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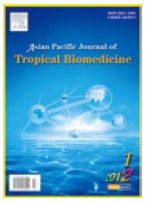

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Exploration of anti-nociceptive and locomotor effects of *Trichosanthes dioica* root extracts in Swiss albino mice

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ABSTRACT

Objective: To explore the anti-nociceptive and locomotor activities of the dichloromethane and methanol extracts of *Trichosanthes dioica (T. dioica)* root in Swiss albino mice. **Methods:** The dichloromethane and methanol extracts of *T. dioica* root were evaluated for antinociceptive activity by acetic acid-induced writhing and tail flick methods in Swiss albino mice. The locomotor activity was tested in mice by using actophotometer. **Results:** In writhing test, dichloromethane extract dose dependently and significantly inhibited writhes at the dose of 75 and 150 mg/kg body weight ($P<0.05$ and $P<0.01$, respectively), while metahnol extract produced significant protection ($P<0.05$) at the dose of 150 mg/kg body weight. In tail flick test, the dichloromethane extract showed significant ($P<0.01$, after 60 min and $P<0.05$, after 120 min) increase in reaction time. In locomotor activity evaluation, the dichloromethane extract in a dose dependent manner significantly ($P<0.05$, at 75 mg/kg, and $P<0.001$, at 150 mg/kg) depressed locomotor activity in mice. The dichloromethane extract was found to be the most active in all the tests. **Conclusions:** The present investigation demonstrates that the root extracts of *T. dioica* exhibited promising anti-nociceptive and depressed locomotor effects in Swiss albino mice.

1. Introduction

Traditional medicine worldwide is being re-evaluated by extensive research on different plant species and their active therapeutic principles. The rich wealth of plant kingdom can represent a novel source of newer compounds with significant therapeutic activity. The major merits of herbal medicine seem to be their perceived efficacy, low incidence of serious adverse effects and low cost.

Trichosanthes dioica Roxb. (Cucurbitaceae) (*T. dioica*), called pointed gourd in English, Potol in Bengali and Patola in Sanskrit, is a dioecious climber found wild throughout the plains of North and North-East India from Punjab to Assam and Tripura states of India. It is also grown and commercially cultivated in India, Pakistan, Bangladesh and Sri Lanka for its fruits, a common culinary vegetable in the Indian subcontinent. In India, all parts of this plant have been traditionally used for various medicinal purposes. According to Ayurveda, the traditional system of Indian medicine, its root is used as a strong purgative. The root of *T. dioica* has been traditionally used in India as purgative and as tonic, febrifuge, in treatment of jaundice, anasarca and ascites[1-4]. However, reports on the experimental pharmacological studies on its root are comparatively scanty. In our earlier studies, we reported anthelmintic effects of leaf and root, antibacterial antimitotic and antitumor activities of the root of *T. dioica*[5-9]. In the present study, we found it necessary to explore the anti-nociceptive and locomotor activities of *T. dioica* root extracts in Swiss albino mice to justify the folkloric attributes.

2. Materials and methods

2.1. Plant material

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The mature tuberous roots of *T. dioica* were collected during December 2008 from Majdia, Nadia district, West Bengal, India. The species was identified by Dr. M. S. Mondal at the Central National Herbarium, Botanical Survey of India, Howrah, West Bengal, India, and a voucher specimen (CNH/I-I/57/2009/Tech.II/493) was deposited at the Pharmacognosy Research Laboratory, Bengal School of Technology (A College of Pharmacy), West Bengal, India. Just after collection, the plant material was washed thoroughly with water and shade dried at room temperature (24-26 ℃), and ground mechanically into a coarse powder.

2.2. Drugs and chemicals

The following drugs and chemicals were obtained from the sources specified: Glacial acetic acid, from Ranbaxy Fine Chemicals Ltd., New Delhi; Ibuprofen and Chlorpromazine hydrochloride, from Recon, Bangalore, India; Morphine sulphate, from Sigma Chemical Co., USA. All the other chemicals were of analytical grade obtained commercially. Doubled distilled water from all-glass still was employed throughout the study.

2.3. Preparation and standardization of extracts

The powdered plant material (750 g) was initially macerated with n-hexane (1 L) overnight, and the air-dried marc was macerated separately with dichloromethane (DCM) and methanol (MeOH), (450 mL each) at room temperature (24-26 ℃), with frequent shaking for 4 days, followed by re-maceration with the solvents, similarly for 3 days. The macerates were combined, filtered and evaporated to dryness in vacuo (at 35 ℃ and 0.8 Mpa) in a Buchi evaporator, R-114 to yield DCM (3.72 %), and MeOH (7.22%), dry extracts, which were denoted as DCTD and METD respectively. All the dry extracts were kept in a vacuum desiccator until use. Preliminary phytochemical analysis and planar chromatographic studies on silica gel pre-coated high performance thin layer chromatography (HPTLC) plates (Silica gel 60 F254 Merck, Germany), revealed the presence of flavonoids, triterpenoids (including cucurbitacin type of triperpenoids) and steroids in DCTD; whereas METD revealed the presence of flavonoids, triterpenoids, steroids, saponins, amino acids, carbohydrates and reducing sugars[10,11].

2.4. Experimental animals

Adult male Swiss albino mice of about 2 months of age weighing 20 to 22 g were maintained under standard laboratory conditions (temperature 25±2 ℃, relative humidity 48%, with dark and light cycle 14/10 h). They were allowed free access to standard dry pellet diet (Hindustan Lever, Kolkata, India) and water ad libitum. The mice were acclimatized to laboratory conditions for 10 days before commencement of the experiments. The mice were fasted for 10 h with water ad libitum before receiving the experimental treatments.

2.5. Pre-screening of animals

A pre-screening test was carried out to exclude the possibility of obtaining false positive results in anti-nociceptive evaluation. The mice were placed on a slowly rotating (~10 rpm) horizontal drum covered with a wire mesh, the animals that fell off were considered as discoordinated and hence they were not used in the present studies[12].

2.6. Acetic acid-induced writhing test

The test was carried out as per previously reported method with minor modifications[13]. The prescreened animals were divided into six groups (n=8). The first group of animals (which served as control) received normal saline 5 mL/kg body weight *p.o.* The second group of animals (which served as reference) received ibuprofen at a dose of 50 mg/kg body weight *p.o.* The remaining four groups received the test extracts (DCTD, METD) at the doses of 75 mg and 150 mg/kg body weight *p.o.* respectively. Thirty minutes after administration of normal saline, ibuprofen and test extracts acetic acid was administered (1% v/v, 0.2 mL, irrespective of body weight, *i.p.*) to all groups. The number of writhing was noted during a period of 10 minutes. The mean writhing scores in each group were calculated and expressed the percentage of protection using the following formula:

(Control mean - Treated mean/ Control mean) × 100%.

2.7. Tail flick test

2.7.1. Determination of basal reaction time

Before treatment the basal reaction time for each mouse to radiant heat (Analgesiometer, Techno) was determined by placing the tip of the tail on the radiant heat source (hot nicrome wire). The strength of the current passing through the naked nicrome wire was kept constant at 6 Amps. The tail withdrawal time in seconds from the heat (flicking response) was considered as nociceptive end point. Any mouse failing to withdraw its tail within 3-5 seconds was excluded from the study[14].

2.7.2. Main test

The study was performed as per the method reported by previous researchers[15]. The prescreened animals (reaction time: 3-4 sec) were divided into six groups (n=8). The first group (which served as control) received normal saline 5 mL/kg body weight p.o. The second group (which served as reference) received morphine sulphate at a dose of 5 mg/kg body weight s.c. The remaining four groups received the test extracts (DCTD, METD) at the doses of 75 mg and 150 mg/kg body weight p.o. respectively. The reactions of all groups of mice were determined after

30, 60 and 120 minutes by tail flick method similarly as mentioned above and the latency times (in seconds) were recorded. The mean latency times for each group were calculated.

2.8. Evaluation of locomotor activity

The CNS depressant activity of the extracts were evaluated by studying locomotor activity of mice using an actophotometer (Techno, India). The mice were divided into five groups (n=8). The equipment was turned on and animals of each group were placed individually in the activity cage for 10 mins and the basal activity score of all the animals were monitored and recorded. Then the first group (which served as reference) received chlorpromazine hydrochloride at the dose of 3 mg/kg body weight i.p. The rest four groups received the test extracts (DCTD, METD) at the doses of 75 mg and 150 mg/kg body weight p.o. respectively. Thirty minutes after administration of chlorpromazine hydrochloride and the test extracts, the animals were again tested for activity scores for 10 mins. Each animal served as its own control[16]. Percent reduction in motor activity was calculated for each animal by using the following formula:

% reduction in motor activity = (Wa −Wb/Wa) × 100%

Where W_a and W_b are mean activity scores before and after treatments, respectively.

2.9. Statistical analysis

The values were expressed as mean ± standard error of mean (SEM). The results were analyzed for statistical significance by One-Way Analysis of Variance (ANOVA) followed by Dunnett's post hoc test for significance using computerized Graph Pad InStat version 3.05, Graph Pad software, USA. P values less than 0.05 (P<0.05) were considered as statistically significant.

3. Results

The results of acetic acid-induced writhing are summarized in Table 1. The DCTD showed significant and dose dependent protection at the dose of 75 and 150 mg/kg body weight (P<0.05 and P<0.01 respectively) as compared with the control group. The METD showed significant (P<0.05) inhibition of writhes, at the dose of 150 mg/kg body weight. The mean writhing score during 30 min observation period

Table 1

Effect of T. dioica root extracts on acetic acid induced writhing in mice (mean±SEM) (n=8).

Treatment	Dose (mg/kg)	Mean writhing scores	Percentage of protection
Control (N. saline)	–	59.67 ± 4.22	–
DCTD	75	44.78 ± 2.69*	24.95
	150	39.38 ± 4.02**	34.00
METD	75	54.20 ± 3.92	9.17
	150	46.04 ± 3.30*	22.84
Ibuprofen	50	32.51 ± 4.70**	45.51

*: P< 0.05, **: P<0.01 when compared to saline control.

Table 2

Effect of T. dioica root extracts on tail flick test in mice (mean±SEM) (n=8).

Treatment	Dose (mg/kg)	Pretreatment reaction time	Mean reaction time (seconds)		
			30 min	60 min	120 min
Control (N. saline)	–	3.50± 0.75	3.70 ± 0.43	3.40 ± 0.80	3.30 ± 0.50
DCTD	75	2.70 ± 0.25	3.50 ± 0.27	3.20± 0.72	3.80 ± 1.51
	150	3.20 ± 0.18	4.30 ± 0.55	6.80 ± 0.63**	5.20 ± 0.29*
METD	75	3.80 ± 0.69	3.50 ± 0.31	3.20 ± 1.44	3.40 ± 0.73
	150	1.70 ± 1.42	2.20 ± 0.20	2.60 ± 0.60	2.10 ± 0.30
Morphine sulphate	5	3.40 ± 0.08	8.70 ± 1.14**	< 10*** ↑	< 10*** ↑

*: P<0.05, **: P<0.01, ***: P< 0.001 when compared to saline control. ↑ A cut off time of 10 sec was taken as maximum analgesic response to avoid damage tail due to heat.

Table 3

Effect of T. dioica root extracts on locomotor activity in mice (mean±SEM) (n=8).

Treatment	Dose (mg/kg)	Mean motor activity scores (in 10 mins)		% reduction in motor activity
		Before treatment	After 30 min of treatment	
Chlorpromazine HCl	3	162.01 ± 12.08	34.67 ± 16.05	78.60***
DCTD	75	166.20 ± 17.24	121.49 ± 14.49	26.90*
	150	134.42 ± 15.84	73.11 ± 11.80	45.60***
METD	75	150.73 ± 10.38	146.96 ± 14.61	2.48
	150	143.31 ± 12.63	141.63 ± 13.57	1.17

*: P<0.05, ***: P<0.001 when compared to control (mice before treatment).

in the normal saline control group was (59.67±4.22) which corresponds to the findings of previous workers[13,17,18].

The results of tail flick test are presented in Table 2. Only the DCTD at the dose of 150 mg/kg exhibited significant (P<0.01, after 60 mins and P<0.05, after 120 mins) increase in reaction time. Peak analgesic effect was observed after 60 min. The mean reaction times in the control group after 60 and 120 min were found to correspond to the reports of earlier researchers[13,15,16].

In locomotor activity study (Table 3), it was found that only the DCTD significantly depressed the locomotor activity in mice in a dose dependent fashion (P<0.05, at 75 mg/kg, and P<0.001, at 150 mg/kg).

4. Discussion

The anti-nociceptive activity of *T. dioica* root extracts was explored by both acetic acid induced writhing method and tail flick method in mice to assess peripheral (non-narcotic) and central (narcotic) type of activities respectively[19].

Acetic acid induced writhing is chemically induced nociception by intraperitonial injection of dilute acetic acid solution to mice. The chemical agents can produce nociceptive reactions in mice. Intraperitoneal injection of phenyl para quinone, bradykinin or dilute acetic acid (1-3% v/v) produces pain reaction that is characterized as writhing response. Constriction of abdomen, turning of trunk (twist) and extension of hind limbs (at least one) are considered as writhing reaction to chemically induced pain[14,19].

Acetic acid induced writhing test is known as a visceral pain model nociception. Several mediators like kinins, actylcholine, substance P, calcitonin-gene-related peptide and different prostaglandins (PG) take part in visceral pain model nocicepotion and transmission of the nociception from the viscera. In this test, both central and peripheral analgesics can be detected. Analgesics of both narcotic (central) e. g. morphine, pentazocin, pethidine and non-narcotic (peripheral) type, e. g. aspirin, ibuprofen, indomethacin can inhibit the writhing response in mice[14,19].

The tail flick test is thermally induced nociception model where radiant heat is used as a source of pain. Here, radiant heat (through a hot nicrome wire) is applied to the tail of mice and the withdrawal of tail from the radiant heat source (hot nicrome wire) is considered as flicking response to the thermally induced pain[20]. The flicking reaction which is the end point of this test may be mediated as a spinal reflex. Analgesics of only central (narcotic) type, e. g. morphine, pethidine, pentazocine etc can increase the tail flick latency period indicating anti-nociception[19,21].

The demonstration of significant and dose dependent peripheral anti-nociceptive actions by DCTD was found to be the most potent. The METD although found less active, exhibited significant peripheral anti-nociceptive actions. The results of tail flick test clearly indicated that the DCTD had significant central (narcotic) anti-nociceptive action that was absent in the METD; but the extent of central analgesic effect was much lower than that of reference morphine sulfate. This means that the DCM extract of *T. dioica* root exerted anti-nociceptive activity involving both peripheral and central mechanisms revealing the involvement of the central nervous system in anti-nociception, whereas the MeOH extract inhibited only the peripheral pain mechanisms in mice. This means that the DCTD exerted anti-nociceptive activity interfering both peripheral and central mechanisms for the transmission of painful messages in mice.

Most of the centrally acting analgesics have certain central nervous system depressant effects. The locomotor activity was evaluated to assess the central nervous system (CNS) depressant property of extracts on the motor activity in mice. Most of the centrally active analgesic agents influence the locomotor activities mainly by reducing the motor activity because of their more or less CNS depressant property[19,22]. Locomotor activity is considered as an index of alertness and a decrease may lead to sedation as a result of reduced excitability of the CNS[23]. The mean basal activity scores of mice were found to comply with the findings of earlier workers[16,24]. The present results showed no significant influence in locomotor activity of mice by the METD, but the DCTD significantly decreased locomotor activity in a dose dependent fashion and hence indicating its CNS depressant property in mice.

Preliminary phytochemical analysis and planar chromatographic studies (HPTLC) revealed the presence of various compounds in METD, whereas the DCTD mainly contained triterpenoids and steroids together with cucurbitacin type triterpenoids. It appears that the presence of triterpenoids and/or steroids was responsible for the enhanced activity of DCTD. In this connection, it is noteworthy to mention here that METD also contained triterpenoids and steroids, along with several other constituents, but the expected synergistic effect, however, was not observed here, as DCTD was more active than METD. The cucurbitacins present in DCTD may be responsible for its enhanced peripheral as well as central actions. Dereplication strategies based on these findings could be helpful for isolation of the active anti-nociceptive and CNS depressant constituents.

In conclusion, from the present preliminary investigation, it can be inferred that both the extracts of *T. dioica* root possessed remarkable anti-nociceptive effect in Swiss albino mice, the methanol extract being only peripherally active and the dichloromethane extract being active both centrally and peripherally. Only the dichloromethane extract exhibited significant depressed locomotor activity in mice, thus revealing its depressant action to the CNS. The triterpenoid especially cucurbitacin content in the dichloromethane extract may be responsible for its central actions. The outcome of the present study can corroborate the traditional and folkloric uses of *T. dioica* in the Indian

subcontinent. Purification of the extracts and further studies can reveal the exact mechanisms and constituents behind the anti-nociceptive and locomotor depressant effects of *T. dioica* root.

Conflict of interest statement

We declare that we have no conflict of interest.

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References

[1] Kirtikar KR, Basu BD. *Indian medicinal plants*. New Delhi: Bishen Singh Mahendra Pal Singh; 1935.p. 1110-1111.

[2] Anonymous. *The wealth of India: raw materials*. New Delhi: Publication and Information Directorate, CSIR; 1976.p. 289-291.

[3] Nadkarni KM. *Indian materia medica*. Bombay: Popular Prakashan; 1976.p. 1236-1237.

[4] Sharma PC, Yelne MB, Dennis TJ. *Database on medicinal plants used in Ayurveda*. New Delhi: Central Council for Research in Ayurveda and Siddha; 2002,p. 269-272.

[5] Bhattacharya S, Haldar PK, Ghosh AK. *In vitro* effects of *Trichosanthes dioica* leaves on annelids and nematodes. *Pharmacologyonline* 2009; **5**: 242-248.

[6] Bhattacharya S, Haldar PK, Ghosh AK. Paralytic and lethal effects of Trichosanthes dioica root extracts in experimental worms. *Pharm Biol* 2010; **48**: 960-965.

[7] Bhattacharya S, Haldar PK. Antibacterial activity of *Trichosanthes dioica* root. *Global J Pharmacol* 2010; **4**: 122-126.

[8] Bhattacharya S, Haldar PK. Evaluation of *in vitro* cytotoxic effect of *Trichosanthes dioica* root. *Phcog Res* 2010; **2**: 355-358.

[9] Bhattacharya S, Prasanna A, Majumdar P, Kumar RBS, Haldar PK. Antitumor efficacy and amelioration of oxidative stress by *Trichosanthes dioica* root against Ehrlich ascites carcinoma in mice. *Pharm Biol* 2011; **49**: 927-935.

[10] Harborne JB. *Phytochemical methods, a guide to modern techniques of plant analysis*. New Delhi: Springer (India) Pvt. Ltd.; 1998, p. 80-185.

[11] Wagner H, Bladt B. *Plant drug analysis, a thin layer chromatography atlas*. Berlin Hiedelberg: Springer Verlag; 1996.p. 94.

[12] Ghosh MN. *Fundamentals of experimental pharmacology*. Kolkata: Hilton & Co.; 2008.p. 132.

[13] Nonato FR, Barros TA, Lucchese AM, Oliveira CE, dos Santos RR, Soares MB, et al. Antiinflammatory and antinociceptive activities of *Blechnum occidentale* L. extract. *J Ethnopharmacol* 2009; **125**: 102-107.

[14] Kulkarni SK. *Hand book of experimental pharmacology*. New Delhi: Vallabh Prakashan; 1999.p. 117-119.

[15] Barua CC, Talukdar A, Begum SA, Lahon LC, Sarma DK, Pathak DC, et al. Antinociceptive activity of methanolic extract of leaves of *Achyranthes aspera* Linn. (Amaranthaceae) in animal models of nociception. *Indian J Exp Biol* 2010; **48**: 817-821.

[16] Bhattacharya S, Haldar PK, Zaman MK. Anti-nociceptive and locomotor activity of *Zanthoxylum nitidum* stem bark extracts in experimental animal models. *J Compl Integr Med* 2010; **7**: 1-8.

[17] Khan S, Mehmood MH, Ali AN, Ahmed FS, Dar A, Gilani AH. Studies on anti-inflammatory and analgesic activities of betel nut in rodents. *J Ethnopharmacol* 2011; **135**: 654-661.

[18] Biswas M, Biswas K, Karan TK, Bhattacharya S, Ghosh AK, Haldar PK. Evaluation of analgesic and anti-inflammatory activities of *Terminalia arjuna* leaf. *J Phytol* 2011; **3**: 33-38.

[19] Vogel HG. *Drug discovery and evaluation, pharmacological assays*. Berlin, Heidelberg: Springer Verlag; 2002.p. 670-723.

[20] Parker AG. Antinociceptive effects of the aqueous extract of *Brugmansia suaveolens* flowers in mice. *Biol Res Nurs* 2007; **8**: 234-239.

[21] Bhattacharya S, Nagaich U. Assessment of anti-nociceptive efficacy of *Costus speciosus* rhizome in Swiss albino mice. *J Adv Pharm Tech Res* 2010; **1**: 34-40

[22] Kasture SB. A handbook of experiments in pre-clinical pharmacology. Pune: Career Publications; 2007,p. 104-105.

[23] Singh N, Kaur S, Bedi PMS, Kaur D. Anxiolytic effects of *Equisetum arvense* Linn. extracts in mice. *Indian J Exp Biol* 2011; **49**: 352-356.

[24] Muthal AV, Chopde CT. Effect of neuropeptide FMRFamide on morphine and amphetamine stimulated locomotor activity. *Indian J Pharmacol* 1993; **25**: 167-169.