

Analgesic, Hypoglycemic and Antidiarrheal Activities Screening of *Canna indica* L.

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Abstract

The rhizome of *Canna indica* L. (family: Cannaceae) was extracted in methanol and two doses- 200 and 400 mg/kg body weight of the extract were employed to reveal central and peripheral analgesic, hypoglycemic and antidiarrheal properties *in vivo*. According to tail immersion technique, both doses significantly rose the time of latent response for 90 minutes compared to control mice group in assessing central analgesia. Acetic acid-induced writhing process demonstrated 37.29% and 64.41% inhibition of mice movement at both doses, respectively which indicated promising and statistically significant peripheral analgesic effect. On the other hand, hypoglycemic and antidiarrheal activities maintained dose and time dependent manner. Moreover, the extract of rhizome displayed statistically significant antidiarrheal activity at 400 mg/kg b.w. dose.

Key words: Central analgesic activity, peripheral analgesic activity, hypoglycemic activity, antidiarrheal activity.

Introduction

The Cannaceae family comprises of a single genus of flowering plants, *Canna* L. which are large tropical and subtropical perennial herbs (Christenhusz and Byng, 2016). Cannas are widely used as garden plants because of their ornamental value. They possess large amount of starch which have been used in agriculture for human as well as animal consumption (Khoshoo and Guha, 1976).

Canna indica L., belonging to the Cannaceae family, is known as Indian shot, edible canna, African arrowroot, etc. in English and Kolaboti in Bengali. This plant is native to the Americas, but now naturalized into other tropical and sub-tropical countries (Al-Snafi, 2015). It is economically and medicinally significant throughout the world. The tubers and rhizomes of this plant are edible and can be consumed raw or cooked. The seeds are utilized as

beads or rosaries, and the leaves are used for wrapping pastries and can be fed to livestock. The roots are the source of starch used to prepare cakes (Yeo, 2012). In addition, *C. indica* has been widely used to treat various ailments traditionally. The plant possesses diuretic, diaphoretic and demulcent effects. Decoction of root is also found to be utilized in dyspepsia, fevers, dropsy, gonorrhoea and amenorrhoea. Extract of seed is administered to alleviate earache. Sterilized leaves and stems are employed as insecticide (Ghani, 1998; Ong and Siemonsma, 1996). Various studies have been conducted to analyze and isolate the phytochemical constituents from various parts of *C. indica*. The plant contains various secondary metabolites, including alkaloids, proteins, carbohydrates, cardiac glycosides, flavonoids, terpenoids, tannins, essential oils, saponins, anthocyanin pigments, steroids, etc. (Al-

Snafi, 2015; Lamaeswari and Ananthi, 2012; Tinoi et al., 2006; Nirmal et al., 2008; Srivastava and Vankar, 2010a; Srivastava and Vankar, 2010b). The present study aimed at screening of analgesic, hypoglycemic and antidiarrheal activities of extract of *C. indica* rhizome in methanol.

Materials and Methods

Collection of plant materials and extraction: The rhizome of *C. indica* L. was gathered and then verified by Bangladesh National Herbarium, Dhaka, Bangladesh. Apart from this, a sample as voucher has been archived for subsequent reference.

The collected rhizomes were made dirt free and shade dried for several days. The dried materials were crushed by a grinder and subsequently the powdered substances (500 g) were soaked in methanol (3 L) for nearly 15 days at standard temperature. At first, the combination was filtered by means of fresh cotton and again filtered by Whatman filter paper number 1. Then a rotary evaporator was employed to concentrate the filtrate and the crude methanol extract was acquired.

Drugs and chemicals: Methanol, glucose, morphine (Reneta Limited, Bangladesh), Tween-80 (BDH Chemicals, UK), castor oil, loperamide (Square Pharmaceuticals Ltd. Bangladesh), diclofenac sodium (Opsonin Pharma Limited, Bangladesh), normal saline (Beximco Pharmaceuticals Ltd. Bangladesh) and glibenclamide (Acme Laboratories Ltd., Bangladesh) were utilized in this study. Apart from these, other reagents used were of analytical grade.

Experimental animals: Both male and female mice of Swiss Albino bred were collected from icddr,b (International Centre for Diarrhoeal Diseases and Research, Bangladesh) which had average weight between 25-35 gm and age 4-5 weeks. The mice were kept for at least 7 days in the animal house situated at State University of Bangladesh before employing to the tests in order to familiarize with the environment. The temperature and relative humidity were maintained at $24.0 \pm 1^\circ\text{C}$ and 55-65%, respectively and 12 hrs light/12 hrs dark cycles were also kept in

the laboratory. They were given rodent feed as well as water following the recommendations and principles of Federation of European Laboratory Animal Science Associations (FELASA) throughout the experiments.

Grouping of mice: Twelve mice were allocated into four groups randomly comprising three of them in each group for bioassays. The groups were denoted as- positive control (STD), negative control (CTL) and two test groups getting crude extract of two doses- 200 (CI 200) and 400 (CI 400) mg/kg of body weight.

Central analgesic activity: The central analgesic effect was studied following tail immersion method according to Pizziketti et al. in 1985. The negative control and standard groups were fed with oral Tween 80 in saline (1%, 0.1 ml/10 mg) and subcutaneous morphine (2 mg/kg b.w.), respectively. On the other hand, the experimental animals received two doses of crude extract orally. The tail tips of the mice were dipped in hot water to stimulate pain. The times needed by the mice to veer off their tails were traced at 30, 60 and 90 minutes after the introduction of the test substances. The percentage of inhibitory activity was estimated as-

$$\text{Pain inhibition percentage (PIP)} = \frac{T_1 - T_0}{T_0} \times 100\%$$

Where, T_1 = Post-drug latency, T_0 = Pre-drug latency

Peripheral analgesic activity: To screen the activity of peripheral analgesia of *C. indica*, acetic acid-induced writhing technique was followed which was portrayed by Koster et al. (1959). At first, the mice were given acetic acid (0.1 mL) intraperitoneally to induce pain sensation. Diclofenac sodium at dose 5 mg/kg b.w. was provided to the standard mice group and the test animals were fed with the extracts at both doses. The percent inhibition of movement was reckoned as-

$$\% \text{ Inhibition of writhing} = \frac{\text{Average of writhing (control)} - \text{Average of writhing (test)}}{\text{Average of writhing (control)}} \times 100\%$$

Hypoglycemic activity: Hypoglycemic activity was ascertained via tail tipping process explained by Durschlag *et al.* in 1996. At zero minute, the plasma glucose levels (mmol/L) of each mouse were determined by means of a glucometer (Bioland G-423 S). Next the standard, negative control and test groups got glibenclamide at 5 mg/kg b.w., Tween 80 in saline and the two doses of methanolic extract orally, respectively. After 30 minutes, every animal were applied with glucose solution (10%, i.e. 2 gm/kg b.w.). Then 60, 120 and 180 minutes after glucose administration, blood samples were drawn from mice tail and the glucose levels were quantified to detect the hypoglycemic effect of *C. indica* rhizome.

Antidiarrheal activity: The anti-diarrheal activity of the rhizome was evaluated in accordance with the technique portrayed by Shoba & Thomas, 2001 which is known as castor oil-induced diarrheal test. All mice were administered with 1 ml of castor oil intending to induce diarrhea. The control group taken Tween-80 in solution of saline; standard group was given oral loperamide (5 mg/kgb.w.) and the extract was administered to the test groups through oral route. The quantity of stools was traced for individual mouse. The inhibitory action was determined as-

$$\% \text{ Inhibition of defecation} = \frac{\text{Mean defecation of control} - \text{Mean defecation of standard or test sample}}{\text{Mean defecation of control}} \times 100\%$$

Statistical analysis: The values are introduced like mean \pm standard error of mean ($M \pm SEM$) and one way ANOVA. Afterwards Dunnett's test was employed to find out the significant difference of the negative control group with standard and test groups, where the p values < 0.05 were deemed to be significant statistically.

Results and Discussion

Table 1 presented the central analgesic activities of the rhizome of *C. indica*. After 30 minutes, both doses (200 and 400 mg/kg b.w.) of the extract displayed the percent of inhibition of 77.15% and

109.11%, respectively, while morphine showed 236.59% inhibition. The response time was elongated in a means which was time and dose dependent and the findings were observed to be statistically significant. For instance, after 90 minutes, the percentages of tail immersion time elongation shown by both the doses were 246.22% and 295.81%, respectively. There are various complex ways by which heat induced pain can be regulated, such as opiate, dopaminergic, serotonergic and descending noradrenergic systems centrally (Argoff, 2011). The potent analgesia produced by *C. indica* extract may operate through central mechanisms relating to the aforementioned receptor systems or via inhibition of leukotrienes, prostaglandins etc.

Statistical assessment of the peripheral analgesic activity revealed that the extract of *C. indica* rhizome possesses significant effect with writhing inhibition of 37.29% and 64.41% at both dosages, respectively when compared to diclofenac sodium with 76.27% inhibition (Table 2). In this test, acetic acid is used to stimulate pain sensation in peripheral origin and the mice respond to the pain through writhing which is helpful for monitoring analgesic properties (Gawade, 2012). Pain induced by acetic acid generates via activation of chemosensitive nociceptors that triggers the discharge of endogenous substances including serotonin, prostaglandins, bradykinins, histamine etc. or via involvement of abdominal constriction (Kabir *et al.*, 2021; Bentley *et al.*, 1983; Schumacher *et al.*, 1940). Since the plant extract decreased the writhing frequency in mice, it can be inferred that *C. indica* possesses significant analgesic activity. The mechanism of this outcome might be associated with the inhibition of release or synthesis of the aforesaid endogenous substances.

The extract of *C. indica* rhizome displayed dose and time dependent diminution in blood glucose level in mice model. The extract maintained dose dependent hypoglycemic effect (Table 3). Hypoglycemic property of plant extract might be exerted due to the stimulation in insulin secretion from β cells of pancreas or activation of the insulin receptors so that the blood glucose can be absorbed

followed by the peripheral glucose utilization (Khan et al., 2014; Joseph et al., 2011).

The crude extract of *C. indica* rhizome demonstrated statistically significant antidiarrheal property at 400 mg/kg b.w. dose (Table 4). Moreover, the activity found to be maintained dose dependent manner (41.67% and 45.83%). Ricinoleic acid is the active constituent of castor oil which can trigger peristalsis in the small intestine resulting in alteration of electrolyte and water permeability of the

membrane of intestine and thereby inflammation of the intestinal mucosa. Eventually it can induce diarrhea owing to prostaglandins secretion (Maniyar et al., 2010). In addition, the inhibition of autacoids and prostaglandin release were triggered due to some phytochemicals, like- diterpenes, sesquiterpenes, flavonoids and terpenoid derivatives, thus antagonize the activity of the oil (Nikiema et al., 2001; Rajput et al., 2011). The antidiarrheal activity of *C. indica* may be linked to the aforementioned mechanism.

Table 1. Central analgesic activity of crude methanol extract of *C. indica* rhizome.

Animal group	After 30 min		After 60 min		After 90 min	
	M ± SEM	% Elongation	M ± SEM	% Elongation	M ± SEM	% Elongation
CTL	2.32 ± 0.08*	-	2.34 ± 0.19*	-	2.25 ± 0.06*	-
STD	6.78 ± 0.32*	236.59	9.56 ± 0.10*	309.13	12.29 ± 0.15*	446.07
CI 200	3.57 ± 0.10*	77.15	5.57 ± 0.15*	138.28	7.79 ± 0.16*	246.22
CI 400	4.21 ± 0.27*	109.11	6.62 ± 0.12*	183.50	8.88 ± 0.30*	295.81

Values are expressed as Mean ± SEM (n=3). *p< 0.05 compared to negative control. CTL = negative control (1% Tween 80 in water), STD = positive control (Morphine at 2 mg/kg b.w.), CI 200 = Methanolic extract of *C. indica* rhizome at 200 mg/kg b.w., CI 400 = Methanolic extract *C. indica* rhizome at 400 mg/kg b.w.

Table 2. Peripheral analgesic activity of crude methanol extract of *C. indica* rhizome.

Animal group	No. of writhing			Number of writhing (Mean ± SEM)	% Inhibition
	M-1	M-2	M-3		
CTL	20	18	21	19.67 ± 0.88	-
STD	4	6	4	4.67 ± 0.67***	76.27
CI 200	9	14	14	12.33 ± 1.67*	37.29
CI 400	9	6	6	7.00 ± 1.00***	64.41

Values are expressed as Mean ± SEM (n=3). ***p< 0.001, *p< 0.05 compared to negative control. CTL = negative control (1% Tween 80 in water), STD = positive control (Diclofenac sodium at 5mg/kg body weight). M1, M2, M3 = Mice 1, Mice 2, Mice 3, respectively.

Table 3. Plasma glucose level (mmol/L) of mice in hypoglycemic activity test.

Animal group	Dose (mg/kgb.w.)	30 min	60 min	120 min	180 min
		M ±SEM (mmol/l)	M ± SEM (mmol/l)	M ±SEM (mmol/l)	M ± SEM (mmol/l)
CTL	-	18.5 ± 3.47	16.40 ± 4.30	6.00 ± 0.45	5.60 ± 0.47
STD	10	17.3 ± 1.31	15.5 ± 1.27	4.07 ± 0.69	3.80 ± 0.31
CI 200	200	16.5 ± 1.33	14.08 ± 1.32	5.4 ± 0.50	4.10 ± 0.18
CI 400	400	12.50 ± 0.54	10.80 ± 1.87	4.80 ± 1.42	3.98 ± 0.57

Values are expressed as Mean ± SEM (n=3). CTL = negative control (1% Tween 80 in water), STD = positive control (Glibenclamide at 5 mg/kg b.w.).

Table 4. Antidiarrheal activity of methanol extract of *C. indica* rhizome.

Animal group	Dose (mg/kgb.w.)	Number of diarrheal feces (Mean \pm SEM)	% Reduction of diarrhea
CTL	10	8.00 \pm 0.58	-
STD	5	1.67 \pm 0.33***	79.17
CI 200	200	3.67 \pm 1.33	41.67
CI 400	400	5.67 \pm 0.33**	45.83

Values are expressed as Mean \pm SEM (n=3). ***p< 0.001, **p< 0.01 compared to control. CTL = negative control (1% Tween 80 in water), STD = Positive control (Loperamide at 5 mg/kg b.w.).

Conclusion

The rhizome extracts of *C. indica* in methanol exhibited significant analgesic and antidiarrheal effects in animal model. Hence, more studies are necessary to isolate phytoconstituents and ascertain their bioactivities.

References

- Al-Snafi, A.E. 2015. Bioactive components and pharmacological effects of *Canna indica*: an overview. *Int. J. Pharmacol. Toxicol.* **5**, 71-75.
- Argoff, C. 2011. Mechanisms of pain transmission and pharmacologic management. *Curr. Med. Res. Opin.* **27**, 2019-2031.
- Bentley, G.A., Newton, S.H. and Starr, J. 1983. Studies on the antinociceptive action of alpha-agonist drugs and their interactions with opioid mechanisms. *Br. J. Pharmacol.* **79**, 125-34.
- Christenhusz, M.J. and Byng, J.W. 2016. The number of known plants species in the world and its annual increase. *Phytotaxa.* **261**, 201-217.
- Crunkhorn, P. and Meacock, S.C.R. 1971. Mediators of the inflammation induced in the rat paw by carrageenin. *Br. J. Pharmacol.* **42**, 392-402.
- Durschlag, M., Wurbel, H., Stauffacher, M. and Holst, D.V. 1996. Repeated blood collection in the laboratory mouse by tail incision-modification of an old technique. *Physiol. Behav.* **60**, 1565-1568.
- Gawade, S.P. 2012. Acetic acid induced painful endogenous infliction in writhing test on mice. *J. Pharmacol. Pharmacother.* **3**, 348.
- Ghani, A. 1998. *Medicinal plants of Bangladesh: chemical constituents and uses*. Asiatic society of Bangladesh, pp. 1-33.
- Joseph, B. and Jini, D. 2011. Insight into the hypoglycaemic effect of traditional Indian herbs used in the treatment of diabetes. *Res. J. Med. Plant.* **5**, 352-376.
- Kabir, F., Jaman, A.U., Rumpa, R.A., Jannat, T., Alam, S., Saha, T., Islam, M.A. and Soma, M.A. 2021. *In vitro* and *in vivo* investigations provide new insights into bioactivities of *Blumea clarkei* Hook. f. leaves. *Bangladesh Pharm. J.* **24**, 149-158.
- Khan, M.F., Khan, Z.I., Uddin, M.R., Rahman, M.S., and Rashid, M.A. 2014. *In vivo* hypoglycemic and alloxan induced antidiabetic activity of *Xeromphis uligonosa* Retz. *Afr. J. Pharm. Pharmacol.* **9**, 363-366.
- Khoshoo, T.N. and Guha, I. 1976. Origin and evolution of cultivated cannas. *Glimpses Pl. Res.* **3**, 1-81.
- Koster, R., Anderson, M. and De Beer, E.J. 1959. Acetic acid for analgesic screening. *Proc. Soc. Exp. Biol. Med.* **18**, 412-415.
- Lamaeswari, G. and Ananthi, T. 2012. Preliminary phytochemical screening and physicochemical characterization of *Canna indica* L. *Int. J. Pharm. Sci. Rev. Res.* **14**, 76-79.
- Maniyar, Y., Bhixavatimath, P. and Agashikar, N.V. 2010. Antidiarrheal activity of flowers of *Ixora coccinea* Linn. in rats. *J. Ayurveda Integr. Med.* **1**, 287-291.
- Mishra, D., Ghosh, G., Kumar, P.S. and Panda, P.K. 2011. An experimental study of analgesic activity of selective COX-2 inhibitor with conventional NSAIDs. *Asian J. Pharm. Clin. Res.* **4**, 78-81.
- Nikiema, J.B., Vanhaelen-Fastré, R., Vanhaelen, M., Fontaine, J., De Graef, C. and Heenen, M. 2001. Effects of antiinflammatory triterpenes isolated from *Leptadenia hastata* latex on keratinocyte proliferation. *Phytother. Res.* **15**, 131-134.
- Nirmal, S.A., Kolhe, N.M., Pal, S.C. and Mandal, S.C. 2008. Nonpolar compounds from *Canna indica* rhizomes. *FU. Phys. Chem. Tech.* **6**, 141-144.

- Ong, H.C. and Siemonsma, J.S. 1996. *Canna indica* L. In: *Plant Resources of South-East Asia 9. Plants Yielding Non-seed Carbohydrate* (Flach, M. and Rumawas, F., Eds.), Backhuys Publishers, Leiden, Netherlands, pp. 63–66.
- Pizziketti, R.J., Pressman, N.S., Geller, E.B., Cowan, A. and Adler, M.W. 1985. Rat cold water tail-flick: a novel analgesic test that distinguishes opioid agonists from mixed agonist-antagonists. *Eur. J. Pharmacol.* **119**, 23-29.
- Rajput, M.S., Nair, V., Chauhan, A., Jawanjal, H. and Dange, V. 2011. Evaluation of antidiarrheal activity of aerial parts of *Vinca major* in experimental animals. *Middle East J. Sci. Res.* **7**, 784-788.
- Schumacher, G.A., Goodell, H., Hardy, J.D. and Wolff, H.G. 1940. Uniformity of the pain threshold in man. *Science* **92**, 110-112.
- Srivastava, J. and Vankar, P.S. 2010a. *Canna indica* flower: New source of anthocyanins. *Plant Physiol. Biochem.* **48**, 1015-1019.
- Srivastava, J. and Vankar, P.S. 2010b. Methylated anthocyanidin glycosides from flowers of *Canna indica*. *Carbohydr. Res.* **345**, 2023-2029.
- Tinoi, J., Rakariyatham, N. and Deming, R.L. 2006. Determination of major carotenoid constituents in petal extracts of eight selected flowering plants in the north of Thailand. *Chiang Mai J. Sci.* **33**, 327-334.
- Yeo, P.F. 2012. *Secondary pollen presentation: form, function and evolution* (Vol. 6). Springer Science & Business Media, Germany.
- Zhao, G.X., Hui, Y., Rupprecht, J.K., McLaughlin, J.L. and Wood, K.V. 1992. Additional bioactive compounds and trilobacin, a novel highly cytotoxic acetogenin, from the bark of *Asimina triloba*. *J. Natu. Prod.* **55**, 347–356.