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Antinociceptive and anthelmintic activity of *Canna indica*

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Dried, coarsely powdered leaves, flowers, rhizomes and seeds of *Canna indica* were successively extracted with benzene and methanol in Soxhlet apparatus. The effect of benzene and methanol extracts of various parts of *C. indica* on nociceptive response using writhing test and hot plate method in mice was examined. All the extracts of *C. indica* showed significant central and peripheral analgesic activity in hot plate method and acetic acid-induced writhing test, respectively, at the dose of 50 mg kg⁻¹ intraperitoneally. Methanolic extract of leaves of *C. indica* showed highest increase in reaction time in hot plate method while benzene extract of leaves of *C. indica* showed more inhibitory effect on writhing induced by acetic acid. Anthelmintic activity of these extracts was evaluated on *Pheritima posthuma*. Results showed that the methanolic extract of rhizomes of the plant took less time to cause paralysis of the earthworms.

Keywords: *Canna indica*; Cannaceae; Antinociceptive; Hot plate method; Writhing test; Anthelmintic; *Pheritima posthuma*

1. Introduction

Canna indica L. (Cannaceae) is commonly known as Indian shot or Canna lily. Several varieties are common all over India and are grown in gardens. It is an upright perennial rhizomatous herb up to 5 feet high, whose leaves are fleshy with thin margins, usually not more than 1 foot long and half as broad, lanceolate to sub-orbicular. Flowers are red or yellow and showy. It encloses a variable number of round, shiny black seeds. In folkloric medicine, root decoction is used for the treatment of fever, dropsy, and dyspepsia. Seed juice is used to relieve earaches. Flowers are said to cure eye diseases [1,2].

The objective of the present study was to evaluate analgesic and anthelmintic potential of the plant.

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2. Results

2.1. Hot plate test

All the extracts of *C. indica* showed significant analgesic activity at 50 mg kg⁻¹, i.p. dose (table 1). Analgesic activity was comparable with standard drug pentazocine. Among all the extracts, methanolic extract of leaf of *C. indica* showed highest increase in reaction time.

2.2. Writhing test

All extracts of *C. indica* at the dose of 50 mg kg⁻¹, i.p., significantly attenuated the number of writhing and stretching induced by intraperitoneal 0.6% acetic acid (table 2). Benzene extract of leaf of *C. indica* showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts as well as standard drug paracetamol. Methanolic extracts of leaves, flowers, rhizomes, and seeds showed minimum inhibitory effect on writhing.

2.3. Anthelmintic activity

Anthelmintic potential of all the extracts was compared with standard drug albendazole. Methanolic extract of rhizomes showed the most potent activity followed by methanolic extract of seeds of *C. indica* (table 3).

3. Discussion

Thermic painful stimuli are known to be selective to centrally active drugs [3]. In the present study, all the extracts showed significant ($p < 0.05$ and $p < 0.0001$) analgesic activity but among all the extracts, methanolic extract of leaves of *C. indica* showed

Table 1. Effect of various extracts of *C. indica* on thermic stimulus-induced pain in mice (hot plate test).

Treatment	Latency to lick the paws (seconds \pm SEM)					
	Pre-drug reaction time	30 min	60 min	90 min	120 min	150 min
Vehicle	3.6 \pm 0.5	3.8 \pm 0.88	4.0 \pm 0.44	3.8 \pm 0.37	3.8 \pm 0.20	3.57 \pm 0.58
Pentazocine	3.5 \pm 0.32	8.0 \pm 0.81*	10.0 \pm 0.85**	14.0 \pm 0.58**	7.5 \pm 0.74*	3.5 \pm 0.65
BL	3.44 \pm 0.57	9.72 \pm 0.27**	7.49 \pm 0.27**	7.23 \pm 0.25**	9.36 \pm 0.12**	7.68 \pm 0.25**
BF	3.82 \pm 0.38	10.86 \pm 0.31**	15.68 \pm 0.24**	8.09 \pm 0.44**	7.17 \pm 0.47**	8.43 \pm 0.19**
BS	3.85 \pm 0.65	7.09 \pm 0.25*	8.62 \pm 0.19**	11.64 \pm 0.25**	8.31 \pm 0.25**	8.06 \pm 0.25**
BR	3.48 \pm 0.38	7.91 \pm 0.11*	8.92 \pm 0.14**	8.77 \pm 0.12**	8.7 \pm 0.12**	9.32 \pm 0.25**
ML	3.36 \pm 0.53	14.0 \pm 0.25**	12.0 \pm 0.81**	20.0 \pm 0.25**	12.5 \pm 0.47**	5.5 \pm 0.38*
MF	3.29 \pm 0.32	7.38 \pm 0.58*	9.3 \pm 0.27**	13.0 \pm 0.12**	7.0 \pm 0.24**	6.63 \pm 0.11*
MS	3.51 \pm 0.81	6.0 \pm 0.74	9.0 \pm 0.20**	10.0 \pm 0.38**	9.0 \pm 0.44**	6.0 \pm 0.14*
MR	3.79 \pm 0.85	12.0 \pm 0.57**	12.33 \pm 0.25**	9.0 \pm 0.31**	6.33 \pm 0.47*	5.67 \pm 0.17*

All the values are expressed as mean \pm SEM; $n = 6$, * $p < 0.05$, ** $p < 0.0001$ significant compared to control. All the extracts and pentazocine were given intraperitoneally at 50 mg kg⁻¹ dose. BL, BF, BS, and BR are benzene extract of leaves, flower, seed, and rhizomes and ML, MF, MS and MR are methanolic extracts of leaves, flower, seed, and rhizomes, respectively.

highest increase in reaction time. Prostaglandins and bradykinins were suggested to play an important role in analgesia [4,5]. Flavonoids and tannins are reported to inhibit prostaglandin synthesis [6]. A number of flavonoids and tannins have been reported to produce analgesic activity [7]. As phytochemical tests showed presence of flavonoids and tannins in methanolic extract of leaves of *C. indica*, they might suppress the formation of prostaglandin and bradykinins or antagonize their action and exert its activity.

Peripheral analgesic activity was assessed by acetic acid-induced writhing test, which showed significant ($p < 0.05$ and $p < 0.0001$) suppression of writhing by all the extracts, but benzene extract of leaves of *C. indica* showed more inhibitory effect on writhing induced by acetic acid as compared to other extracts and standard drug paracetamol (table 2). It was observed that the onset of writhing was delayed and duration of

Table 2. Effect of various extracts of *C. indica* on acetic acid-induced writhing in mice.

Treatment	Number of writhing
Vehicle	61.0 ± 2.15
Paracetamol	6.5 ± 1.17**
BL	5.47 ± 1.09**
BF	22.62 ± 0.78**
BS	36.66 ± 1.05**
BR	11.12 ± 0.37**
ML	53 ± 1.02*
MF	54.5 ± 1.9*
MS	53 ± 2.1*
MR	40.5 ± 0.97**

All the values are expressed as mean ± SEM; $n = 6$, * $p < 0.05$, ** $p < 0.0001$ significant compared to control. All the extracts and paracetamol were given intraperitoneally at 50 mg kg⁻¹ dose. All the extracts and pentazocine were given intraperitoneally at 50 mg kg⁻¹ dose. BL, BF, BS, and BR are benzene extract of leaves, flower, seed, and rhizomes and ML, MF, MS, and MR are methanolic extracts of leaves, flower, seed, and rhizomes, respectively.

Table 3. Anthelmintic activity of various parts of *C. indica*.

Treatment	Time to paralysis (min)
BL	2.3 ± 1.34
BF	1.49 ± 1.26
BS	2.27 ± 1.08
BR	2.10 ± 1.04
ML	3.35 ± 1.12
MF	2.45 ± 1.51
MS	1.38 ± 0.21
MR	1.24 ± 1.32
Albendazole	1.50 ± 1.69

Results are expressed as mean ± SEM from six observations at the dose of 20 mg mL⁻¹; Control worms were alive up to 24 h of observation. BL, BF, BS, and BR are benzene extract of leaves, flowers, seeds, and rhizomes and ML, MF, MS, and MR are methanolic extracts of leaves, flowers, seeds, and rhizomes, respectively.

writhing was shortened. Acetic acid is known to trigger the production of noxious substances within the peritoneum, which induces the writhing response [8]. The effect of the extracts against the noxious stimulus may be an indication that it depressed the production of irritants and thereby reduction in number of writhes in the animals.

Methanolic extract of rhizomes showed most potent anthelmintic activity followed by methanolic extract of seeds of *C. indica*. So it can be concluded that active principle responsible for anthelmintic activity is a polar compound present in rhizomes and seeds of *C. indica*.

Overall we can say that leaves of *C. indica* possess better antinociceptive activity and rhizomes have better anthelmintic activity.

4. Experimental

4.1. Plant material

Leaves, flowers, rhizomes, and seeds of *C. indica* L. were collected from Ahmednagar district and get authenticated from Botanical Survey of India, Ministry of environment and forest, Government of India. A voucher specimen was deposited. (Voucher specimen No: 63492).

4.2. Preparation of the extract

Separately dried and coarsely powdered leaves, flowers, rhizomes, and seeds (500 g) of *C. indica* were subjected to successive solvent extraction in Soxhlet extractor using benzene and methanol as solvent, to obtain nonpolar and polar fractions, respectively. The extracts were concentrated by vacuum distillation and then dried in open air [9].

4.3. Animals

Animals were procured from National Toxicological Center, Pune. Male Swiss Albino mice, weighing between 25 and 30 g were used for all the experimental protocols. The animals were housed for at least one week in the laboratory animal room prior to testing. Food and water were given *ad libitum*. All procedures described were reviewed and approved by Institutional Animal Ethical Committee.

Indian adult earthworms (*Pheretima posthuma*) collected from moist soil and washed with normal saline to remove all the faecal matter, were used for the anthelmintic study. The earthworms of 3–5 cm in length and 0.1–0.2 cm in width were used for all the experimental protocol due to its anatomical and physiological resemblance with the intestinal roundworm parasites of human beings [10,11].

4.4. Drugs and chemicals

The following drugs were used. Drugs: pentazocine lactate injection (Ranbaxy, Ahmedabad), paracetamol injection (Heilenlab, Goa), albendazole (BANDY, Mankind Pharma Ltd. New Delhi), Chemicals: acetic acid (AR Grade, PCL, Pune), benzene and methanol (AR Grade, PCL, Pune), dimethyl sulphoxide (DMSO)

(PCL, Pune), saline water (Claris Lifesciences Ltd. Ahmedabad). Benzene and methanol extracts of various parts of *C. indica* were suspended into minimum volume of DMSO and then volume is adjusted with water for injection. All drug solutions were prepared immediately before starting the experiment.

4.5. Analgesic activity

4.5.1. Hot plate test. Central analgesic activity was evaluated using hot plate method as per described by Woolfe and MacDonald [12]. Mice were divided into ten groups of six animals each. The first group served as control and received only vehicle, second group was administered standard drug pentazocine (50 mg kg^{-1} , i.p.). The animals of third to sixth group were treated with benzene extracts (50 mg kg^{-1} , i.p.) of leaf, flower, rhizomes and seeds of *C. indica*, respectively. The animals of seventh to tenth group were treated with methanolic extracts (50 mg kg^{-1} , i.p.) of leaf, flower, rhizomes, and seeds of *C. indica*, respectively. Mice were placed individually on the hot plate maintained at $55^\circ\text{C} \pm 1^\circ\text{C}$ and latency of nociceptive response such as licking, flicking of a hind limb or jumping was noted. The readings were taken at 30, 60, 90, 120, and 150 min after administration of extracts. The experiment was terminated 20 s after their placement on the hot plate to avoid damage to the paws.

4.5.2. Writhing test. Peripheral analgesic activity was evaluated using acetic acid-induced writhing test [13,14]. Mice were divided into ten groups of six animals each. The animals received benzene extracts (50 mg kg^{-1} , i.p.) of leaf or flower or rhizome or seed or methanolic extracts (50 mg kg^{-1} , i.p.) of leaf or flower or rhizome or seed or standard drug paracetamol (50 mg kg^{-1} , i.p.) or vehicle, 30 min before intraperitoneal injection of 0.1 mL of 0.6% solution of acetic acid. Mice were placed individually into glass beakers after administration of acetic acid and 5 min were allowed to elapse. The mice were then observed for the period of 30 min and then number of writhes recorded for each animal.

4.6. Anthelmintic activity [15]

All the extracts of *C. indica* were dissolved into minimum amount of DMSO and then volume is adjusted to 10 mL with saline water. Nine groups, of six earthworms each were released into 10 mL of desired formulations as follows; vehicles (5% DMSO in normal saline), Albendazole (20 mg mL^{-1}) or benzene extracts of leaves or flower or rhizomes or seeds or methanolic extracts of leaves or flower or rhizomes or seeds of *C. indica* (20 mg mL^{-1} , each) in normal saline containing 5% DMSO.

Observations were made for the time taken for paralysis of individual worm. Paralysis was said to occur when the worms did not revive even in normal saline.

4.7. Statistical significance

The results were analyzed for statistical significance using students *t*-test. $p < 0.05$ and $p < 0.0001$ were considered as significant.

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