



Research Article

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ANTI-INFLAMMATORY ACTIVITY OF *LEPIDAGATHIS CRISTATA* FLOWER EXTRACTS

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ABSTRACT

The *Lepidigathis cristata* Willd belong to the family of Acanthaceae. In the present study the Anti-inflammatory activity of flower extracts were performed. The methanol, ethyl acetate, chloroform extracts were prepared by soxhlet extraction method and were used for Anti-inflammatory activity in two dose level that is 200 and 400 mg/kg body weight in two screening methods, one is carrageenans induced paw edema method (n = 5), another is Formalin induced paw edema method (n = 5). The flower chloroform extracts showed maximum activity in both models with 50 and 43.4 % of protection at 120 and 180 minutes intervals at the dose of 400 mg/kg body weight respectively.

Keywords: *Lepidigathis cristata*, Anti-inflammatory, carrageenan, Formalin, Methanol, Ethyl acetate, Chloroform.

INTRODUCTION

The plant *Lepidigathis cristata* is a common herb in the eastern plains Karnataka, India. The leaves, roots, flowers, seeds and the whole plant of *Lepidigathis cristata* are medicinally useful¹. The roots of the herb are particularly considered in stomachic and dyspepsia. In present study *Lepidigathis cristata* willd (Family: Acanthaceae) locally known as nakkapidi, lankapidi by yanadi tribal people at Andhra Pradesh, India was selected. This yanadi tribal population is much widely distributed in Cuddapah, Chittoor, Nellore and Prakasham districts and they use this plant for burns, wounds and the tuber ash mixed with coconut oil and used as a lotion. In other parts of India it is known as Kollchechutar (Bombay), karappanundu (Malayalam), Bhuyaterada (marathi), and Otdhomo (Santhal)². It is a perennial herb with procumbent braches of 20 cm long and the leaves are oblong up to 2.5 cm in length and 0.8 cm in width. Spikes capitate mostly crowded on lower nodes bracts pubescent, white margined, spinescent, calyx lobes bract like with a short spine, corolla hairy, white, lower lip with middle lobe larger and pinkish³. The plant was dried under shade is used for anti-inflammatory activity.

MATERIAL AND METHODS

The plant was collected from sheshachala hills near by Rajiv Gandhi Institute of Medical Sciences Kadapa, Andhra Pradesh, India. The plant was identified by Assistant professor Dr. Madhusudana Reddy Department of Botany, Yogivemana University, Kdapa, Andhra Pradesh, India. The specimen of the plant was stored in the department of the pharmacology (specimen. No. TPCP/LC/09/2011). The collected whole plant was dried at room temperature under shade for fifteen days then the flower part was separated manually. The separated plant material was powdered by using mechanical mixer and sieved (mesh.no.40) material used for extraction.

Extractions were carried out by using soxhlet of 1000 ml capacity round bottom flask. The extractions were carried with methanol, ethyl acetate, and chloroform until the solvent becomes colorless in the soxhlet and the extracts were concentrated under reduced pressure. The percentage yield of prepared extracts was found to be 8.6 % w/w, 6.2 % w/w, and 4.7 w/w. The Albino Wistar rats of either sex obtained from Sree Venkateshwara Enterprises Bangalore, India were used for the study. The animals were acclimatized for a week and maintained under standard laboratory conditions, given free access to standard pelleted feed (M/s Pranava agro industries Sangli, Maharastra, India) and U.V. treated purified and filtered water, *ad libitum*. The *Lepidagathis cristata* Willd, flower extracts were made into suspension by using Tween-80 1 % and dissolved in 0.1 % DMF and further made it into suspension by using 1 % tragacanth in water. Diclofenac was used in the dose of 12.5 mg/kg as standard drug.

Drugs and chemicals used

Diclofenac, Formaldehyde 10 % v/v, (Source S.D. Fine chemicals) Carrageenan (Source: Himedia, Lot No 0000112386 RM-1576, Mfg date, 04-2011).

Acute toxicity studies

The maximum lethal dose was found to be 2000 mg/kg body weight orally. The determination of acute toxicity by adapting fixed dose, the guidelines of CPCSEA and 1/10th and 1/5th of LD₅₀, cut of values of the extracts were taken as a screening doses i.e. 200 and 400 mg/kg body weight.

Pharmacological screening

The Wistar rats (100-150 g) of either sex were used for the study. They were acclimatized to normal laboratory conditions for one week under 12 h light and dark cycle

and the given pellet diet and U.V. treated purified filtered water. The extracts were administered orally and the dose was selected between the minimum effective dose and maximum non lethal dose. All the experimentations were performed according to the protocols and recommendations of the institutional animal ethics committee (IAEC) with approved reference number Rc.No.413/Acad/2011-2012.

Anti-inflammatory activity

Carrageenan induced paw edema model in rats

Overnight fasted, albino Wistar rats weighing between 100 and 150 g were randomly divided into eight groups of five animals each, Group I positive control and received normal saline, Group II served as standard diclofenac and Group III to VIII received the test extracts at 200 and 400 mg/ kg through oral route. After 30 minutes of drug administration, 0.1 ml of 1 % w/v suspension of carrageenan was injected into the sub-plantar region of the right hind paw of each rat. The paw volume was measured plethysmographically immediately after injection and at 30, 60,120, 180 and 240 minutes interval^{4,5}. The values are depicted in Table 1.

Table 1: Anti-inflammatory activity of *Lepidagathis cristata* flower extracts on Carrageenan induced paw edema in rats

Group	Dose (mg/kg)	Paw volume, ml after different time interval					
		0 minute	30 minute	60 minutes	120 minutes	180 minutes	240 minutes
Carageenan		0.84 ± 0.03	1.43 ± 0.05	1.49 ± 0.02	1.53 ± 0.04	1.60 ± 0.04	1.64 ± 0.07
Diclofenac	12.5	0.80 ± 0.03	1.06 ± 0.05 ^a	1.12 ± 0.03 ^b	1.17 ± 0.06 ^b	1.11 ± 0.08 ^c	0.90 ± 0.03 ^c
FME	200	0.92 ± 0.03	1.22 ± 0.08 ^a	1.23 ± 0.04 ^b	1.20 ± 0.07 ^c	1.16 ± 0.04 ^c	1.15 ± 0.05 ^c
FME	400	0.85 ± 0.02	1.14 ± 0.01 ^a	1.35 ± 0.02	1.35 ± 0.09 ^a	1.33 ± 0.08 ^b	1.24 ± 0.10 ^c
FCE	200	0.91 ± 0.05	1.35 ± 0.03	1.43 ± 0.05	1.45 ± 0.03	1.38 ± 0.06 ^b	1.32 ± 0.07 ^b
FCE	400	0.73 ± 0.06	1.20 ± 0.05	1.33 ± 0.03	1.46 ± 0.09 ^b	1.38 ± 0.12 ^b	1.30 ± 0.14 ^b
FEE	200	0.85 ± 0.02	0.96 ± 0.03 ^c	1.08 ± 0.04 ^c	1.14 ± 0.08 ^c	0.98 ± 0.05 ^c	0.88 ± 0.11 ^c
FEE	400	0.82 ± 0.73	1.22 ± 0.04 ^a	1.23 ± 0.08 ^a	1.33 ± 0.11 ^a	1.34 ± 0.08 ^b	1.30 ± 0.10 ^c

Table 2: Percentage protection of the *Lepidagathis cristata* extracts by carrageenan induced paw edema in rats

Group	Dose (mg/kg)	% protection				
		30 minutes	60 minutes	120 minutes	180 minutes	240 minutes
Control	-	-	-	-	-	-
Diclofenac		24.5	28.5	31.6	27.9	11.1
FME	200	24.5	25.2	23.3	20.6	20
FME	400	25.4	37	37	36	31.4
FCE	200	32.5	36.3	37.2	34	31
FCE	400	39	45	50	47	43.8
FEE	200	11.4	21.2	25.4	13.2	3
FEE	400	32	33.8	38.3	38.3	36.9

Formalin induced paw edema model in rats

Overnight fasted, albino Wistar rats weighing between 100 and 150 g were randomly divided into eight groups of five animals each. Group I was positive control and received normal saline, Group II served as standard diclofenac and, Group III to VIII received the test extracts at 200 and 400 mg/ kg through oral route. After 30 minutes of drug administration 0.1 ml of 10 % v/v solution of formalin was injected into the sub-plantar region of the right hind paw of each rat. The paw volume is measured plethysmographically immediately after

injection, again at 30, 60,120 and 180 minutes interval^{5,6}. The values are depicted in Table 2.

- Group-1: Carrageen Control-1 % /Formalin 0.1 ml
- Group-2: Standard control-12.5 mg/kg (Diclofenac)
- Group-3: Flower Methanol Extract (FME)-200 mg/Kg
- Group-4: Flower Methanol Extract (FME)-400 mg/Kg
- Group-5: Flower Chloroform Extract (FCE)-200 mg/kg
- Group-6: Flower Chloroform Extract (FCE)-400 mg/kg
- Group-7: Flower Ethyl acetate Extract (FEE)-200 mg/kg
- Group-8: Flower Ethyl acetate Extract (FEE)-400 mg/k

Table 3: Anti-inflammatory activity of *Lepidagathis cristata* flower extracts on Formalin induced paw edema in rats

Group	Dose (mg/kg)	Paw volume, ml after different time interval				
		0 minute	30 minutes	60 minutes	120 minutes	180 minutes
Formalin		1.21 ± 0.16	1.69 ± 0.10	1.64 ± 0.13	1.77 ± 0.09	1.75 ± 0.02
Diclofenac	12.5	1.20 ± 0.08	1.53 ± 0.09 ^a	1.57 ± 0.05	1.51 ± 0.05 ^b	1.41 ± 0.05 ^b
FME	200	1.22 ± 0.08	1.46 ± 0.24	1.47 ± 0.05	1.70 ± 0.05 ^a	1.50 ± 0.07 ^a
FME	400	1.07 ± 0.06	1.21 ± 0.15 ^b	1.50 ± 0.06	1.51 ± 0.10 ^a	1.49 ± 0.09 ^a
FCE	200	1.14 ± 0.07	1.49 ± 0.04	1.65 ± 0.02	1.64 ± 0.01	1.62 ± 0.09
FCE	400	1.14 ± 0.05	1.57 ± 0.14	1.83 ± 0.18	1.9- ± 0.11	1.71 ± 0.15
FEE	200	1.24 ± 0.06	1.50 ± 0.24	1.47 ± 0.05	1.49 ± 0.05 ^a	1.60 ± 0.07
FEE	400	1.12 ± 0.05	1.67 ± 0.14	1.93 ± 0.18	1.98- ± 0.11	1.81 ± 0.15

Values are expressed in MEAN ± S.E.M. for five animals each group. ANOVA followed by Tukeys multiple comparison test. Values are statically ^ap < 0.05, ^bp < 0.01, and ^cp < 0.001 when compared with 0 minute interval

Table 4: Percentage protection of the *Lepidagathis cristata* extracts by on Formalin induced paw edema in rats

Group	Dose (mg/kg)	% protection			
		30 minutes	60 minutes	120 minutes	180 minutes
Control		-	-	-	-
Diclofenac		21	23.5	20.5	14.8
FME	200	16.4	17	28.2	18.6
FME	400	11.5	28.6	29.1	28.1
FCE	200	23.4	30.9	30.4	29.6
FCE	400	27.3	37.7	40	33.3
FEE	200	17.3	15.6	16.7	22.5
FEE	400	32.9	41.9	43.4	38.1

RESULTS

The different extracts of *Lepidagathis cristata* of flower were screened for anti-inflammatory activity and all the compounds showed pharmacological response. The values are expressed in MEAN \pm S.E.M for five animals each group and values are depicted in the Table 1 and 2. The percentage protection was calculated and the values are expressed in the Table 3 and 4.

DISCUSSION

The plant extracts did not exhibit any mortality up to the dose level 2000 mg/kg body weight hence doses were selected 1/5th and 1/10th that is 400 and 200 mg/kg body weight p.o.

Carrageenan induced paw edema model in rats

The flower methanolic extract (FME) of *Lepidagathis cristata* showed 37 % (1.35 \pm 0.09) protection at 400 mg/kg, FCE 50 % (1.46 \pm 0.09) (P < 0.01) at 400 mg/kg and the FEE 38.3 % (1.34 \pm 0.08) (P < 0.01), 400 mg/kg at 180 minutes and all the compounds showed maximum activity at 120 minutes with graded dose response.

Formalin induced paw edema model in rats

The FMC showed 29.1 % (1.51 \pm 0.10) (P < 0.05) at 400 mg/kg, FCE 40 % (1.9- \pm 0.11) and FEE 43.4 % protection on 120 minutes at the dose of 400 mg/kg body weight. The values were significant when compared with zero intervals. The chloroform extract of *Lepidagathis cristata* was found to demonstrate highest anti-inflammatory activity at 400 mg/kg body weight. There is evident that compounds inhibiting the carrageenan induced edema have been found effective against the cyclooxygenase enzymes⁷. Based on these reports it can be inferred that the inhibitory effect of tested plant extracts on the carrageenan induced inflammation were maximum at 120 minutes and they were effective till 180 minutes due to possible mediation by arachidonic acid metabolites, which produce an edema dependent on neutrophil mobilization. In the above extracts flower chloroform extract (FCE) showed highest activity. The formalin injection into rat paw produces localized inflammation and pain. The development of edema in the

rat paw after the injection of formalin with nociceptive effect is biphasic in nature, an early neurogenic component followed by a later tissue-mediated response⁸. The first phase is mediated through the release of histamine, serotonin and kinins where as the second phase is related to release of prostaglandin and slow reacting substances which are at their peak at 3 h. Inhibition of edema observed in the formalin model may be due to the ability of the extracts to inhibit these chemical mediators of inflammation. All the extracts showed significant inhibition in formalin induced rat paw edema.

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