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Evaluation of analgesic and neuropharmacological properties of the aerial part of *Tinospora cordifolia* Miers. in mice

Md. Mokarram Hossain[1*], S. M. Raquibul Hasan[1], Raushanara Akter[1], Md. Nurul Islam[2], Md. Jahidur Rashid[2], Moni Rani Saha[1], Md. Ehsanul Hoque Mazumder[2] , Sohel Rana[2]

Department of Pharmacy, Stamford University Bangladesh[1]
51 Siddeswari Road, Dhaka-1217
Department of Pharmacy, Jahangirnagar University [2]
Savar, Dhaka-1342

***Corresponding Author**

Md. Mokarram Hossain
Lecturer, Dept. of Pharmacy
Stamford University Bangladesh
Contact no.: +8801727348428
E-mail: smmh1123@yahoo.com

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ABSTRACT

Tinospora cordifolia Miers. is used in the Ayurvedic system of medicine for the treatment of jaundice, diabetes, and rheumatoid arthritis, and is also used as memory enhancer. But still there is no report of analgesic and neuropharmacological activities of the plant. For this reason, the present study was designed to evaluate analgesic and neuropharmacological activities of methanol extract of the aerial parts of *Tinospora cordifolia.* Analgesic activity of the crude extract at the dose of 200 and 400 mg/kg b.w. was evaluated for its central and peripheral pharmacological actions using hotplate and tail flick tests and acetic acid-induced writhing test respectively in mice. The extract produced a significant (p < 0.05-0.001) increase in pain threshold in hotplate and tail flick tests in a dose dependent manner. In acetic acid-induced writhing test the extract at both doses produced significant (p < 0.001) inhibition of writhing reaction but maximum inhibition (65.01%) of writhing was found at 400 mg/kg dose compared to the reference drug Diclofenac-Na at the dose of 10 mg/kg b.w. (77.07%). Neuropharmacological activity of the extract was also evaluated using rodent behabioural models; hole cross to evaluate motor activity, open field to evaluate exploratory behaviour and thiopental sodium-induced sleeping time to evaluate sedative potential of the extract. The extract significantly (p < 0.05-0.001) decreased motor activity and exploratory behavior of mice in hole cross and open field test respectively. The extract also produced rapid onset and maximized the duration of sleeping time when administered with thiopental sodium. Results of this study suggest that the aerial part of *T. cordifolia* possesses significant analgesic and CNS depressant activity.

Key words: *Tinospora cordifolia*, Menispermaceae, Analgesic, Neuropharmacological.

INTRODUCTION

Each type of modern drugs currently used for the management of pain and inflammatory conditions (opioids or non-narcotics, salicylates and corticosteroids e.g. hydrocortisone) presents well known adverse effects. Moreover, synthetic drugs are very expensive to develop since, for the successful development of a new product usually costs in the range of 0.5 to 5 million dollars. On the contrary, many medicinal herbs have been used as a form of therapy for the relief of pain throughout history (Almeida et al., 2001) without any adverse effects. It is therefore essential that efforts should be made to introduce new medicinal plants to develop drugs which are cheaper, safer and more effective. Plants still represent a massive untapped source of structurally novel compounds that might serve as lead for the development of novel drugs (Ahmad et al., 1992). *Tinospora cordifolia* (Willd.) Miers. is a large, glabrous, deciduous climbing shrub belonging to the family Menispermaceae (Nadkarni and Nadkarni, 1976). It is distributed throughout Bangladesh, other parts of tropical Indian subcontinent and China. The stems of *Tinospora cordifolia* are succulent with long filiform fleshy aerial roots from the branches. The bark is creamy white to grey, leaves are membranous and cordate, flowers are small and yellow or greenish yellow, seeds are curved and fruits are fleshy and single seeded. Flowers grow during the summer and fruits during the winter (Kirtikar and Basu, 1975; Anonymous, 1976). *T. cordifolia* is widely used in folk medicine and Ayurvedic system of medicine as a tonic, vitalizer and as a remedy for diabetes and metabolic disorders (Nadkarni, 1954; Chopra et al., 1958). Scientific reports describing immuno-modulatory, antidiabetic, cardioprotective and hypolipidemic activities (Wadood et al., 1992; Desai et al., 2002; Rao et al., 2005) are available. Antioxidant activity of *T. cordifolia* and inhibition of lipid peroxidation have been reported (Prince and Menon, 1999). Aerial part of the plant contains a

number of chemical constituents- alkaloids, steroids, glycosides, terpenoids, flavonoids and polysaccharides (Singh et al., 2003). The potential therapeutic value of *T. cordifolia* in the management of pain and other inflammatory conditions is yet to be investigated. Therefore, as part of our ongoing investigations on local medicinal plant of Bangladesh (Hossain et al., 2008) in this paper we are reporting analgesic and neurophrmacological activity of the aerial part of *T. cordifolia*.

MATERIALS AND METHODS

Chemicals and drugs

Diclofenac-Na and Diazepam were collected from Square Pharmaceuticals Ltd., Bangladesh; Nalbuphine and Thiopental Sodium were bought from local market of Bangladesh.

Plant material

The aerial part of the plant was collected from Dinajpur, Bangladesh in February 2008 and was identified by Dr. Abdul Ghani, Professor of Phytochemistry, Stamford University Bangladesh. The plant part was thoroughly washed with water, cut into small pieces and dried in hot air woven at 55˚C for 3 days followed by at 40˚C for the next 4 days.

Extraction

After drying the pieces of aerial part were then coarsely powdered using a grinding mill. 85 gm powder was extracted with a mixture of methanol: water (7:3, v/v) by a Soxhlet apparatus at 65◦C. The solvent was completely removed and obtained 8.6 gm (yield = 10.11%) dried crude extract which was used for investigation.

Animal

For the experiment male Swiss albino mice, 3-4 weeks of age, weighing between 20-25 gm, were collected from the animal research branch of the International Center for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: 24.0 ± 1.0˚C, relative humidity: 55-65% and 12hrs light/12 hrs dark cycle) and had free access to feed and water ad libitum. The animals were acclimatized to laboratory condition for one week prior to experiments. All protocols for animal experiment were approved by the institutional animal ethical committee.

Analgesic activity

Hot plate method

The animals were divided into four groups with five mice in each group. Group I animals received vehicle (1% Tween 80 in water, 10 ml/kg body weight), animals of Group II received Nalbuphine at 10 mg/kg body weight while animals of Group III and Group IV were treated with 200 and 400 mg/kg body weight (p.o.) of the crude extract of T. cordifolia. The animals were placed on Eddy's hot plate kept at a temperature of 55±0.5 0C. A cut off period of 15 s (Franzotti et al., 2000), was observed to avoid damage to the paw. Reaction time was recorded when animals licked their fore or hind paws, or jumped prior to and 0, 30, 60 and 90 min after oral administration of the samples (Eddy and Leimback, 1953; Kulkarni, 1999; Toma et al., 2003).

Tail immersion test

The procedure is based on the observation that morphine like drugs selectively prolongs the reaction time of the typical tail withdrawal reflex in mice (Toma et al., 2003). The animals were treated as discussed above. 1 to 2 cm of the tail of mice was immersed in warm water kept constant at 55℃. The reaction time was the time taken by the mice to deflect their tails. The first reading was discarded and the reaction time was recorded as a mean of the next three readings. A latency period of 20 s was defined as complete analgesia and the measurement was then stopped to avoid injury to mice. The latent period of the tail-flick response was determined before and 0, 30, 60 and 90 min after the administration of drugs.

Acetic acid-induced writhing test

The analgesic activity of the samples was also studied using acetic acid-induced writhing model in mice. Test samples and vehicle were administered orally 30 min before intraperitoneal administration of 0.7% acetic acid but Diclofenac-Na was administered intraperitonially 15 min before injection of acetic acid. After an interval of 5 min, the mice were observed for specific contraction of body referred to as 'writhing' for the next 10 min (Ahmed, 2004).

Neuropharmacological activity

Thiopental Sodium-induced sleeping time

A sub-hypnotic dose of thiopental sodium (10 mg/kg) was i.p. injected to mice 20 min after a similar injection of vehicle or the drug. Sleeping time was determined as the interval between the loss and the recovery of the righting reflex (Ferrini et al., 1974). Groups of male mice (n=5) were injected with sodium thiopental (10 mg/kg i.p) 15 minutes after administration of either normal saline or T. cordifolia extract (200 and 400 mg/kg), and the time interval between losing and regaining of righting reflex was measured as sleeping time.

Hole cross test

The method was adopted as described by Takagi et al. (1971). A steel partition was fixed in the middle of a cage having a size of 30 × 20 × 14 cm. A hole of 3 cm diameter was made at a height of 7.5 cm in the centre of the cage. The number of passage of a mouse through the hole from one chamber to other was counted for a period of 3 min at 0, 30, 60, 90 and 120 min after oral administration of the extract.

Open field test

This experiment was carried out as described by (Gupta et al., 1971). The animals were divided into control and test groups containing five mice each. The test group received T. cordifolia extract at the doses of 200 and 400 mg/kg body weight orally whereas the control group received vehicle (1% Tween 80 in water).The floor of an open field of half square meter was divided into a series of squares each alternatively coloured black and white. The apparatus had a wall of 40 cm height. The number of squares visited by the animals was counted for 3 min at 0, 30, 60, 90 and 120 min after oral administration of the test drugs.

Statistical Analysis

Statistical analysis for animal experiment was carried out using one-way ANOVA followed by Dunnet's multiple comparisons. The results obtained were compared with the vehicle control group; $p < 0.05$, 0.001 were considered to be statistically significant.

RESULTS

Hot plate method

Result of hotplate test is presented in Figure 1. Both doses of the extract produced a dose dependent increase in latency time when compared with the vehicle. The result was found to be statistically significant ($p < 0.05$-0.001).

Tail immersion test

The tail withdrawal reflex time following administration of the extract of *T. cordifolia* was found to increase with increasing dose of the sample. The result was statistically significant ($p < 0.05$-0.001) and was comparable to the reference drug Nalbuphine (Figure 2).

Acetic acid-induced writhing test

Table 1 shows the effects of the extract of on acetic acid-induced writhing in mice. The oral administration of both doses of *T. cordifolia* extract significantly ($p < 0.001$) inhibited writhing response induced by acetic acid in a dose dependent manner.

Thiopental sodium induced sleeping time

Result of thiopental sodium induced sleeping time test is presented in table 2. Both doses of the extract produced a dose dependent decrease in onset of sleep and increase in duration sleep. The result was found to be statistically significant ($p < 0.05$-0.001).

Hole cross test

The number of hole crossed from one chamber to another by mice of the control group was similar from 30 minutes to 120 minutes (Figure 3). Hole cross test of *T. cordifolia* showed significant decrease of movement from its initial value at 0 to 120 minutes. The result was statistically significant ($p < 0.05$-0.001).

Open field test

In the open field test *Tinospora cordifolia* showed significant dose dependent decrease of movement from its initial value at zero minute to 120 minutes (Table 3). The result was statistically significant ($p < 0.001$).

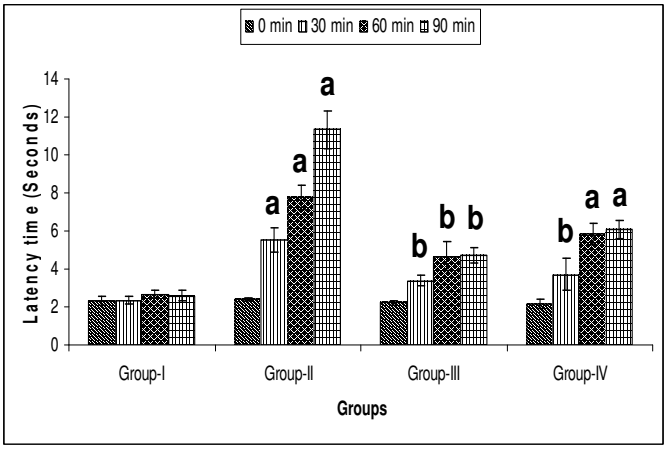

Figure 1: Effects of methanol extract of the aerial part of *Tinospora cordifolia* on latency to hotplate. [Values are mean ± SEM, (n = 5); [a] and [b] denote $p < 0.001$ and $p < 0.05$ respectively. Dunnet test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Nalbuphine 10 mg/kg body weight (i.p.), Group III and Group IV were treated with 200 and 400 mg/kg body weight (p.o.) of the crude extract of *T. cordifolia*]

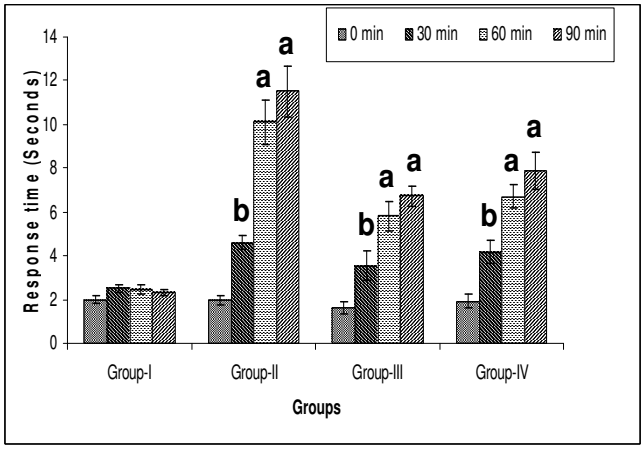

Figure 2: Effects of methanol extract of the aerial part of *Tinospora cordifolia* on tail withdrawal reflex of mice. [Values are mean ± SEM, (n = 5); [a] and [b] denote $p < 0.001$ and $p < 0.05$ respectively. Dunnet test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Nalbuphine 10 mg/kg body weight (i.p.), Group III and Group IV were treated with 200 and 400 mg/kg body weight (p.o.) of the crude extract of *T. cordifolia*]

Table 1: Effects of methanol extract of the aerial part of *Tinospora cordifolia* on acetic acid-induced writhing in mice.

Groups	Dose (mg/kg)	No. of writhing	% protection
Group-I	Vehicle	42.3±1.27	-
Group-II	10	9.7±0.94 [a]	77.07
Group-III	200	26.5±0.99 [a]	37.35
Group-IV	400	14.8±0.91 [a]	65.01

Values are mean ± SEM, (n = 5); [a] denotes $p < 0.001$, Dunnet test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Diclofenac-Na 10 mg/kg body weight (i.p.), Group III and Group IV were treated with 200 and 400 mg/kg body weight (p.o.) of the crude extract of *T. cordifolia.*

Table 2: Effects of methanol extract of the aerial part of *Tinospora cordifolia on* Thiopental sodium induced sedative test.

Groups	Onset of Sleep (min)	Duration of Sleep (min)
Group-I	10.76±0.236	80.2±2.22
Group-II	16.43±0.05[a]	95.8±0.96[a]
Group-III	14.62±0.13[a]	120.4±0.84[a]

Values are mean ±SEM, (n = 5); [a] denotes $p < 0.001$, Dunnet test as compared to control. Group I animals received Thiopental sodium 10 mg/kg body weight (i.p.), Group II and Group III were treated with Thiopental sodium 10 mg/kg (i.p.) plus 200 and 400 mg/kg body weight (p.o.) respectively of the crude extract of *T. cordifolia.*

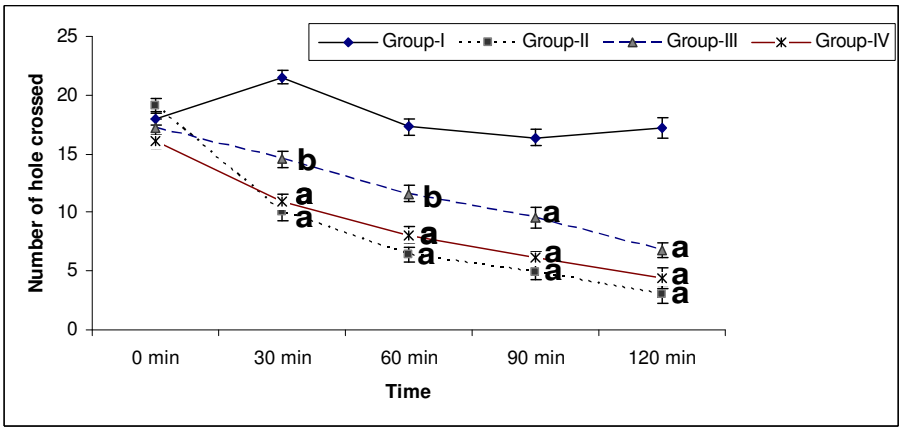

Figure 3: Effects of the methanol extract of *Tinospora cordifolia on* Hole cross test. [Values are mean ±SEM, (n = 5); [a] and [b] denote $p < 0.001$ and $p < 0.05$ respectively. Dunnet test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Diazepam 1 mg/kg body weight (i.p.), Group III and Group IV were treated with 200 and 400 mg/kg body weight (p.o.) of the crude extract of *T. cordifolia.*

Table 3: Effects of the methanol extract of *Tinospora cordifolia on* Open field test.

Groups	Observations				
	0 min	30 min	60 min	90 min	120 min
Group-I	95.2±1.08	86.2±1.08	84.8±1.74	81.4±1.51	80.4±1.58
Group-II	96.5±1.86	34.3±2.14[a]	18.7±2.51[a]	8.6±2.34[a]	4.2±2.35[a]
Group-III	97.6±2.45	56.2±2.42[b]	41.4±2.06[a]	24.2±2.55[a]	18.5±2.22[a]
Group-IV	94.4±2.56	48.7±3.34[a]	30.8±2.72[a]	15.4±3.58[a]	10.6±2.24[a]

Values are mean ±SEM, (n = 5); [a] and [b] denote $p < 0.001$ and $p < 0.05$ respectively. Dunnet test as compared to control. Group I animals received vehicle (1% Tween 80 in water), Group II received Diazepam 1 mg/kg body weight (i.p.), Group III and Group IV were treated with 200 and 400 mg/kg body weight (p.o.) of the crude extract of *T. cordifolia.*

DISCUSSION

Acetic acid induced writhing test is suitable for detecting both central and peripheral analgesia, whereas hot plate and tail flick tests are most sensitive to centrally acting analgesics. Intraperitoneal administration of acetic acid releases prostaglandins and sympathomimetic mediators like PGE_2 and $PGF_{2\alpha}$ and their levels increase in the peritoneal fluid of the acetic acid induced mice (Deraedt *et al*., 1980). The abdominal constrictions produced after administration of acetic acid is related to sensitization of nociceptive receptors to prostaglandins. It is therefore possible that the extract exerts its analgesic effect by inhibiting the synthesis or action of prostaglandins.

Thermally induced nociception indicates narcotic involvement (Besra et al., 1996). The centrally acting analgesics generally elevate the pain threshold of mice towards heat. The extract significantly delayed the response time to thermal pain sensation in both hot plate and tail flick method indicating narcotic involvements. Moreover, since the extract inhibited both peripheral and central mechanisms of pain, it is possible that the extract acted on opioid receptor (Elisabetsky et al., 1995; Pal et al., 1999).

Extensive review of literature on *T. cordifolia* confirms the presence of alkaloids, glycosides, flavonoids, steroids and terpenoids in the aerial part of the plant. So, the observed analgesic activity may be attributed to any of these phytoconstituents. There are also reports of analgesic activity of flavonoid which is mediated by inhibiting the production of prostaglandins.

While evaluating neuropharmacological activities of *T. cordifolia,* it was found that the plant extract possesses central nervous system depressant activity as indicated by decreased exploratory behaviour in mice. The extract also displayed a marked sedative effect as indicated by the reduction in gross behaviour and potentiation of thiopental sodium induced sleeping time. It is generally accepted that the sedative effect of drugs can be evaluated by measurement of spontaneous motor activity and barbiturate induced sleeping time in laboratory animal model (Lu, 1998). This result confirms those of Fujimori (1995) who proposed that the enhancement of barbiturate induced hypnosis is a good index of CNS depressant activity.

Results of the present investigation suggest that the aerial part of *T. cordifolia* possesses strong analgesic and CNS depressant activity and provide a scientific basis for the use of the plant in traditional system of medicine in the treatment of inflammatory disorders.

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