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EVALUATION OF PHYTOCHEMICAL SCREENING AND ANTINOCICEPTIVE PROPERTIES OF THE ETHANOLIC EXTRACT OF *TREMA CANNABINA* LOUR LEAVES

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ABSTRACT

The objective of study is to investigate the possible antinociceptive activity of ethanolic extract of the leaves of Trema cannabina Lour (family: Cannabaceae) growing in Bangladesh. Method used in which antinociceptive activity has been tested by hot plate test, tail immersion test & acetic acid-induced writhing test. It is resulted that the phytochemical analysis of the ethanolic extract of the leaves of Trema cannabina (T. cannabina) indicated the presence of reducing sugar, tannins, steroid & alkaloid types of compounds. Crude extracts of T. cannabina (500 mg/kg dose) showed maximum time needed for the response against thermal stimuli (6.79±0.15 seconds) which is comparable to diclofenac sodium (8.26±0.14 seconds) in the hot plate test. Hot tail immersion test also showed similar results as in hot plate test. The leaves extracts at 500 and 250 mg/kg showed significant reduction in acetic acid induced writhing in mice with a maximum effect of 47.56% reduction at 500 mg/kg dose. The effect produced by the alcoholic extract at the highest dose was comparable to that of diclofenac sodium at 25 mg/kg (67.07%). Therefore, it can be concluded that the obtained results tend to suggest the antinociceptive activity from the ethanolic extract of the leaves of T. cannabina and thus provide the scientific basis for the traditional uses of this plant part as a remedy for pain.

KEYWORDS : *Trema cannabina, antinociceptive, hot plate test, tail immersion test and acetic acid induced writhing model.*

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INTRODUCTION

Bangladesh possesses rich floristic wealth and diversified genetic resources of medicinal plants. It has a widely ranging tropical and the agro climatic conditions, which are conducive for introducing and domesticating new and exotic plant varieties. The use the plants, plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds. *Trema cannabina* is one of the common medicinal plants grown in Indian subcontinent. Different parts of this plant have been used in traditional medicine.

T. cannabina is a tree and belongs to the Cannabaceae family. The plant is distributed in almost all districts of Bangladesh and is used in traditional medicine by the rural people and possesses various interesting pharmacological activities [1]. The root of the plant is used in the treatment of diarrhoea, asthma and passing of blood in urine; the bark is used as poultice in muscular pain; the roots, barks and leaves are used in epilepsy [1-2]. In African folk medicine, it is used in many diseases including dysentery, hypertension, etc [3] Fruit, leaves, bark, stems, twigs and seeds are also used in traditional medicine. The leaves are used to treat cough and sore throats and bark are used to make cough syrups. Other reported uses include remedies for bronchitis, gonorrhea, malaria, yellow fever, toothache and intestinal worms [1, 4].

Since no literature is currently available to substantiate antinociceptive activity from ethanolic leaf extract of *T. cannabina*, therefore the present study is a part of our on-going pharmacological and chemical screening of selected *T. cannabina* and designed to provide scientific evidence for its use as a traditional folk remedy by investigating the antinociceptive activity.

MATERIALS AND METHODS

Collection and identification of plant materials

T. cannabina was collected from Khulna University, Khulna, Bangladesh. A specimen copy was deposited to Bangladesh National Herbarium for identification & the accession number was DACB-34739.

Preparation of ethanolic extract

The leaves of *T. cannabina* were freed from any of the foreign materials. Then the leaves were air-dried under shed temperature followed by drying in an electric oven at 40º C. The dried plant materials were then ground into powder. About 150g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 700ml of 95% ethanol. The container with its contents was sealed and kept for a period of 7 days accompanying occasional shaking and stirring. The whole mixture then underwent a coarse filtration by a piece of clean, white cotton material. Then it was filtered through whatman filter paper (Bibby RE200, Sterilin Ltd., UK) which was concentrated with rotary evaporator at bath temperature not exceeding 40˚ to have gummy concentrate of extract (yield approx. 2.77%).

Test Animals & Drug

Young Swiss-albino mice either sex, 3-4 weeks of age, weighing 20 -25 g were used for in vivo pharmacological screening. Mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). They were housed in standard environmental conditions and fed with rodent diet and water ad libitum. All experimental protocols were in compliance with BCSIR Ethics Committee on Research in Animals as well as internationally accepted principles for laboratory animal use and care.

The standard drug Diclofenac Na and Loperamide were used for this study and purchased from Square Pharmaceuticals Ltd, Bangladesh.

Phytochemical screening

The freshly prepared crude extract was qualitatively tested for the presence of chemical constituents, by using the following reagents and chemicals, for example, alkaloids were identified by the dragendorff's reagent, flavonoids with the use of Mg and HCl, tannins with ferric chloride and potassium dichromate solutions, and steroids with

Libermann-Burchard reagent. Reducing sugars with benedict's reagent[5-7].

MATERIAL & METHODS

ANALGESIC ACTIVITY

Hot Plate Test

Albino mice were placed in aluminum hot plate kept at a temperature of 55 ± 0.5 0C for a maximum time of 10 second [8]. Reaction time was recorded when animals licked their fore, hind paws and jumped at before and at 0, 15, 30 and 45 min followed by oral administration of crude extract (250 and 500 mg/kg). Diclofenac sodium 25 mg/kg was used as a reference drug.

Tail immersion test

Mice were treated with diclofenac sodium (25 mg/kg) and two doses of the crude extract (250 and 500 mg/kg). Antinociceptive effect of the test substances was determined by the tail immersion test method described by Sewell and Spencer [9]. One to two centimeter of the tail of mice was immersed in warm water kept constant at 50°C. The reaction time was the time taken by the mice to deflect their tails. The first reading is discarded and the reaction time was taken as a mean of the next two readings. The latent period of the tail-flick response was taken as the index of antinociception and was determined at 0, 30 and 60 min after the administration of drugs. The maximum reaction time was fixed at 10 seconds.

Acetic Acid-Induced Writhing Test

The Analgesic activity of the crude ethanolic extract of leaves of *T. cannabina* was studied using acetic acid induced writhing model in mice [10-11]. The animals were divided into control, positive control and test groups with five mice in each group. The animals of test groups received test substance at the dose of 250 and 500 mg/kg body weight. Positive control group was administered with Diclofenac Na (standard drug) at the dose of 25 mg/kg body weight and vehicle control group was treated with 1% Tween 80 in water at the dose of 10ml/kg body weight. Test samples, standard drug and control vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid. After an interval of 15 min, the mice were observed writhing (constriction of abdomen, turning of trunk and extension of hind legs) for 5 min.

RESULTS

Chemical group test

Results of different chemical tests on the ethanolic extract of leaves of *T. cannabina* showed the presence of reducing sugar, tannins, steroid & alkaloid (Table 1).

Table 1: Results of different group tests of ethanolic extract of leaves of *T. cannabina*

Phytoconstituents	Ethanol extract of *T. cannabina*
Alkaloid	+
Reducing sugars	+
Tannins	+
Gums	-
Flavonoids	-
Saponin	-
Steroid	+

+: Positive result; - : Negative result;

Analgesic activity

Hot Plate Test

Two doses of ethanolic extract of leaves of *T. cannabina* increased the reaction time in a dose dependent manner to the thermal stimulus which was summarized in Table- 2. The highest nociceptive inhibition of thermal stimulus was exhibited at a higher dose 500 mg/kg of crude extract which has maximum time needed for the response against thermal stimuli (6.79±0.15 seconds) which is comparable to diclofenac sodium (8.26±0.14 seconds) and found statistically significant.

Table 2: Effect of ethanolic extract of leaves of *T. cannabina* on Hot plate test in mice

Treatment	Dose (mg/kg, p.o.)	Response Time (sec)			
		0 min (Latency)	15 min	30 min	45 min
Control(1% aq. Tween 80)	10 ml/ kg	1.80±0.08	2.56±0.25	2.96±0.21	3.13±0.16
Diclofenac-Na	25	2.08±0.06	5.84±0.31*	6.75±0.17*	8.26±0.14*
Et. of leaves of *T. cannabina*	250	1.96±0.04	3.92±0.16*	4.11±0.10*	4.90±0.28*
	500	2.02±0.09	5.19±0.21*	6.20±0.21*	6.79±0.15*

*Values are expressed as mean±SEM (Standard Error Mean); Et.: Ethanolic; *indicates P < 0.001; one-way ANOVA followed by Dunnet's test as compared to control; p.o.: per oral.*

Tail immersion test

Two doses of ethanolic extract of leaves of *T. cannabina* increased the reaction time in a dose dependent manner to the thermal stimulus as seen in the hot plate test which was summarized in Table-3. The highest nociceptive inhibition of thermal stimulus was exhibited at a higher dose 500 mg/kg of crude extract (8.14±011 seconds), which is comparable to diclofenac sodium (8.17±0.11 seconds) and was statistically significant.

Table 3: Effect of ethanolic extract of leaves of *T. cannabina* on Tail immersion test in mice

Treatment	Dose (mg/kg, p.o.)	Response Time (sec)		
		0 min	30 min	60 min
Control(1% aq. Tween 80)	10 ml/ kg	2.22±0.22	3.19±0.19	3.76±0.20
Diclofenac-Na	25	4.20±0.26*	7.89±0.16*	8.17±0.22*
Et. of leaves of *T. cannabina*	250	3.21±0.11*	4.89±0.17*	5.79±0.16*
	500	4.04±0.33*	7.07±0.35*	8.14±0.11*

*Values are expressed as mean±SEM (Standard Error Mean); Et.: Ethanolic; *indicates P < 0.001; one-way ANOVA followed by Dunnet's test as compared to control; p.o.: per oral.*

Acetic Acid-Induced Writhing Test

Table 4 showed the effect of the ethanolic extract of leaves of *T. cannabina* on acetic acid induced writhing in mice. At the dose of 250 mg/kg & 500 mg/kg of body weight, the extract produced 34.15% & 47.56 %writhing inhibition in test animals respectively. The results were statistically significant (P <0.01 & P <0.001) and was comparable to the standard drug Diclofenac Na, which showed 67.07% at a dose of 25 mg/kg weight.

Table 4: Effects of the ethanolic extract of leaves of *T. cannabina* on acetic acid induced writhing of mice (n=5)

Group	Treatment and Dose	Number of writhes (% Writhing)	% Writhing Inhibition
Control	1% tween 80 solution 10 ml/kg, p.o.	16.4± 1.72 (100)	---
Positive Control	Diclofenac Na 25 mg/kg, p.o.	5.4 ± 0.68 ** (32.93)	67.07
Test Group- 1	Et. Extract of *T. cannabina* 250 mg/kg, p.o.	10.80± 0.58 * (65.85)	34.15
Test group- 2	Et. Extract of *T. cannabina* 500 mg/kg, p.o.	8.60 ± 0.68 ** (52.44)	47.56

*Values are expressed as mean±SEM (Standard Error Mean); Et.: Ethanolic; *indicates P < 0.01 & **indicates P < 0.001; one-way ANOVA followed by Dunnet's test as compared to control; n = Number of mice; p.o.: per oral*

DISCUSSION

A number of natural products are used in various traditional medical systems to treat relief of symptoms from pain. The crude extracts of leaves of *T. cannabina* demonstrated significant anti-nociceptive activity at two different dose levels in various animal models of pain. Acetic acid-induced writhing response elucidated peripheral activity, while the hot plate tests, hot tail flick test investigated both peripheral and central activity [12-13]. Nociceptive reaction towards thermal stimuli in hot plate test and tail immersion in hot water test using mice is a well-validated model for the detection of opiate analgesic as well as several types of analgesic drugs from spinal origin [14-15]. Nociceptive pain inhibition was noticed highest in both the test at 45 minutes after administration of the extracts and the response time is increased from 2.02 seconds to 6.79 seconds in hot plate test at dose 500mg/kg while it was also increased from 4.04 seconds to 8.14 seconds in tail flick test at the same dose level. Other doses used in this study also increases the latent period significantly with the time being in both tests. Acetic acid-induced writhing test has been used as a model of chemonociceptive induced pain, which increases PGE2 and PGF2a peripherally. The crude ethanolic extract of leaves of *T. cannabina* showed significant reduction of abdominal contraction in mice. Local peritoneal receptors were postulated to be partly involved in the abdominal constriction (writhing) response [15-16]. The method has been associated with prostanoids in general, i.e. increased levels of PGE2 and PGF2α in peritoneal fluids[16] as well as lipoxygenase products by some researchers[13]. In the present study, the reduction of the antinociceptive process obtained within the first hour is probably related to reduction in the release of preformed inflammatory agents, rather than to a reduced synthesis of the inflammatory mediators by inhibition of cyclooxygenases and/or lipoxygenases (and other inflammatory mediators). Thus the anti-nociceptive activity shown by crude extracts of leaves of *T. cannabina* in hot plate, hot tail-flick and acetic acid induced writhing test indicate that alcoholic extracts of the plant might possess centrally and peripherally mediated antinociceptive properties.

Furthermore, phytochemical screening of the ethanol extract of *T. cannabina* reveals the presence of steroid, tannin, reducing sugars & alkaloid. Inhibition of pain is associating with presence of steroidal constituents [17]. Tannins also play a role in antinociceptive and anti-inflammatory activities in some studies [18]. Because tannins inhibit prostaglandin synthesis by modifying the production of cyclooxygenase (cox – 1& cox – 2) and lipooxygenase (lox) involved in the prostaglandin synthesis [19-20]. Besides, alkaloids are well known for their ability to inhibit pain perception [21]. So these phytoconstituents might be responsible for its analgesic activity.

CONCLUSION

In conclusion, it can be revealed that the crude ethanolic extract of *T. cannabina* plant possess significant antinociceptive activity. The potential of the extract of *T. cannabina* as antinociceptive agent may be due to the presence of phytoconstituents like tannins, alkaloids etc and might be responsible for its activity and justify its use as a traditional folk remedy. However, a more extensive study is necessary to determine the exact mechanism(s) of action of the extract and its active compound(s).

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