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Antinociceptive and anti-inflammatory effects of *Cleome chelidonni* Linn. roots in experimental animals

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ABSTRACT

The effect of *Cleome chelidonni* Linn (Family: Capparidaceae) methanolic methanolic root extract was studied for the antinociceptive and anti-inflammatory activity in experimental animals. *C. chelidonni,* 25 – 100 mg/kg administered orally for 3 days exhibited graded dose response equivalent to 21.95% - 89.90% protection in the tail flick latent test in rat. The *C. chelidonni* methanolic root extract (50 and 100 mg/kg, p.o X 3 days) was effective in hot plate reaction time (64.05% and 112.97%, p< 0.01 and p< 0.001), analgesymeter induced mechanical pain (28.17% and 54.42%, p < 0.01 and p < 0.001) and acetic acid- induced writhing (26.68% and 51.79%, p < 0.5 and p < 0.05) in mice. The methanolic root extract of *C. chelidonni* potentiated the analgesic activity with pentazocine (10 mg/kg, i.p.) and aspirin (25 mg/kg, i.p.). In the carrageenan- induced paw edema *C. chelidonni* methanolic root extract (50 and 100 mg/kg, p.o X 3 days) decreased paw volume significantly (26.68% and 51.79%) and dose dependent anti-inflammatory activity in 1-3 hour time interval and potentiated with nimesulide (50 mg/kg, p.o.). In summary, this study demonstrates that methanolic methanolic root extract of *C. chelidonni* has significant antinociceptive and anti-inflammatory activity.

KEY WORDS: *Cleome chelidonni*, root, pain, inflammation, Antinociceptive

INTRODUCTION

Cleome chelidonni Linn. a plant belonging to the family of Capparidaceae is grows as weed in most tropical countries. It is widely distributed throughout the India is also called "Polanisia chelidonni". Infusion of this plant used in gingivitis and in skin diseases. The Preliminary Phytochemical studies reveal the presence of Glucocapparin and Glucocleomin. But no scientific work has been carried out on the roots (1). The Lambadi tribals of north telangana districts of Andhra Pradesh use this plant for the treatment of pain, gastro intestinal disorders and infectious diseases. . The ethnic tribal communities have been using the *C. chelidonni* from many generations and information regarding the efficacy remains primarily anecdotal. There is no previous record and research work available on the traditional medicinal values of *C. chelidonni* . Most of the ancient knowledge systems continued to survive by oral communication from generation to generation in rural as well as in tribal communities. Therefore, the present study was undertaken to demonstrate scientifically the antinociceptive and anti-inflammatory activities of the standardized methanolic root extract of *C. chelidonni* leaves in experimental animals.

MATERIALS AND METHODS

Plant material

Cleome chelidonni L. roots were collected freshly in and around our university campus, Visakhapatnam, South India. The plant was identified by the botanist Dr. P. Jayababu, department of botany, A.M.L degree college, Anakapally. A voucher specimen of the collected sample was also deposited in the herbarium of our college for the future reference.

Alcoholic extraction

Alcoholic extract was prepared from a powder of the root of *C. chelidonni* prepared in electric grinder. The

750 g powder was extracted with alcohol (95%v/v) in soxlate apparatus. The extract was evaporated to dryness under vacuum and dried in vacuum desiccator (14.5%w/w).

Test animals

Charles-Foster (CF) albino rats (110-125gm) and Wistar strain mice (16-18 gm) of either sex were obtained from the animal house of Mahaveer enterprises, Hyderabad. They were kept in the departmental animal house at 25 ± 2°C and relative humidity 45 – 51.5%, light and dark cycles of 10 and 14 h respectively for 1 week before and during the experiments. The animals were provided with standard rodent pellet diet (Hind lever) and water was allowed *ad libitum*. Rearing up of animals in the experimental period and there upkeep during the entire experimental span confirmed to ethical guidelines laid down by Institutional Animal Ethical Committee.

Drug treatment

The essential methanolic root extract of the leaves *C. chelidonni* (suspended in 0.5% carboxy methyl cellulose in distilled water) in doses of 25 - 100 mg/kg was administered once daily for three consecutive days. Nimesulide (Cipla, India) in the dose of 50 mg/kg, p.o was used as the standard anti-inflammatory agent, where as pentazocine (Ranbaxy, India) 10 mg/kg, i.p. and aspirin (Astra – IDL Ltd, India) 25 mg/kg, i.p. were used as standard analgesic agents. All the reference drugs were administered 30 minutes before the experiment. Control group of animals received suspension of 0.5% carboxy methyl cellulose in distilled water. Experiments were conducted on day 3, one hour after last drug or vehicle administration.

Pharmacological tests

Antinociceptive activity

Tail flick latent period

The technique described by Davies et al., (2) was adopted, using a techno analgesiometer. The rat was placed in a rat holder with its tail coming out through a slot in the lid. The tail was kept on the bridge of the analgesiometer, called jacket with an electrically heated nichrome wire underneath. The tail received radiant heat from the wire, heated by passing current by 6 mA. The time taken for the withdrawal of the tail after switching on the current, was taken as a latent period, in seconds of "tail flicking response and was considered as the index of nociception. The cut off time for determination of latent period was taken at 30 seconds to avoid injury to the skin (3). Three tail flick latencies were measured per rat at each time interval and the means of the tail flick latencies were used for statistical analysis. Pentazocine (10 mg/kg, i.p.) was used as a standard reference.

Hot plate reaction time in mice

Mice were screened by placing them on a hot plate maintained at 55 ± 1°C and recording the reaction time in seconds for fore paw licking or jumping. Only mice which reacted within fifteen seconds and which did not show large variation when tested on four separate occasions, each fifteen minutes apart, were taken for the test. Pentazocine (10 mg/kg i.p.) was used as reference standard. The time for fore paw licking or jumping on the heated plate of the analgesiometer was taken as a reaction time (4).

Analgesy-metre induced pain

The analgesic effect of *C. chelidonni* was tested in mice of either sex using an Ugo Basile analgesy metre. This method involves the application of force to the paw of the mice using the analgesy-metre which exerts a force that increases at a constant rate. The mice were gently placed between the plinth and plunger. The instrument was switched on and constant motor rate was used to drive the plunger on to the paw of mice. When the mice struggles the instrument is switched off and force at which the animal felt pain was read on a scale calibrated in gram X 10 by pointer.

Acetic acid induced writhing response in mice

Acetic acid solution at a dose of 10ml/kg (0.6%) was injected i.p. and the number of writhes during the following 15 minutes period was observed (5). Significant reductions in number of writhes by drug treatment as compared to vehicle treatment animals were considered as a positive analgesic response. The percent inhibition of writhing was calculated.

Anti-inflammatory activity

Carrageenan-induced paw edema

Rats were injected with 0.1 ml of 1 % λ carrageenan into the subplantar region of the left hind paw (6). The paw was marked with ink at the level of lateral malleolus and dipped in perspex cell up to the mark. The paw volume was measured with Ugo Basile Plethysmometer (No: 6142, 7140 Comerio-varese, Italy) before and 60, 120 and 180 minute's after injecting the λ carrageenan suspension.

Statistical analysis

The values are expressed as mean ± SEM. Statistical significance of the differences between control and treated groups was calculated using unpaired Students't test followed by Mann-Whitney U-test (two tailed). A value of $p < 0.05$ was considered to be significant.

RESULTS

Tail flick latent period

The methanolic root extract of *C. chelidonni* at the dose levels of 25, 50, 100 mg/kg exhibited graded dose response equivalent to 21.95% - 89.90% protection. Pretreatment with pentazocine significantly potentiated the antinociceptive effect of *C. chelidonni* at the dose of 50 and 100 mg/kg producing 115.21% and 144.48% protection (Table 1).

Hot plate reaction time in mice *C. chelidonni* (50 and 100 mg/kg) significantly increased the reaction time and the percent protection is equivalent to 64.05% and 112.97% respectively. Pentazocine increased the reaction time of *C. chelidonni* to 238.20% and 257.30% at 50 and 100 mg/kg (Table 2).

Analgesy-meter induced pain

The data (Table 3) indicates that the methanolic root extract of *C. chelidonni* treated mice exhibited resistance against mechanical pain after 30 minutes. The weight that indicates pain after treatment was dose dependent and significantly synergies the activity of aspirin.

Acetic acid induced writhing- The methanolic root extract of *C. chelidonni* showed a significant decrease in writhing response induced by acetic acid and the degree of percent inhibition was 26.68% and 51.79% at 50 and 100 mg/kg. Under the same experimental condition the analgesic effect of *C. chelidonni* potentiated the analgesic activity of aspirin as shown by further decrease in the writhing response and prevented the abdominal cramping, when given in combination (Table 4).

Carrageenan induced paw edema

Treatment with different doses of *C. chelidonni* methanolic root extract at 50 and 100 mg/kg showed a significant and dose dependent anti-inflammatory activity with time interval 1-3 hours. The effect was similar to nimesulide and significantly potentiated the activity of *C. chelidonni* (Table 5).

DISCUSSION

C. chelidonni showed significant anti-nociceptive and anti-inflammatory effects on the experimental animal models. The methanolic root extract of *C. chelidonni* was found to increase significantly the tail flick reaction time. This test is useful for discriminating between centrally acting opiate and non opiate analgesics, giving positive response with the former only. The essential methanolic root extract of *C. chelidonni* exhibited analgesic activity in rats and potentiated with the analgesic activity with pentazocine. Hot plate reaction time in mice method was originally described by Woolfe and MacDonald (4). This test has been found to be suitable for evaluation of centrally but not of peripherally acting analgesics. The validity of this test has been shown even in the presence of substantial impairment of motor performance (7). The significant results indicate that *C. chelidonni* may be acting centrally. Analgesy-meter induced pain is the force applied to the paw by the plinth increases at a constant rate, being the motor synchronous with mains frequency, its speed (60rpm) is constant, unaffected by friction and wear. The force measured on the scale is in 10 gram steps by a pointer riveted to the slide. *C. chelidonni* significantly alleviated the pain threshold. This offers new perspectives in the treatment of pain, as there is evidence that a symptom of vital pain varies in intensity with central and peripheral somato sensory pathways. In the acetic acid induced writhing response, *C. chelidonni* significantly inhibited the abdominal constriction and potentiated the activity of aspirin in mice. Acetic acid causes an increase in peritoneal fluids of PGE_2 and $PGF_2\alpha$ involving in part, peritoneal receptors (8, 9) and is very sensitive method of screening anti-nociceptive effect of compounds (10).

To assess the antiinflammatory activity, *C. chelidonni* was evaluated by using the carrageenan-induced paw edema method. Carrageenan-induced paw edema as an in *vivo* model of inflammation is a screening procedure in which the involvement of the cyclooxygenase products of arachidonic acid metabolism and the production of reactive oxygen species are well established (11). Development of edema induced by carrageenan is commonly correlated with the early exudative stage of inflammation, one of the important processes of inflammatory pathology (12, 13). The results in table 5 show that *C. chelidonni* (50 and 100 mg/kg) caused significant ($p < 0.05$) inhibition of the carrageenan-induced paw edema. These inhibitions were similar to that produced by the non-steroidal antiinflammatory drug used as a positive control (50 mg/kg Nimesulide, p.o.) at 3 h after induction. In the beginning of carrageenan injection, there was a sudden elevation of paw volume in relation to histamine mediators (14). After 1 h the inflammation increased gradually and was elevated during the later 3 h. This second phase could be due to the prostaglandins and kinins liberated, which accompanies leukocyte migration. *C. chelidonni* could be antiinflammatory by inhibiting the cyclooxygenase

Table 1. *Effect of C. chelidonni methanolic root extract on tail flick latent period in rats*

Treatment	Dose (mg/kg)	Mean latent period of tail flick response (sec)	
		Initial	After 30 min
Control	_	5.05 ± 1.01	7.01 ± 1.32
C. chelidonni	25	6.09 ± 1.42	7.92 ± 1.01
C. chelidonni	50	5.59 ±1.18	10.91 ± 1.61[a]
C. chelidonni	100	4.87 ± 1.35	14.25 ± 1.92[b]
Pentazocine	10	5.72 ±1.05	15.90 ± 1.41[b]
C. chelidonni + Pentazocine	50 + 10	5.73 ± 1.03	19.53 ± 1.09[c]
C. chelidonni + Pentazocine	100 + 10	5.10 ± 1.41	23.05 ± 1.25[c]

Values are mean ±SEM for six rats
p: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to control group

Table 2. *Effect of C. chelidonni methanolic root extract on hot plate reaction time in mice*

Treatment	Dose (mg/kg)	Mean latent period (sec)	
		Initial	After 30 min
Control	_	9.96 ± 1.10	10.10 ± 1.12
C. chelidonni	50	10.15 ± 1.15	17.21 ± 2.15[a]
C. chelidonni	100	10.93 ±1.32	22.64 ± 2.89[b]
Pentazocine	10	10.45 ± 1.39	31.33 ± 4.10[c]
C. chelidonni + Pentazocine	50 + 10	9.40 ± 1.05	36.54 ± 3.54[c]
C. chelidonni + Pentazocine	100 + 10	9.69 ± 1.19	38.66 ± 3.93[c]

Values are mean ±SEM for six mice
p: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to control group

Table 3. *Effect of C. chelidonni methanolic root extract on force induced pain in mice*

Treatment	Dose (mg/kg)	Weight causing pain (g)	
		Before administration	After administration
C. chelidonni	50	84.9 ± 4.72	109.1 ± 5.99[a]
C. chelidonni	100	85.0 ± 5.61	131.8 ± 7.05[b]
Aspirin	10	85.2 ±6.20	130.4 ± 7.07[b]
C. chelidonni + Aspirin	50 + 25	82.3 ± 4.35	141.0 ± 7.13[b]
C. chelidonni + Aspirin	100 + 25	84.1 ± 5.17	147.0 ± 8.32[b]

Values are mean ±SEM for six mice
p: [a]< 0.01 and [b]< 0.001 compared to respective before administrative group

Table 4. *Effect of C. chelidonni methanolic root extract on acetic acid-induced writhing in mice*

Treatment	Dose (mg/kg)	Number of writhing	% Inhibition
Control	-	25.41 ± 3.11	-
C. chelidonni	50	18.63 ± 2.33	26.68
C. chelidonni	100	12.25 ± 2.85[a]	51.79
Aspirin	10	10.55 ± 1.94[b]	58.48
C. chelidonni + Aspirin	50 + 25	8.97 ± 1.51[c]	64.69
C. chelidonni + Aspirin	100 + 25	7.69 ± 1.21[c]	69.74

Values are mean ±SEM for six mice
p: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to control group

Table 5. *Effect of C. chelidonni methanolic root extract on λ carrageenan induced paw edema in rats*

Treatment	Dose (mg/kg)	Paw volume (ml) at		
		60 min	120 min	180 min
Control	-	0.81 ± 0.03	1.35 ± 0.04	1.16 ± 0.03
C. chelidonni	50	0.85 ± 0.03	0.84 ± 0.03	0.79 ± 0.02[c]
C. chelidonni	100	0.73 ± 0.02	0.78 ± 0.02[b]	0.53 ± 0.01[c]
Nimesulide	50	0.65 ± 0.02[b]	0.54 ± 0.02[c]	0.52 ± 0.01[c]
C. chelidonni + Nimesulide	50 + 50	0.78 ± 0.01[a]	0.42 ± 0.02[c]	0.38 ± 0.01[c]
C. chelidonni + Nimesulide	100 + 50	0.67 ± 0.01[b]	0.37 ± 0.01[c]	0.31 ± 0.01[c]

Values are mean ± SEM for six rats
p: [a]< 0.05, [b]< 0.01 and [c]< 0.001 compared to respective control group

pathway, considering that the mechanism involved in the genesis of the carrageenan induced edema could cause the release of prostaglandins and kinins, among other substances (15, 16). In conclusion, the methanolic root extract of *C. chelidonni* significantly antagonized acetic acid-induced writhing and significantly attenuated the nociception produced by hot plate thermal stimulation as well as reducing the inflammation induced by carrageenan. The exact mechanism of action and the active principles responsible for such activities remain to be elusidated, but taking all these *in vivo* results together it can be suggested that *C. chelidonni* was able to prevent the production of proinflammatory mediators, especially those related to the lipoxygenase and cyclooxygenase pathways.

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