CFM) and Butanol fraction of *Momordica cymbalaria* (BFM) were evaluated for its anti-inflammatory activity by Cotton pellet induced granuloma method.

Drugs and Reagents

All chemicals used for the experiments were of analytical grade and were purchased from HiMedia and Qualigens Fine Chemicals; Mumbai (India).

In-vivo pharmacological studies

Experimental Animals

Wistar rats of either sex weighing 100–160 g was used in the study and fed with standard laboratory pellet diet; Provimi limited (India), provided water ad libitum and were maintained at 23–25°C, 35 to 60% humidity, and 12 h light/dark cycle. The rats were acclimatized to the laboratory conditions for a period of 7 days prior to experiment. The experimental protocol (1468/PO/a/11/CPCSEA, June 8th, 2011) was duly approved by institutional animal ethics committee (IAEC). Before the experiment, food was withdrawn overnight but adequate water was given to the rats. The test was performed using the cotton pellet induced granuloma method. The rats were divided into eleven groups (n = 6). The rats were anesthetized under light ether and an incision was made on the lumbar region by blunted forceps, a subcutaneous tunnel was made and a sterilized cotton pellet (100 $\pm$ 1 mg) was inserted in the groin area. All the animals received either CEE/CFM/BFM or Indomethacin or vehicle (1% Carboxymethylcellulose (CMC) orally depending upon their respective grouping for seven consecutive days from the day of cotton pellet insertion, on the 8th day animals were anesthetized again, and cotton pellets were removed and dried to constant mass.

% Inhibition = $\frac{\text{Weight of pellet (control)} - \text{Weight of pellet (test)}}{\text{Weight of pellet (control)}} \times 100$

Animal Grouping

The animals were divided into 11 groups of six animals each.

Group –I: Control group received CMC.

Group –II: Received Indomethacin 10mg /kg body weight (Standard group)

Group-III: Received 50mg /kg body weight of CEE.

Group-IV: Received 100 mg /kg body weight of CEE.

Group –V: Received 200mg/ kg body weight of CEE.

Group –VI: Received 50mg /kg body weight of CFM.

Group –VII: Received 100mg /kg body weight of CFM.

Group –VIII: Received 200mg/ kg body weight of CFM.

Group –IX: Received 50mg/ kg body weight of BFM.

Group –X: Received 100mg/kg body weight of BFM.

Group –XI: Received 200mg/kg body weight of BFM.

STATISTICAL ANALYSIS

Results of anti-inflammatory activity were expressed as Mean $\pm$ SD. Results were analyzed using one-way ANOVA. Differences were considered as statistically significant at $p < 0.05$ are compared to control.

RESULTS

The results of the effect of CEE, CFM and BFM on Granuloma formation in the cotton pellet method are

presented in Table 1 and Figure 1. CEE treatment at the test doses 50, 100 and 200 mg/kg body weight caused 18.00%, 38.97%, 41.87% inhibited respectively. There was no significant effect by CEE at 50 mg/kg treatment but at 100 and 200 mg/kg body the effect was significant. The standard drug indomethacin caused 57.33% inhibition as compared to the control.

The CFM at the test doses 50, 100 and 200 mg/kg body weight reduced the gangrene formation by 40.00%, 43.96% and 45.94% as compared to control. On the other hand, BFM showed 43.82%, 50.25% and 50.68% reduction in granuloma formation respectively at these doses. The effects of CFM at test dose 100 and 200 mg/kg were significant as compared to standard, but at 50 mg/kg effects were not significant. The effects of BFM were significant at every test dose as compared to standard. The BFM showed almost same activity as that of standard at a relatively low dose level (100 mg/kg) when compared to CEE and CFM. This is another indication that BFM possess maximum activity as compared to CEE and CFM.

Table.1: Effect of CEE, BFM and CFM on cotton pellet Granuloma in rats.

Treatment	Dose	Weight of dry cotton pellet(mg)	% Inhibition
Control	Vehicle	84.66 $\pm$ 4.33	--
Indomethacin	10 mg/kg	36.12 $\pm$ 1.84*	57.33
Crude ethanolic extract(CEE)	50 mg/kg	69.45 $\pm$ 4.15	18.00
	100 mg/kg	51.66 $\pm$ 3.11*	38.97
	200mg/kg	49.21 $\pm$ 2.88*#	41.87
Chloroform fraction(CFM)	50 mg/kg	50.88 $\pm$ 2.22*	40.00
	100 mg/kg	47.44 $\pm$ 2.11*#	43.96
	200 mg/kg	45.76 $\pm$ 1.98*#	45.94
Butanol fraction(BFM)	50 mg/kg	47.56 $\pm$ 2.45*#	43.82
	100 mg/kg	42.11 $\pm$ 1.98	50.25

* $p \leq 0.05$ - As compared to control; # $p \leq 0.05$ - As compared to Indomethacin treated group.

Fig.1: Effect of treatment on cotton pellet Granuloma in rats.

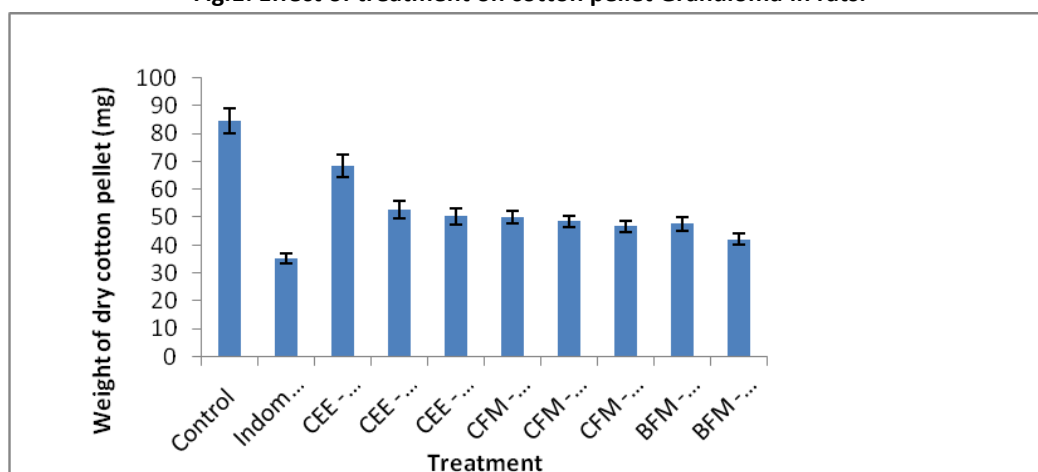
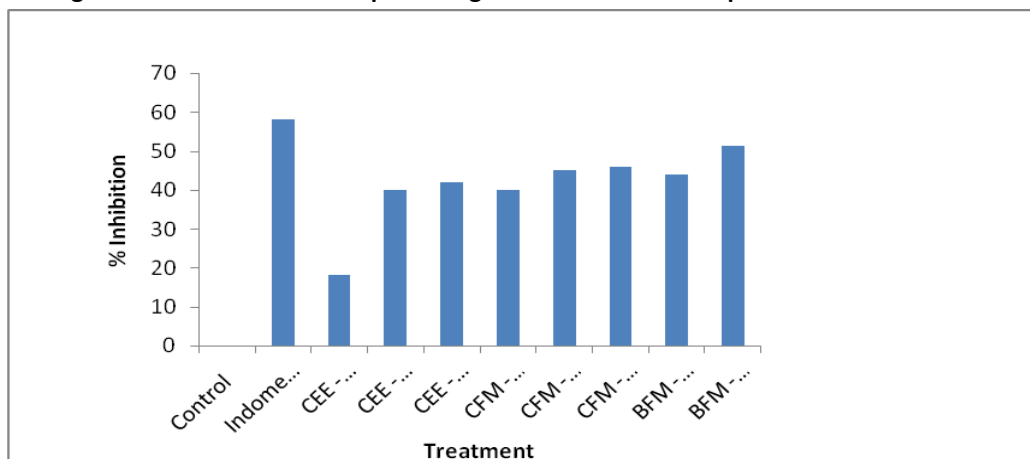


Fig.2: Effect of treatment on percentage inhibition on cotton pellet Granuloma in rats



DISCUSSION

Inflammation is the biological response of vascular tissues to harmful stimuli, such as pathogens, damaged cells or irritants characterized by redness, swollen joints, pain in joints, its stiffness and loss of joint function. NSAIDs are the drug of choice for treatment of pain and inflammation. Unfortunately, these drugs are associated with increased risk of blood clot resulting in strokes and heart attacks. Therefore, the developments of potent anti-inflammatory drugs from the plant origin are now under considerations. The cotton pellet Granuloma model was used to determine the anti-inflammatory activity of CEE, CFM and BFM on the proliferative segment of inflammation. CEE, CFM and BFM exhibited reduced effect on Granuloma development. The wet and the dry weight of the pellet relate transuda and granulomatous tissues (Lowry *et al*, 1951; Castro *et al*, 1968). Chronic inflammation associated with development of proliferate cells. NSAIDS reduce the volume of granuloma, reduce infiltration, inhibits the growth of collagen fibers and decreases mucopolysaccharides (Della Loggia *et al*, 1968; Alcaraz *et al*, 1988). The CEE, CFM and BFM showed best anti-inflammatory activity in chronic inflammatory circumstances, which reflected its worth in decrease in rise of fibroblasts and formation of collagen and mucopolysaccharides (Bhattacharyam *et al*, 1992; Swingle, 1974). In this model the CEE, CFM and BFM significantly decreased infiltration of neutrophils and monocytes (Anderson *et al*, 1971; Shen.1967; Weissmann *et al*, 1967; Jannoff *et al*, 1964). These results indicate that the CEE, CFM and BFM change the activity of chemical mediators which are participated at the site of inflammation.

CONCLUSION

The results obtained in the present investigation revealed the anti-inflammatory activity of *M. cymbalaria* *in vivo* against cotton pellet induced granuloma in wistar rats. Out of three fractions butanol fraction of *Momordica cymbalaria* had shown potent anti-inflammatory activity. Further detailed molecular investigation is necessary to delineate the underlying protective mechanism of *Momordica cymbalaria* against inflammation.

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CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

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