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Full Paper

Anti-nociceptive activity of *Cansjera rheedii* J. Gmelin (Opiliaceae)

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Abstract : The ethanolic extract of aerial parts of *Cansjera rheedii* J, Gmelin (Opiliaceae) was screened for its anti-nociceptive property using both chemical and thermal methods of nociception in mice. In the chemical method, acetic acid-induced writhing test, and in the thermal method, tail-flick test was performed. The extract at doses of 250 mg and 500 mg/kg/ip inhibited the abdominal constriction induced by acetic acid and also increased the pain threshold of mice towards thermal source. The activity exhibited by the extract was comparable to that of the standard drugs (Pentozocine, 5 mg/kg/ip, for tail-flick test and Piroxicam, 10 mg/kg/ip, for acetic acid-induced writhing test). From the results it was concluded that the ethanolic extract of the plant exhibits anti-nociceptive activity by central and peripheral mechanisms.

Keywords: *Cansjera rheedii*, anti-nociceptive, writhing test, tail-flick test

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Introduction

Medicinal herbs have been used as a form of therapy for the relief of pain throughout history [1]. Natural products in general, and medicinal plants in particular, are believed to be an important source of new chemical substances with potential therapeutic efficacy. Taking into account that the most important analgesic prototypes (salicylic acid and morphine) were originally derived from plant sources, the study of plant species traditionally used as pain killers should still be seen as a fruitful research strategy in the search of new analgesic drugs. In an earlier study, we have reported analgesic activities of *Toddalia asiatica* Linn [2] and *Helichrysum bracteatum* [3]. The present study was taken up to evaluate the anti-noceptive activity of ethanolic extract of aerial parts of *Cansjera rheedii* J. Gmelin by using acetic acid-induced writhing and tail-flick method.

C. rheedii (Opiliaceae), a climbing shrub, sometimes armed, commonly known as Kalimanakeerai in Tamil, is generally found in India through Malaya to Hong Kong and northern Australia [4, 5]. The tribes of Nilgiris in Tamil Nadu (India) use the plant extract for the treatment of post-natal pain [6] and intermittent fever [7]. The extract of *C. rheedii* has been reported to have a hepatoprotective effect [8], cytotoxic effect [9], anthelminthic activity [10], anti-inflammatory and membrane stabilising properties [11], antipyretic activity [12] and hypnotic activity [13]. Preliminary phytochemical screening of the ethanolic extract of *C. rheedii* reveals the presence of alkaloids, phytosterols, saponins, flavonoids, glycosides, phenolic compounds and tannins [14]. The literature survey revealed that there are no scientific studies carried out regarding anti-nociceptive activity on the aerial parts of *C. rheedii* to substantiate its therapeutic claim, hence the present examination of the plant for its anti-nociceptive property.

Materials and Methods

Collection of plant material

The aerial parts of the plant (*C. rheedii*) were collected in and around Auroville, Puducherry in the month of June 2006, and it was identified and authenticated by Auro Herbarium Sakthi Botanical Survey Department, Auroville. A voucher specimen (VS-12) has been kept in our laboratory for future reference. The aerial parts were cut into small pieces, shade-dried and powdered. The coarse powder was subjected to continuous hot extraction with ethanol (95% v/v) in a Soxhlet extractor. The ethanol was removed by distillation under reduced pressure. The remaining extract was dissolved in water and used for the experiment.

Animals

Wister albino mice (20±5 g) of both sexes were procured from Adhiparasakthi College of Pharmacy, Melmaruvathur, Chengalpet district, Tamil Nadu, South India. They were fed on commercial diet (Hindustan Lever Ltd., Bangalore) and water ad libitum. All the animals were acclimatised for a week before use. The room temperature was maintained at 22±2° C. The institutional Animal Ethical Committee had approved the experimental protocol.

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Drugs and chemicals

Piroxicam (Dr. Reddy's lab, Hyderabad) and Pentazocine (Pure Pharma Pvt. Ltd., Mumbai) were used as reference standards. Ethanol (95%v/v), chloroform (AR), petroleum ether (LR) and acetic acid (AR) were purchased from Ranbaxy Laboratories Ltd., Punjab.

Acute toxicity study

Acute toxicity study was performed according to guidelines 425 of the Organisation for Economic Co-operation and Development (OECD) [15]. No adverse effect or mortality was detected in Swiss albino mice up to 2 g/kg /peroral (po) of the extract during a 24-h observation period.

Anti-nociceptive activity

Two models, viz. acetic acid-induced writhing response (chemical method) and tail-flick assay (thermal method) using albino mice, were employed to study the anti-nociceptive effect [16-18]. The animals were divided into four groups of six animals each. Group I served as normal control and received distilled water (10 ml/kg/po). Group II served as reference group and received Pentazocine (5 mg/kg/ip) in tail-flick method and Piroxicam (10 mg/kg/ip) in acetic acid-induced writhing method. Groups III and IV served as treatment groups and received the ethanolic extract of *C. rheedii* (250 and 500 mg/kg/ip respectively).

Chemical method (acetic acid-induced writhing test)

Acetic acid (1%v/v) was administered intraperitoneally to all the groups at the dose of 1 ml/kg body weight 30 minutes after the administration of the test compounds. Anti-nociception was recorded by counting the number of writhes after the injection of acetic acid for a period of 20 minutes. A writhe is indicated by abdominal constriction and full extension of the hind limb. The pain inhibition percentage (PIP) [19] was calculated according to the following formula: $PIP = \{(T_1-T_0)/T_0\}\times 100$, where T_1 and T_0 are post-drug and pre-drug latency respectively.

Thermal method (tail-flick test)

Basal reaction time of animals to radiant heat was recorded by placing the tip (last 1-2 cm) of the tail on the current source of an analgesiometer (INCO). The tail withdrawal from the heat (flicking response) was taken as the end point. The animals which showed a flicking response within 3-5 sec were selected for the study. A cut-off period of 15 sec was observed to avoid damage to the tail. The measurement of withdrawal time using the tail-flick apparatus was conducted at 0, 15, 30, 60, 90, 120 and 180 minutes after administration of the drugs.

Statistical analysis

The data were expressed as mean $\pm$ SEM of 6 animals. Results were analysed statistically by one-way analysis of variance (ANOVA) test between two groups: test and control groups, followed by student's t-test. P values less than 0.05 ($P<0.05$) were considered indicative of significance.

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Results and Discussion

The anti-nociceptive activity of the ethanolic extract of *C. rheedii* was evaluated in mice using both chemical and thermal methods of nociception. These methods are used to detect central and peripheral analgesia, whereas hot-plate and tail-flick tests are most sensitive to centrally acting analgesics. Intraperitonial administration of acetic acid releases prostaglandins such as PGE_2 and $PGF_{2\alpha}$ and their levels were increased in the peritoneal fluid of the acetic acid-treated mice [20]. Thermal induced nociception indicates involvement of narcotic receptors [21]. Thermal nociceptive tests are more specific to opioid μ receptors and non-thermal tests are to opioid κ receptors [22-23]. Both doses (250 mg and 500 mg) of the plant extract significantly ($P<0.001$) reduced the number of abdominal constrictions and stretching of hind limbs induced by the injection of acetic acid, exhibiting a writhing inhibition percentage of 65.07 and 69.85 respectively (Table 1), which was comparable with that of the standard drug Piroxicam (71.08 %). The abdominal constriction produced after administration of acetic acid is related to sensitisation of nociceptive receptors to prostaglandins. It is therefore possible that the extract exerts its analgesic effect by inhibiting the synthesis or action of prostaglandins.

Table1. Effect of *C. rheedii* on acetic acid-induced nociception

Group	Dose (mg/kg)	No. of writhes (mean ± SEM)	% Inhibition (PIP)
Control	10 ml/kg	81.6 ± 0.89	-----
Standard (Piroxicam)	10	23.6* ± 2.23	71.08
Extract	250	28.5*± 1.78	65.07
Extract	500	24.6*± 2.12	69.85

Note: Values are mean ± SEM (n=6); *$p<0.001$ as compared to control

The centrally acting analgesics generally elevate the pain threshold of mice towards heat. Both doses of the ethanolic extract of *C. rheedii* significantly ($P<0.05$, 0.01 and 0.001) increased the reaction time of the animals towards the thermal source. In the tail-flick test, the drugs showed greatest activity at 60-90 minutes of drug administration (Table 2). The extract at 250 and 500 mg/kg exhibited activity comparable to that of the standard drug Pentazocine at 5 mg/kg.

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Table 2. Effect of *C. rheedii* extracts in tail-flick test in mice

Group	Response time after drug treatment (sec)						
	0 min	15 min	30 min	60 min	90 min	120 min	180 min
Control (2ml/kg)	4.3± 0.72	3.7± 0.72	4.3± 0.47	4.7± 0.27	3.7± 0.55	4.3± 0.73	4.0± 0.47
Standard Pentazocin (5 mg/kg)	4.3± 0.72	14^{*}± 0.03	14^{*}± 0.03	13^{*}± 0.82	12.3^{*}± 0.98	9.7^{*}± 0.27	7.7^{*}± 0.27
Extract (250 mg/kg)	4.7± 0.72	8.7^{***}± 1.52	12^{*}± 0.33	14^{*}± 0.03	13.3^{*}± 0.55	12.7^{*}± 1.09	7.0^{**}± 0.47
Extract (500 mg/kg)	3.7± 0.72	6.3^{***}± 1.19	11.3^{**}± 1.36	13.7^{*}± 0.27	14^{*}± 0.03	11.7^{*}± 0.91	7.7^{***}± 1.19

Note: Values are mean ± SEM (n=6); $^{*}P<0.001$, $^{**}P<0.01$, $^{***}P<0.05$ as compared to control

Conclusions

From the above results it can be preliminarily concluded that the crude extract of *C. rheedii* exhibits anti-nociceptive activity. As indicated by increase in tail-flick latency and decrease in the number of writhing movements following the extract treatment, this extract may possibly have a central as well as peripheral analgesic action.

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