



EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF MOMORDICA CYMBALARIA AGAINST CARRAGEENAN INDUCED INFLAMMATION IN WISTAR RATS

Papagatla Poli Reddy^{1*}, Jupally Venkateshwar Rao² and Vinitha Edula³

¹Associate Professor, Department of Pharmacology, Swami Ramananda Tirtha Institute of Pharmaceutical sciences, Nalgonda, Telangana, India, 500084

²Principal & Professor, Talla Padmavathi college of pharmacy, Urus, Kareemabad, Warangal, Telangana, India.

³Assistant Professor, Department of Pharmacology, Swami Ramananda Tirtha Institute of Pharmaceutical sciences, Nalgonda, Telangana, India, 500084

*Corresponding Author Email: polireddy0004@gmail.com

ABSTRACT

Inflammation is a protective mechanism of the body, evoked by various stimuli such as disease-causing organisms, ecological factors, ischemia, immunological reactions, biological factors and free radicals. *Momordica cymbalaria* (Cucurbitaceae) had been used widely for its reported biological activities in ayurvedic system of medicine. The present work is an attempt to investigate anti-inflammatory activity of various fractions of *Momordica cymbalaria* fruit on carrageenan-induced inflammation in wistar rats. The anti-inflammatory activity was found to be dose dependent in carrageenan induced paw edema. Butanol fraction of *Momordica cymbalaria* (BFM) was found to significantly ($p < 0.05$) reduce the carrageenan induced paw edema (55.4%) as compared to carrageenan control. The percentage inhibition of standard anti-inflammatory drug indomethacin was (55.6%). The results of the present study demonstrate that the butanol fraction of *Momordica cymbalaria* possess significant anti-inflammatory activity. Further detailed investigation is currently underway to characterize the active agents responsible for the observed effects and to estimate pro/anti-inflammatory mediators to confirm the mechanism.

KEY WORDS

Inflammation, Ischemia, Paw edema, Carrageenan,

INTRODUCTION

Inflammation is a complex non-specific biological response of vascular tissues to tissue injury caused by pathogens, irritants and damaged cells. Inflammation is characterized by increased movement of plasma, neutrophils and macrophages from the blood into the site of injury resulting in increased blood supply, cellular metabolism, vasodilatation and extravasation of fluids (Ferrero-Miliani et al., 2007). On entry of pathogen into the body the cell membrane activates phospholipase A2 it acts on phospholipids and releases arachidonic acid. It is highly reactive further metabolized by

cyclooxygenase enzyme (COX) and results in production of prostaglandins and leukotrienes, the major components that are involved in induction of pain and inflammation (Higgs et al., 1984; Vane, 1971). The most widely used drugs throughout the world for inflammation include the non-steroidal anti-inflammatory drugs, but they are associated with undesirable side effects on gastric mucosa, kidney, bronchus and cardiovascular system (Wallace and Vong, 2008), and have limited use (Burke et al., 2006). The current research trend is on investigation of medicines from plant origin because of their easy ability and accessibility with minimal side effects (Ibrahim et al.,

2012). Natural products such as flavonoids, steroids, polyphenols, coumarins, terpenes, stearic acid and alkaloids possess anti-inflammatory and analgesic activities with minimal side effects (Shah and Alagawadi, 2011; Shukla et al., 2010). Inflammation induced by cutaneous injection of carrageenan in rodents is reliable, acute, nonimmune, and highly reproducible method. Cardinal signs of inflammation such as edema, hyperalgesia, and erythema develop immediately in carrageenan model due to release of proinflammatory agents such as bradykinin, histamine and reactive oxygen and nitrogen species. Hence in the present investigation inflammation is induced by carrageenan. Several of the botanical species belonging to the genus *Momordica* are used in folk medicine and among them *Momordica charantia* is used in various systems of 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). Recently, it has been demonstrated that *Momordica charantia* is effective for the treatment of anxiety and depression (Arunachalam et al., 2008). Recent studies reported that *Momordica grosvenori* Swingle possess anti-inflammatory, anti-oxidative, anti-diabetic, and nephroprotective activities (Min-Hsiung et al, 2009). 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 carrageenan-induced inflammation 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 Carrageenan induced rat paw edema method.

Drugs and Reagents

All chemicals used for the experiments were of analytical grade and were purchased from Hi Media 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.

Animal Grouping

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

Group –I: Control group received acacia (5% of 10ml/ kg i.e. only vehicle).

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.

All the drugs were given orally half an hour before the administration of carrageenan suspension with the help of an oral catheter. Carrageenan (1%) suspension (i.e. 10 mg/ml) in 5% acacia was prepared and 0.1 ml was

injected in sub plantar region of left hind paw of each rat. The paw volume was measured immediately after injection (i.e.0.0 h) and after 1, 2, 3 and 4 h with the help of Plethysmometer. The average paw swelling in the group of extract treated rats was compared with control group and the standard group.

The percent change in edema was calculated by the formula:

$$\% \text{ Edema Inhibition} = [1 - (V_t/V_c)] \times 100$$

V_t and V_c are edema volume in the drug treated and control groups respectively.

STATISTICAL ANALYSIS

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

RESULTS

The crude ethanolic extract of *M. cymbalaria* i.e., CEE, CFM and BFM were evaluated for anti-inflammatory activity in acute and chronic experimental animal models and the results are summarized in Table: 1. The result obtained indicates that the extract possesses significant ($p < 0.05$) anti-inflammatory activity in rats. The CEE at the test doses 50, 100 and 200 mg/kg body weight reduced the edema induced by carrageenan by 31.00%, 37.83%, 43.20%, 29.72%, 36.48% and 43.5% respectively at 2h and 3h, whereas the standard drug showed 59.45% and 55.6% of inhibition respectively at 2h and 3h as compared to the control group. The test doses 50, 100 and 200 mg/kg body weight reduced the edema by 35.54%, 39.18%, 41.89%, 34.00%, 39.18% and 43.24 % respectively at 2 h and 3h. Whereas BFM showed 50.00%, 55.40%, 56.74%, 49.00%, 55.40% and 55.40% reduction in edema volume respectively at 2 h and 3 h at these doses. These results indicate that the BFM possess strongest anti-inflammatory activity as compared to CEE and CFM at every hour after pretreatment. The effects of BFM at 100 and 200 mg/kg body weight at 2 h and 3 h were comparable to that of standard drug Indomethacin.

The pretreatment with CEE, CFM and BFM resulted in a significant and dose-dependent reduction in carrageenan induced paw edema in rats. The percent inhibition was comparatively less at 1 hr after treatment

with CEE and CFM at all the doses when compared to the effect of Indomethacin and BFM. The maximum inhibition of paw volume was observed at 2 and 3 hr after treatment with CEE, CFM and BFM at each dose but the activity was almost consistent from 2 to 3 h and it decreased after 3 h. however, the reduction at 4 h after BFM treatment (100 and 200 mg/kg) was also close to the effect of Indomethacin (Table:1; Fig:1&Fig:2).

DISCUSSION

The present experimental investigation revealed that Butanol fraction of *Momordica cymbalaria* (BFM) possessed significant anti-inflammatory activity in experimental animals at a dose of 100 and 200 mg/kg. Carrageenan induced acute inflammation is one of the reliable procedure to screen anti-inflammmtory drugs. These models are sensitive even to cyclooxygenase inhibitors and are used to evaluate the effect of non-steroidal anti-inflammatory agents, which primarily inhibit the cyclooxygenase involved in prostaglandin synthesis (Selbert et al., 1994). The edema development in carrageenan induced edema occurs in two phases (Vinegar et al., 1969). In the first phase inflammation is seen within an hour of injection of carrageenan which occurs partly due to trauma at the site of injection and partly due to serotonin and histamine components (0-2h), while plateau phase is maintained by a kinin like substance (3 h) and a second accelerating phase of swelling is attributed to Prostaglandin release (>4 h) (Winter et al., 1962). As shown there is a significant percentage inhibition ($p < 0.05$) of paw edema at the 3rd hour with BFM (55.4%). The percentage inhibition of standard anti-inflammatory drug indomethacin was (55.6%). Our results divulged that dispensing of CEE, CFM and BFM reduced the edema from the 1h and remaining all phases, which is mostly due to prevention of various phases and chemical factors of inflammation. Therefore, it can be inferred that the possible inhibitory effect of Butanol fraction of *Momordica cymbalaria* (BFM) in carrageenan induced inflammation may be due to inhibition of lysosomal enzymes, stabilizing the membrane or by altering the action of endogenous factors that are involved in the migration of these substances to the site of inflammation or by inhibition of cyclooxygenase leading to inhibition of prostaglandin synthesis.

CONCLUSION

The results obtained in the present study have shown the anti-inflammatory activity of *M. cymbalaria* in vivo. Out of three fractions butanol fraction of *Momordica cymbalaria* appeared to possess the strongest anti-inflammatory activity, providing a scientific basis for its ethnobotanical uses for alleviating pain and treating inflammatory disorders. Further detailed investigation is currently underway to characterize the active agents responsible for the observed effects and to estimate

pro/anti-inflammatory mediators to confirm the mechanism.

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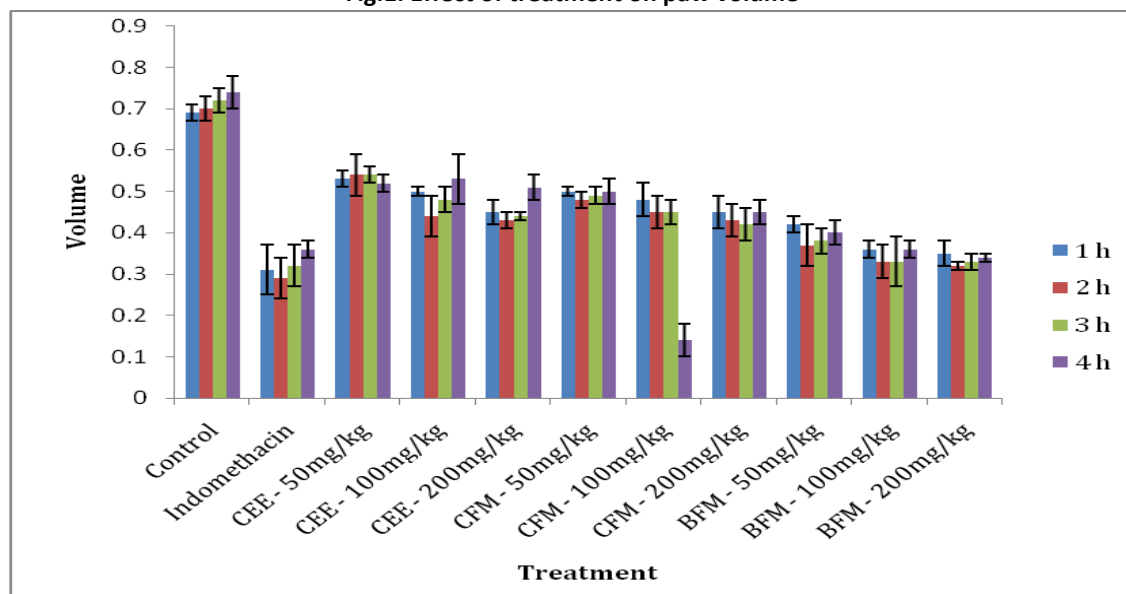
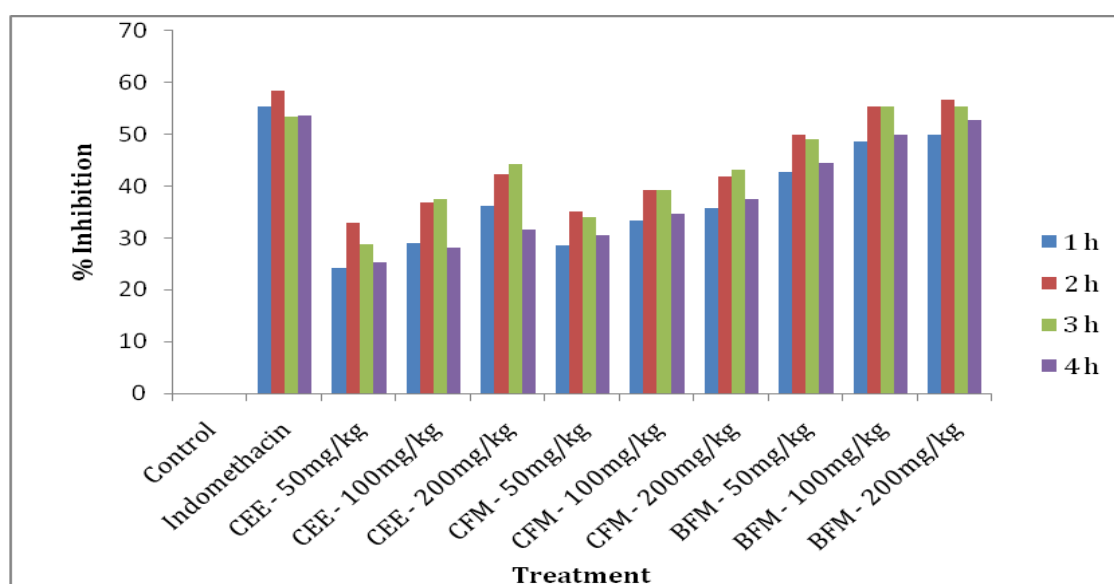
CONFLICT OF INTEREST

Authors have no conflicts of interest to declare.

Table.1. Effect of CEE, BFM and CFM on carrageenan induced rat paw edema

Treatment	Dose	Paw volume							
		1 hr		2 hr		3hr		4 hr	
		ml	% inhibition	ml	% inhibition	ml	% inhibition	ml	% inhibition
Control	vehicle	0.70±0.02	--	.074±0.03	--	0.74±0.03	--	0.72±0.04	--
Indomethacin	10mg/kg	0.32±0.06*	54.30	0.30±0.05*	59.45	0.33±0.05*	55.40	0.34±0.02*	52.77
Crude ethanolic extract(CEE)	50mg/kg	0.54±0.02	23.10	0.51±0.05	31.00	0.52±0.02	29.72	0.53±0.02	26.38
	100mg/kg	0.49±0.01	30.00	0.46±0.05*	37.83	0.47±0.03*	36.48	0.51±0.06	29.16
	200mg/kg	0.44±0.03*#	37.12	0.42±0.02*#	43.20	0.42±0.01*#	43.20	0.50±0.03	30.55
Chloroform fraction(CFM)	50mg/kg	0.50±0.01	28.57	0.48±0.02*	35.13	0.49±0.02*	34.00	0.50±0.03*	30.55
	100mg/kg	0.48±0.04*#	33.33	0.45±0.04*#	39.18	0.45±0.03*#	39.18	0.14±0.04*#	34.72
	200mg/kg	0.45±0.04*#	35.71	0.43±0.04*#	41.89	0.42±0.04*#	43.24	0.45±0.03*#	37.50
Butanol fraction(BFM)	50mg/kg	0.42±0.02*#	42.85	0.37±0.05*#	50.00	0.38±0.03*#	49.00	0.40±0.03*#	44.44
	100mg/kg	0.36±0.02*#	48.57	0.33±0.04*#	55.40	0.33±0.06*#	55.40	0.36±0.02*#	50.00
	200mg/kg	0.35±0.03*#	50.00	0.32±0.01*#	56.74	0.33±0.02*#	55.40	0.34±0.01*#	52.77

**p*< 0.05- As compared to control; #*p*< 0.05- As compared to Indomethacin treated group.

Fig.1. Effect of treatment on paw volume

Fig.2. Effect of treatment on percentage inhibition of paw volume


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Corresponding Author:*Papagatla Poli Reddy***Email: polireddy0004@gmail.com