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Analgesic effects of methanolic extract of *Capparis zeylanica* Linn. roots

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ABSTRACT-

The present study was aimed to investigate the analgesic effects of the methanolic extract of *Capparis zeylanica* Linn. (MECZ) roots in rats and mice. The MECZ (250- 500 mg/kg; p.o.) was evaluated for its analgesic activity by employing acetic acid-induced writhing test, hot plate test and tail immersion tests i.e. in hot and cold water. MECZ (250- 500 mg/kg; p.o.) showed significant (P<0.01) reduction in the number of writhing induced by acetic acid, increased reaction time in hot plate test and elevated pain threshold in hot and cold water tests. MECZ exhibited the dose-dependent analgesic effects. In addition, the acute toxicity studies revealed that MECZ has good margin of safety and did not show the lethal effects on the animals up to the doses of 4 g/kg.

KEYWORDS- *Capparis zeylanica*, Analgesic activity, Writhing, Hot plate, Tail immersion tests

INTRODUCTION

Medicinal herbs are an indispensable part of the traditional medicine practiced all over the world due to low costs, easy access and ancestral experience (1). *Capparis zeylanica* Linn. (Family: Capparidaceae), is commonly known as Indian caper, a climbing shrub found throught India and has been used as a 'Rasayana' drug in the traditional Ayurvedic system of medicine. In northern India, the leaves are widely used as counter- irritant, febrifuge and as a cataplasm in swellings and piles (2). *Capparies zeylanica* has been reported to possess anthalmintic and antimicrobial (3), and immunostimulant activity (4). Modern phytochemical screening of the plant has shown the presence of fatty acids (5), flavonoids (6) and alkaloids (7).

An attempt was, therefore, made to evaluate the analgesic activity of the methanolic extract of *Capparis zeylanica* Linn. roots in different models of pain in albino rats and mice. The extract was also studied for its acute toxicity studies and preliminary phytochemical screening.

MATERIALS AND METHODS

Extraction of the plant material

The roots of *Capparis zeylanica* were collected from the local areas of Wardha district, Maharastra, India and were authenticated by the authority of Botany Department, Nagpur University, Nagpur, India where a voucher specimen (no. 6581) is deposited for the future reference. The roots (500 g) were cleaned, shade dried, coarsely powdered (sieve number: 12) and extracted with methanol using Soxhlet apparatus. The extract was evaporated in *vacuo* (50^{o}C) up to the dryness. Extractive value of the dried methanol extract was found to be 10.82 % w/w.

Animals

Albino Rats (Wistar strain) weighing between 200- 250 g and Swiss albino mice weighing between 20-25g of either sex were used and were maintained at 25±3°C. They were kept in well ventilated animal house under natural photoperiodic condition in large polypropylene cages and were fed standard rat chow and water *ad libitum*. The animal experimental protocol was approved by Animal Ethical Committee of Institute.

Acute toxicity studies

The 50 % lethal dose of the MECZ was estimated by the employment of up- and –down stair case method in mice (8). Doses were adjusted by a constant multiplicative factor viz. 4, for this experiment. The dose for each successive animal was adjusted up and down depending on the previous outcome.

Evaluation of analgesic activity

Acetic acid induced writhing test (Chemical stimulation)

The methanol extract was evaluated for its analgesic activity by acetic acid-induced writhing model described by Siegmund et al (9) and modified by Koster et al (10). Swiss albino mice were divided into four groups. First group was used as negative control and received 5 % gum acacia (5 ml/kg), an hour before

injection (i.p.) of 0.6 % v/v acetic acid (10 ml/kg). Second group served as positive control and received aspirin 300 mg/kg, while the third and fourth groups were administered orally with divided doses of MECZ i.e. 250 and 500 mg/kg, respectively, an hour before acetic acid injection. The number of abdominal constrictions (writhing) and stretching with a jerk of the hind limb was counted for 15 minutes after administering acetic acid. Percent protection against writhing movement was taken as index of analgesia.

Hot Plate method (Thermal stimulation)

In the hot plate method (11) albino rats (200- 250 g) were divided into 4 groups each consisting of six animals. First group served as negative control (received 5 ml/kg of 5 % Gum acacia). The second group served as positive control (received pentozocin 5mg/kg) while the third and fourth group received MECZ (250-500 mg/kg; p.o.). The basal reaction time was noted before 1, 2, 3 hrs after the administration of the drugs.

Tail immersion methods

Hot tail flick test

Swiss albino mice were screened by exposure to the thermal stimulus. The mice showing positive response were divided into four groups of six animals each. The animals of first and second groups were treated, respectively, with 5 % w/v Gum acacia, pentazocine (5 mg/kg; p.o.). The animals of third and fourth groups were administered orally with divided doses of MECZ i.e. 250 and 500 mg/kg, respectively. About 5 cm of the tail of mice was dipped in warm water, kept constant at 50±0.7°C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cutoff time being 60 sec. The latent period of the tail flick response was taken as the index on antinociception and was determined immediately after injection (12).

Cold tail flick test

Swiss albino mice of either sex were employed in this test and selected by exposure to cold stimulus. The mice showing positive response were divided into four groups. Before the experimentation, all the animals were fasted for 24 h (only water *ad libitum* was givrn). The animals of first and second groups were treated, respectively, with 5 % w/v Gum acacia, pentazocine (5 mg/kg; p.o.). Similarly, the animals of third and fourth groups were administered orally with divided doses of MECZ i.e. 250 and 500 mg/kg, respectively. About 5 cm of the tail of mice was dipped in a cold 1:1 mixture of water and ethylene glycol kept constant at 10±0.7°C. The time taken to withdraw the tail clearly out of water was considered as the reaction time with the cutoff time being 60 sec. The latent period of the tail flick response was taken as the index on antinociception and was determined immediately after injection (12, 13).

Statistical analysis

Results of all the above methods are expressed as Mean ± SEM. Total variation present in a set of data was estimated through one-way analysis of variance (ANOVA) followed by Dunnett's *t*-test. Values of $P<0.01$ were considered statistically significant.

RESULTS AND DISCUSSION

This study establishes the central and peripheral analgesic activity of methanolic extract of *C. zeylanica* roots. Acetic acid-induced writhing and hot water test is used to study the action on peripheral nervous system. Cold water test and hot plate method was used to study the action on central nervous system (14). The effect of MECZ (250- 500 mg/kg; p.o.) on acetic acid induced writhing is demonstrated in Table 1. The extract administered at the doses of 250 and 500 mg/kg orally showed the significant ($P<0.01$) reduction in the number of writhes induced by acetic acid in a dose dependent manner. Aspirin (300 mg/kg, p.o.) was used as a standard drug for comparison of results which exhibited significant ($p<0.01$) inhibitory effect on the writhing responses. Acetic acid causes analgesia by liberating endogenous substances including serotonin, bradykinin, histamine and prostaglandin which may stimulate pain nerve ending (15). Therefore, MECZ might inhibit the synthesis and/or release of these endogenous substances.

The result of hot plate test indicated a significant increase ($P<0.01$) in reaction time at 1, 2 and 3 hours as comparable to the reference drug Pentazocin (5 mg/kg; p.o.) which is showed in Table 2. The results obtained from hot and cold tail flick experiments are shown in Table 3, in both the models, administration of MECZ (250- 500 mg/kg; p.o.) showed significant ($P<0.01$) protection against the pain induction.

The result from hot and cold tail flick test also gave additional evidences for the analgesic activity of the root extract. The activity may be attributed due to the presence of flavonoids, alkaloids and other bioactive compounds. In conclusion, the present study demonstrated that MECZ has intrinsic analgesic activity which needs to be investigated with more information on the bioactive principles responsible for the action. The results indicate that the MECZ possesses significant

Table 1: Effects of MECZ on Acetic acid-induced writhing response in mice

Group(s)	*Treatment*	*Dose (mg/kg)*	*Mean number of writhing (15 min)*	*Inhibition of writhing (%)*
I	Control (5 % Gum acacia)	5 ml/kg	85.55±1.63	---
II	Aspirin	300	31.87±0.88[a]	62.74
III	MECZ	250	39.56±1.08[a]	53.75
IV	MECZ	500	44.12±0.51[a]	48.42

Values are expressed as mean±SEM (n=6). [a]P<0.001, very significant with respect to the control group (ANOVA followed by Dunnett t-test). MECZ: Methanolic Extract of Capparis zeylanica

Table 2: Effects of MECZ on thermal stimulus induced pain (Hot Plate Test) in Rats

Group(s)	*Treatment*	*Dose (mg/kg)*	*Reaction time in seconds*			
			0 h	*1 h*	*2 h*	*3 h*
I	Control (5 % Gum acacia)	5 ml/kg	4.98±0.18	5.33±0.16	5.24±0.13	5.08±0.16
II	Pentazocin	5	5.32±0.12	10.08±0.44[a]	11.23±0.58[a]	8.87±0.13[a]
III	MECZ	250	5.15±0.62	6.54±0.36[a]	8.63±0.24[a]	8.63±0.24[a]
IV	MECZ	500	5.14±0.12	8.23±0.93[a]	11.00±1.23[a]	10.66±1.32[a]

Values are expressed as mean±SEM (n=6). [a]P<0.01, very significant with respect to the control group (ANOVA followed by Dunnett t-test). MECZ: Methanolic Extract of Capparis zeylanica

Table 3: Effects of MECZ on pain threshold in hot and cold tail flick test

Group(s)	*Treatment*	*Dose (mg/kg)*	*Analgesic effects (%)*	
			Hot tail flick test	*Cold tail flick test*
I	Control (5 % Gum acacia)	5 ml/kg	8.34±0.78	17.04±0.33
II	Pentazocin	5	58.89±0.13[a]	77.89±0.40[a]
III	MECZ	250	15.23±1.08[a]	39.63±1.58[a]
IV	MECZ	500	21.78±0.58[a]	59.46±0.58[a]

Values are expressed as mean±SEM (n=6). [a]P<0.01, very significant with respect to the control group (ANOVA followed by Dunnett t-test). MECZ: Methanolic Extract of Capparis zeylanica

analgesic activity. In addition, the acute toxicity studies revealed that MECZ has good margin of safety and did not show the lethal effects on the animals up to the doses of 4 g/kg.

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REFERENCES

1. G.B. Marini-Bettolo. Present aspects of the use of Medicinal plants in traditional medicine. *J. Ethnnopharmacol*. **2**: 5-7 (1980).
2. K.R. Kirtikar, *Indian Medicinal Plants*, (Oriental Enterprises, Dehradun, India, 1987) pp.200-2001.
3. R.G. Mali and J.C. Hundiwala. Evaluation of *Capparis decidua* for Anthelmintic and Antimicrobial activities. *Ind. J. Natural Product* **(4)10**: 10-12 (2004).
4. B.V. Ghule, G. Murugnanthan, P.D. Nakhat and P.G. Yeole. Immunostimulant effects of *Capparis zeylanica* Linn. leaves. *J. Ethnopharmacol.* **108**: 311-315 (2006).
5. S.R. Chaudhary, M.J. Chavan and R.S. Gaur. Antiinflammatory and analgesic activity of Capparis zeylanica root extracts. *Ind. J. Natural Products* **20(1)**: 36-39 (2004).
6. M.E. Haquid, M.M. Rahaman, M.A. Mossadik and S.D. Sarkar. E-octadec-7-en-ynoic acid from the roots of *Capparis zeylanica*. *Fitoterapia* **74(2)**: 130-133 (2004).
7. M.A. Saraf. Flavonoids of four Cleome and three Capparis species. Biochemcal systematic ecology **25(2)**: 161-166.
8. R.D. Bruce. An up- and –down procedure for acute toxicity testing. *Fundam. Appl. Toxicol* **5**: 151-157 (1985).
9. E. Siegmund, R. Cadmus, G. Lu. A method for evaluating both nonnarcotic and narcotic analgesics. *Pro. Soc. Experetl. Bio. Med.* **95**: 729-731 (1957).
10. R. Koster, N. Anderson and E.J. Debber. Acetic acid for analgesic screening. Federation Proceedings **18**: 412 (1958).
11. N.B. Eddy and B. Leimbach. Synthetic analgesic: 11-Dithienylbutenyl and dithinylbutylamines. *J. Pharmacol. Expertl.Therapeutics* **2**: 385-393 (1953).
12. K. Jibon, D. Bedabati and D. Niren. Pharmacological studies of *Clerodendron Colebrookianum* Walp- A potent hypotensive plant. Ind. J. Physiol. Pharmacol **3(49)**: 289-296 (2005).
13. R.J. Pizziketti, N.S. Pressma, E.B. Geller and M.W. Alder. Rat cold water tail flick test: A novel analgesic test that distinguishes opiod-antagonist. *Eur. J. Pharmacol.* **119**: 23-29 (1985).
14. S. Bolton and C. Bon. *Pharmaceutical Statistics* (Marcel Dekker Publication, Blacksburg 2004) pp.437-449.
15. V.B. Costa, C.S. Cobe, B.G. Marinho, M.E. Matheus, S.G. Leitao and P.D. Fernandes. Anti-inflammatory and analgesic activity of *Buochea fluminensis*. *Fitoterapia* **74**: 364-371 (2003).