



Evaluation of anti-inflammatory, antinociceptive and antipyretic effects of methanol extract of *Cleome chelidonii*

S. PARIMALAKRISHNAN^{*}, AKALANKA DEY, A. ANTON SMITH and R. MANAVALAN

Department of Pharmacy, Annamalai University, Annamalai Nagar – 608 002, India.

^{*}Corresponding author, E-mail: kalki.vijay@yahoo.co.in

ABSTRACT

The methanol extract of *Cleome chelidonii* whole plant was investigated for its anti-inflammatory, antinociceptive and antipyretic activities at the doses of 50, 100 and 200 mg/kg of body weight. The experimental paradigms used were carrageenan-induced pedal edema for anti-inflammatory activity; acetic acid-induced writhing and hot plate methods to assess antinociceptive activity and yeast-induced hyperpyrexia to evaluate the antipyretic activity. In acute phase inflammation, at dose of 200 mg/kg of body weight after 3 hours treatment with methanol extract of *C. chelidonii* in carrageenan showed a maximum inhibition of 54.6% ($P < 0.05$) when compared with standard drug aspirin (10 mg/kg), which showed decreased formation of granuloma tissue by 60.6% ($P < 0.01$). The methanol extract possesses significant ($P < 0.05$) antinociceptive activity in both paradigms. In rats a significant ($P < 0.01$) reduction in hyperpyrexia was also produced by the extract. This study exhibits that the methanol extract of entire plant of *C. chelidonii* possesses anti-inflammatory, antinociceptive and antipyretic activities.

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Keywords: *Cleome chelidonii*, anti-inflammatory, antinociceptive, antipyretic.

INTRODUCTION

Inflammation is a pathophysiological response of living tissue to injuries that leads to the local increase of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can induce, maintain or aggravate many diseases (Dagmar et al., 2004). However, research work has been going on in inflammatory diseases and the side effects of the currently available anti-inflammatory drugs pose a major problem during their clinical use (Seibert et al., 1994). Therefore, development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary.

The management and treatment of pain is probably one of the most widespread and yet the most difficult aspect to find out a remedy. Analgesic therapy is currently dominated by two major classes of analgesic

drugs, namely opioids and non steroidal anti-inflammatory drugs (NSAIDs). Both classes of analgesic drugs produce serious adverse effects, such as GI disturbances, renal damages (with NSAIDs drugs), respiratory depression and possibly dependence (with opioids). It is understandable that proposition of analgesic agents with fewer adverse effects is desirable. One of the ways to achieve this aim is the use of medicinal plants, which are a rich source of new potentially effective compounds (Mahmood et al., 2004).

Cleome chelidonii (L.) Linn var. (CC) (synonym/other Latin name is *Polanisia chelidonii* DC) is a traditional plant belonging to the family Capparaceae. It is grown as perennials through out dry seasons. It has a pink or white colour flowers. The leaves of CC are claimed to have medicinal properties. CC is generally known for the treatment of colic, dysentery, headache, otitis, and rheumatism (Kirtikar et al., 1987). It has also

been found to possess multiple therapeutic properties like vermifuge and skin diseases (Chopra et al., 1992). CC contains glucocapparin and glucocleomin (Songsak et al., 2002). However, no scientific data are available to prove the effects of the folklore claim. Hence this study was undertaken to establish scientific data for the a) anti-inflammatory activity using carrageenan induced paw edema in rats b) analgesic potential of the methanol extract of CC on acetic acid-induced writhing test and hot plate reaction time in albino mice and c) antipyretic activity of the methanol extract of CC using yeast-induced pyrexia on rats.

MATERIALS AND METHODS

Preparation of the extract

The samples (entire plant) of *Cleome chelidonii* were collected from Sengottai of Tamilnadu, India. The plant material was taxonomically identified and authenticated by the Department of Botany, Annamalai University, Annamalinagar, India. The samples were cleaned, dried under shade, powdered by a mechanical grinder and stored in an airtight container. Methanol was used as solvent in the extraction process. The methanol extract was prepared by using dried coarse material of the whole plant and methanol (yield 2.78%), in a soxhlet apparatus. The methanol extract of *Cleome chelidonii* was suspended in 1% gum acacia and was administered to the animals at the doses of 50, 100 and 200 mg/kg of body weight for each group except the control group in all the experiments.

Animals

Swiss albino mice (25-30 g) and Wistar albino rats (150-200 g) of both sexes were procured from the central animal house of the institute. They were kept in standard polypropylene cages maintained under standard room temperature (20 ± 4 °C; relative humidity 60-70%) in a 12 hours dark-light cycle. The animals were fed with standard pellet diet and water *ad libitum* as usual. The experiments were conducted after getting the prior approval from the IAEC permission.

Chemicals and Drugs used

The chemicals and drugs used were carrageenan (Sigma – Aldrich), acetic acid

(Sigma – Aldrich). Aspirin (Vikash Pharma), paracetamol (Calpol) and morphine (Modi Mundi Pharma) were used as standard drugs.

Anti-inflammatory Models

The anti-inflammatory activity was done by the method of Mahat et al. (2007) and Gupta et al. (2005). The animals were divided into 5 groups (n = 6 per group). Acute inflammation was produced by subplantar injection of 0.1ml of 1% suspension of carrageenan with 2% mucilage in normal saline, in the right hind paw of the rats. After the administration of the carrageenan, the paw volume was measured plethysmometrically at 0 and 3rd hour. Difference between the two readings was taken as the volume of edema and the percentage of anti-inflammatory activity was calculated. The ratio of the anti-inflammatory effect of methanol extract of *C. chelidonii* was calculated by the following equation:

$$\text{AIA (\%)} = (1 - P / SC) \times 100$$

where,

AIA = Anti-inflammatory activity;

P represents the percentage difference of paw volume after administration of drugs;

SC represents the percentage difference of paw volume in the control groups.

Aspirin 100 mg/kg, p.o suspended in 2% mucilage was used as the standard drug.

Acetic acid-induced writhing test

The antinociceptive effect was determined by the method of Gupta et al. (2005) and Huo et al. (2007). Acetic acid in distilled water (0.6%) at the dose of 10 ml/kg of body weight was injected intraperitoneally (i.p.). The number of writhes after 30 minutes was counted. Aspirin in dose of 100 mg/kg, suspended in 2% mucilage was used as the standard drug. Then the percentage inhibition of writhing was calculated.

Hot plate reaction time in mice

The antinociceptive activity was estimated by the method of Huo et al. (2007) and Prashant et al. (2005). Mice were prescreened by placing them on a hot plate maintained at 55 ± 1 °C and the reaction time (in seconds) for hind paw licking or jumping was recorded. Mice which reacted within 15 seconds and which did not show large variation when tested on four separate

occasions (each 15 minutes apart) were used for the study. Morphine (5 mg/kg, i.p.) was used as a standard drug. The time for hind paw licking or jumping on the heated plate of analgesiometer was taken as the reaction time.

Induction of Yeast-induced pyrexia

The antipyretic property was measured by the method reported by Nisar et al. (2007). 20 ml/kg of 20% aqueous suspension of sterilized brewer's yeast powder was administered to the rats subcutaneously. After 24 hours, animals showing an increase of rectal temperature >0.5 °C were selected. Animals were divided into five groups of six rats in each group and were trained to remain quiet in a restraint cage. Normal saline was administered to control group; while experimental group received doses of 50, 100 and 200 mg/kg of body weight of the methanol extract. The fifth group of animals received the standard drug paracetamol (150 mg/kg of body weight) orally. Rectal temperature was measured at 30 minutes before administration and at 30 minutes, 1, 2 and 4 hours after administration by using thermal-probe.

Statistical analysis

Values in the test and tables are represented as Mean \pm SEM. The results were analyzed for statistical significance using one-way ANOVA followed by Dunnet's test. A P value < 0.05 was considered significant.

RESULTS

Anti-inflammatory studies

The methanol extract of *C. chelidonii* (50, 100 and 200 mg/kg) against various experimental animal models exhibited significant (P < 0.05) anti-inflammatory

activity. The effects of methanol extract of *C. chelidonii* and aspirin on the inflammation (induced by carrageenan which forms granuloma) are summarized in figure 1. The methanol extract of *C. chelidonii* showed maximum inhibition of 54.6% at the dose of 200 mg/kg after 3 hours of treatment in carrageenan-induced paw edema, whereas the standard drug (aspirin 10 mg/kg) showed 70.4% of inhibition (P < 0.05).

Acetic acid-induced writhing in mice

The results presented in table 1 showed 15.5%, 25.8% and 45.8% significant (P < 0.01) inhibition of control writhes by methanol extract of *C. chelidonii* at the doses of 50, 100 and 200 mg/kg of body weight respectively and 64.0% significant (P < 0.01) inhibition by aspirin (100 mg/kg).

Hot plate reaction time in mice

The result presented in table 2, showed that the methanol extract of *C. chelidonii* has produced (P < 0.01) antinociceptive activity at all the tested doses when compared to that of control group.

Yeast-induced Hyperpyrexia

The results of the antipyretic activity of the extracts are presented in table 3. The subcutaneous injection of yeast suspension marked elevated rectal temperature after 24 hours of administration. All the doses of the extracts showed a significant antipyretic effect like the standard drug paracetamol. The result obtained from both the standard drug and methanol extract of *C. chelidonii*-treated rats were compared with the control group and a significant (P < 0.01) reduction in rectal temperature of yeast-induced rats was observed.

Table 1: Effects of methanol extract of *C. chelidonii* and aspirin on writhing induced by acetic acid in mice.

Treatment	Dose (mg/kg)	Number of writhes (per 30 min) ^a	Inhibition (%)
Control	--	29.33 \pm 2.24	--
CCME	50	25.00 \pm 1.97	15.50
CCME	100	23.43 \pm 1.04 ^b	25.80
CCME	200	17.55 \pm 1.59 ^b	45.80
Aspirin	100	15.00 \pm 0.90 ^b	64.00

^a The results given are mean \pm S.E.M.; n = 6 animals in each group

^b P < 0.01 , Experimental groups were compared with control

CCME: Methanol extract of *C. chelidonii*

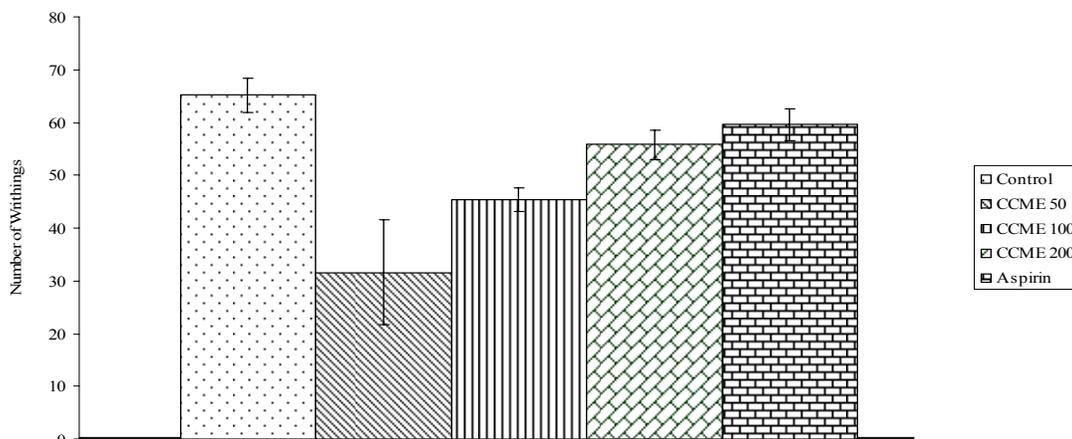


Figure 1: Effect of CCME and aspirin on carrageenan-induced rat paw edema. Difference of means of edema volume (ml) between control and treatment values at different doses \pm S.E.M. Variation compared to the control animals. CCME: Methanol extract of *C. chelidonii*

Table 2: Effects of methanol extract of *C. chelidonii* and morphine on hot plate reaction time in mice.

Treatment	Dose (mg/kg)	Mean latent time ^a	
		Initial	After 30 min
Control	--	6.64 \pm 0.42	6.14 \pm 0.36
CCME	50	7.53 \pm 0.56 ^b	11.34 \pm 1.93
CCME	100	7.87 \pm 0.79 ^b	12.25 \pm 1.82
CCME	200	7.20 \pm 0.63	13.74 \pm 1.06
Morphine	5	7.94 \pm 0.77 ^b	17.17 \pm 1.03

^a The results given are mean \pm S.E.M.; n = 6 animals in each group;

^b P < 0.05, Experimental groups were compared with the control group;

CCME: Methanol extract of *C. chelidonii*

Table 3: The effect of methanol extract of *C. chelidonii* and paracetamol on yeast induced pyrexia in rats.

Treatment	Rectal temperature ($^{\circ}$ C) ^a					
	After yeast injection at		After drug administration at			
	0hr	24hr	1hr	2hr	3hr	4hr
Control	37.6 \pm 0.02	39.9 \pm 0.03	39.8 \pm 0.02	38.2 \pm 0.05	38.9 \pm 0.05	39.9 \pm 0.04
CCME 50 mg/kg	37.7 \pm 0.07	37.8 \pm 0.03 ^b	37.5 \pm 0.02	38.7 \pm 0.02 ^b	39.4 \pm 0.04 ^b	38.1 \pm 0.06 ^b
CCME 100 mg/kg	38.6 \pm 0.02	37.7 \pm 0.04	37.8 \pm 0.05 ^b	38.3 \pm 0.05	39.1 \pm 0.03 ^b	37.9 \pm 0.06 ^b
CCME 200 mg/kg	38.7 \pm 0.04	39.9 \pm 0.06	37.7 \pm 0.02 ^b	39.9 \pm 0.03 ^b	38.7 \pm 0.05	37.9 \pm 0.04 ^b
Paracet. 150 mg/kg	37.8 \pm 0.02	39.9 \pm 0.03 ^b	38.9 \pm 0.02	38.5 \pm 0.02 ^b	39.3 \pm 0.04 ^b	39.7 \pm 0.03

^a The results given are mean \pm S.E.M.; n = 6 animals in each group

^b P < 0.01, Experimental groups were compared with the control group.

CCME: Methanol extract of *C. chelidonii*; Paracet.: Paracetamol

DISCUSSION

Carrageenan-induced rat hind paw edema has been used as a standard inflammation model in order to investigate the acute inflammatory effect of the drug. There are two phases of carrageenan-induced

inflammatory reaction: early or first phase and later or second phase. The early phase results from histamine; serotonin and bradykinin liberation and the second phase is associated with the release of prostaglandin and slow reacting substances (Lee et al., 2001).

In one of the previous study *Cleome* species *C. viscosa* methanol extract at 100, 200 & 400 mg/kg of body weight has shown a promising analgesic activity in mice using the acetic acid writhing, tail flick, tail clip and tail immersion methods (Parimaladevi et al., 2003). When compared to this the whole plant of *C. chelidonii* methanol extract has produced less anti-inflammatory effect.

In another work, the methanol extract of the leaves of *C. gynandra* exhibited significant anti-inflammatory activity in adjuvant-induced arthritic rats. A significant decrease in paw edema with a remarkable increase in body weight was observed following oral administration of the leaf extract (150 mg/kg of body weight). These results demonstrated that the plant extract has no harmful effect and exerts *in vivo* anti-inflammatory properties against adjuvant-induced arthritis (Ogonowski et al., 1997). When compared to this the whole plant of *C. chelidonii* methanol extract has produced less anti-inflammatory effect.

In some other study, antipyretic activity of a methanol extract of *C. viscosa* was investigated for its potential on yeast-induced pyrexia in albino rats, at doses of 200, 300, and 400 mg/kg body weight p.o. and showed significant reduction in normal body temperature (Bouriche et al., 2005). When compared to this the whole plant of *C. chelidonii* methanol extract has less significant reduction in antipyretic effect.

The effects of *C. arabica* leaf extract, rutin and quercetin on soybean lipoxygenase (Lox) activity and on calcium ionophore (A23187) stimulated generation of the leukotriene B₄ and prostaglandin E₂ by human neutrophils were examined. Results indicate that rutin; quercetin and an extract of *C. arabica* containing these compounds inhibit Lox activity, consequently decreasing LTB₄ production. These compounds and extracts containing them may be beneficial for the treatment of inflammatory conditions, particularly those characterized by excessive leukotriene generation (Narendhirakannan et al., 2005).

In this present study, there is an increase in the hind paw volume following carrageenan administration in the control group (0.50 ± 0.03 ml) and aspirin treated group (0.21 ± 0.01 ml). A significant anti-

inflammatory activity was observed for methanol extract of *C. chelidonii* in carrageenan model. In carrageenan-induced paw edema the methanol extract of *C. chelidonii* showed maximum inhibition of 54.6% at the dose of 200 mg/kg after 3 hours of drug treatment. The methanol extract of *C. chelidonii* produced dose-dependent and significant inhibition of carrageenan-induced paw edema. The inhibition produced was less significant when compared to that of the standard drug.

The abdominal constriction may be related to sensitization of nociceptive receptors to prostaglandins induced by acetic acid, which is a sensitive procedure to establish peripherally acting analgesics. The number of writhing movements during a 30 minutes observation in the control group was 29.33 ± 2.24 , which corresponds with findings of other workers (Parimaladevi et al, 2003).

In the hot plate method, the test drug in different doses increased the pain threshold significantly during the period of observation and this indicates the involvement of higher center.

Fever may be a result of infection or one of the sequelae of tissue damage, inflammation, graft rejection or other diseases. Antipyretic drugs reduce elevated body temperature. Regulation of body temperature requires a delicate balance between production and loss of heat, and the hypothalamus regulates the set point at which body temperature is maintained. The result obtained from both the standard drug and methanol extract of *C. chelidonii* treated rats were compared with the control group and found to possess significant reduction in the yeast induced rats. In fever this set point is elevated and a drug like paracetamol helps in keeping the body temperature normal when it is elevated by factors such as exercise or increases in ambient temperature (Goodman and Gilman, 1996).

Conclusion

Based on results of the present study it can be concluded that the methanol extract of *C. chelidonii* has promising activity against acute phase of inflammation at a dose range of 50-200 mg/kg body weight and among the three doses the dose of 200 mg/kg is found to be more potent and efficacious against anti-

inflammatory effect. However, the analgesic activity of methanol extract of *C. chelidonii* was found to have more significant effect on the acetic acid-induced model than the hot plate method and thus it appears that the extract inhibits predominantly the peripheral pain mechanism. The antipyretic activity also showed a less significant influence when compared with the anti-inflammatory and antinociceptive activity. More detailed phytochemical studies are, however, necessary to identify the active principle(s) and exact mechanism of action.

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