Analgesic, anti-inflammatory and anti-diarrheal activities of ethanolic leaf extract of *Typhonium trilobatum* L. Schott

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ARTICLE INFO

Article history:

Received 15 February 2012

Received in revised form 27 February 2012

Accepted 23 April 2012

Available online 28 September 2012

Keywords:

Analgesic

Anti-inflammatory

Anti-diarrheal

Typhonium trilobatum

Toxicity

ABSTRACT

Objective: To explore the efficacy of ethanolic leaf extract of *Typhonium trilobatum* L. Schott in treating diarrhea, pain and inflammation using experimental models. **Methods:** In the present study, acetic acid-induced writhing, xylene-induced ear edema and castor oil-induced diarrheal model were used to evaluate the analgesic, anti-inflammatory and anti-diarrheal activities, respectively. Acute toxicity test was carried out to fix the safe doses of the plant extract. **Results:** The plant extract demonstrated a significant inhibition of writhing ($P < 0.01$) compared with the control group in acetic acid-induced writhing test in mice. The extract also significantly inhibited the xylene induced ear edema formation ($P < 0.05$). In anti-diarrheal test, the extract significantly decreased the frequency of defecation and increased the mean latent period ($P < 0.01$) in castor oil-induced diarrheal model mice at the doses of 250 and 500 mg/kg body weight. **Conclusions:** These results suggest that the extract possesses significant analgesic, anti-inflammatory and anti-diarrheal activities that support to the ethnopharmacological uses of this plant.

1. Introduction

Diseases related with pain and inflammations are the most important symptoms for bringing the patient to physician. Pain is frequently associated with inflammation. It is an ill-defined, unpleasant sensation and may be caused by nociceptive or inflammatory agents. There are several kinds of drugs currently available as anti-inflammatory and anti-nociceptive agents to relieve pain but unfortunately, the uses of these drugs are not fully satisfactory in all cases due to their adverse side effects, for example gastric lesions, ulcers, hypertension and cardiac abnormalities are caused by non-steroidal anti-inflammatory drugs and opiates[1,2]. Therefore, development of newer, more powerful and relatively cheap anti-inflammatory and analgesic drugs with lesser side effects is necessary for human welfare. Diarrhea is another common disease in developing countries especially in children and is responsible for the death of millions of people each year[3]. Medicinal plants are a gifted

source especially for anti-diarrheal drugs[4]. For this motive, to combat the problem of diarrhea in developing countries, the World Health Organization in its Diarrheal Disease Control programme has encouraged to use traditional folklore medicines in the treatment and prevention[3,5].

Typhonium trilobatum (L.) (*T. trilobatum*) Schott belongs to the family Araceae, is a small to moderate sized perennial herb, commonly known as *Bengal arum*, *Ghatkanchu* or *Ghatkol* in Bangladesh. It is widely grown in India, Bangladesh, China, Thailand, Vietnam, Malaysia, and Sri Lanka for its rhizomes, leaves and petioles. It contains thiamine, niacin, carotene, folic acid, sterols and β -sitosterol[6]. The rhizome of this plant is traditionally prescribed for the treatment of skin eruption, gastric ulcer, asthma, headache, swelling, excessive expectoration, traumatic injury, lymph tuberculosis, chronic bronchitis, vomiting, cough, pyogenic sore throat, rheumatism, abscess and snake-bite[7,8], diarrhea and dysentery[9], stimulant and menstrual troubles[10]. Leaves and tubers are cooked as vegetables and given to the patient suffering from piles and rheumatism[11,12]. The uses of this plant as traditional medicine confirms that it may possess some important biological activities. Previous scientific investigations have reported that different parts of this plant possess antimicrobial[8], nematocidal[13], larvicidal activity[7], but

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analgesic, anti-inflammatory and anti-diarrheal activity has not yet been reported. Therefore the present study was undertaken to carry out the possible analgesic, anti-inflammatory and anti-diarrheal activity of ethanolic leaf extract of *T. trilobatum* (L.) Schott in experimental animal.

2. Materials and methods

2.1. Plant material collection and extraction

The leaves of *T. trilobatum* (L.) Schott (Family: Araceae) were collected from the village areas of Bagerhat district, Bangladesh in September 2010. The plant was identified by Bangladesh National Herbarium, Mirpur, Dhaka (Accession No-34477) and the voucher specimen was deposited in the Biotechnology and Genetic Engineering Discipline, Khulna University, Bangladesh. The leaves were shade dried, ground into coarse powder and 150 g of powdered material was taken in a clean, flat-bottomed glass container and soaked in 900 mL of 70% aqueous ethanol at 55 °C for 14 days. Occasionally it was agitated for maximum wetting and extraction. The extract was filtered and evaporated to dry using rotary evaporator. Then the extract was stored at 4 °C until used.

2.2. Experimental animal

Swiss-albino mice (both sexes) weighing between (18–25 g) and Wistar rats of the either sex (180–200 g) were used for the present study collected from International Center for Diarrheal Diseases and Research, Bangladesh (ICDDR, B), Dhaka, Bangladesh. They were maintained under standard environmental conditions and were fed with standard diet from ICDDR, B and had free access to tap water. Guidelines of Institutional Animal Ethics Committee were followed to carry out this study^[14].

2.3. Chemicals and drugs

Glacial acetic acid and xylene were purchased from Sigma chemicals, USA. Diclofenac sodium was collected from Square pharmaceuticals Ltd., Bangladesh. All other chemicals were of analytical grade.

2.4. Preliminary phytochemical analysis

Preliminary phytochemical analysis of ethanolic leaf extract of *T. trilobatum* was carried out by employing standard procedure^[15].

2.5. Acute toxicity test

The acute toxicity test for ethanolic leaf extract of *T. trilobatum* was carried out to evaluate any possible toxicity using the method of Lorke, 1983^[16]. Different doses of extract were injected intraperitoneally into groups of 20 mice. The given maximum dose was 800 mg/kg, while the control group

only received distilled water. The number of deaths was counted at 48 h after treatment.

2.6. Analgesic activity

The ethanolic leaf extract of *T. trilobatum* was tested for analgesic activity using acetic acid-induced writhing method followed by Ahmed *et al*^[17]. Mice were divided into four groups of five animals in each. Group I used as control and was administered vehicle (1% Tween 80 in water, 10 mL/kg body weight). Diclofenac sodium as standard drug was administered to Group II mice at the doses of 10 mg/kg while Group III and IV received the sample extract at two different doses (250 and 500 mg/kg) orally 30 minutes before intraperitoneally administration of acetic acid solution (0.6%) at a dose of 10 mL/kg body weight. A period of 5 minutes was given to each animal to ensure bio-availability of acetic acid, following which period, the number of writhing was counted for 10 minutes. The formula for calculating the percentage inhibition was: average writhes in the control group minus average writhes in the test group divided by average writhes in the control group multiplied by 100^[18].

2.7. Anti-inflammatory activity

Xylene-induced ear edema in rats were used to assess anti-inflammatory activity of the plant extract followed by the method of Tang *et al*^[19]. Experimental animals were administered vehicle (1% Tween 80 in water, 10 mL/kg body weight), standard drug Diclofenac sodium 10mg/kg body weight and two different doses of plant extract (250 mg/kg and 500 mg/kg body weight). One hour later, 0.01 mL of xylene was used to the anterior and posterior surfaces of the right ear of each mouse. After one hour of xylene application, rats were sacrificed and both treated and untreated ears were taken circular sections using a 7 mm diameter cork borer and weighed. The weight difference between untreated and treated ear section was calculated.

2.8. Anti-diarrheal activity: Castor oil-induced diarrhea in mice

The method as described by Jebunnessa *et al*^[3] was used to evaluate anti-diarrheal activity of the plant extract. Twenty mice were divided into four groups of five animals in each. Group I served as control and received vehicle (1% Tween 80 in water, 10 mL/kg body weight) while Group II (positive control group) was administered with the standard antidiarrheal drug loperamide at a dose of 5 mg/kg body weight. Group III and IV (test groups) were given crude leaf extract of *T. trilobatum* at the doses of 250 mg/kg and 500 mg/kg, respectively. One hour later, mice were fed castor oil orally at a dose of 0.5 mL per mouse and individual animal of each group was placed in separate cages having a papers placed below for collection of fecal matters and examined for the presence of diarrhea every hr for 6 h study after castor oil administration. Number of stools or any fluid material

that stained the adsorbent paper and latent period of each mouse were counted during the 6 h period.

2.9. Statistical analysis

The data were presented as mean $\pm$ SEM ($n=5$). Results were analysed by one way analysis of variance (ANOVA) followed by Dunnett's t -test for multiple comparisons. For the comparison between two groups, Student's t -test was employed. The significant difference was considered at $P<0.05$.

3. Results

3.1. Preliminary phytochemical analysis

Preliminary phytochemical analysis of ethanolic leaf extract of *T. trilobatum* showed the presence of reducing sugars, alkaloids, flavonoids, tannins, steroids, gums and glycosides.

3.2. Analgesic activity

In acetic acid-induced writhing model mice, the plant extract significantly reduced the number of writhing in a dose related manner. It showed 49.33% ($P<0.001$) and 65.33% ($P<0.01$) writhing inhibition at the doses of 250 and 500 mg/kg body weight respectively, which was comparable to the standard drug diclofenac-sodium that caused 70.67% ($P<0.001$) at the dose of 10 mg/kg body weight (Table 1).

3.3. Anti-inflammatory activity

In xylene-induced ear edema model rat, it was found that the plant extract exhibited a significant inhibition on ear edema formation in a dose-related manner. It caused 15.0% ($P<0.05$) and 27.5% ($P<0.001$) at the doses of 250 & 500 mg/kg body weight respectively in comparison with the standard drug Diclofenac sodium where the inhibition was 30.0% ($P<0.001$) at the dose of 10 mg/kg body weight (Table 2).

3.4. Anti-diarrheal activity

The extract caused about 30.71% ($P<0.05$) and 58.16% ($P<0.001$) prolongation of the time for castor oil-induced

Table 1

Effects of ethanolic leaf extract of *T. trilobatum* on acetic acid induced writhing in mice (mean $\pm$ SEM, $n=5$).

Group	Number of writhing	Inhibition rate (%)
Blank control (AA 10 mL/kg, <i>i.p.</i> + vehicle 10 mL/kg, <i>p.o.</i>)	15.00 $\pm$ 0.83	0.00
Positive control (AA 10 mL/kg, <i>i.p.</i> + diclofenac sodium 25 mg/kg, <i>p.o.</i>)	4.40 $\pm$ 0.51**	70.67
Test 1 (AA 10 mL/kg, <i>i.p.</i> + plant extract 250 mg/kg, <i>p.o.</i>)	7.60 $\pm$ 0.92**	49.33
Test 2 (AA 10 mL/kg, <i>i.p.</i> + plant extract 500 mg/kg, <i>p.o.</i>)	5.20 $\pm$ 1.88*	65.33

* $P<0.01$, ** $P<0.001$, significant compared to blank control; AA: acetic acid; *i.p.*: intraperitoneally; *p.o.*: per oral.

Table 2

Anti-inflammatory effect of *T. trilobatum* on xylene-induced ear edema model mice (mean $\pm$ SEM, $n=5$).

Group	Increased weight (mg)	Inhibition rate
Blank control (Xylene 0.01 mL injection + vehicle 10 mL/kg, <i>p.o.</i>)	8.00 $\pm$ 0.31	0.00
Positive control (Xylene 0.01 mL injection + diclofenac sodium 10 mL/kg, <i>p.o.</i>)	5.60 $\pm$ 0.24**	30.00
Test 1 (Xylene 0.01 mL injection + plant extract 250 mg/kg, <i>p.o.</i>)	6.80 $\pm$ 0.37*	15.00
Test 2 (Xylene 0.01 mL injection + plant extract 500 mg/kg, <i>p.o.</i>)	5.80 $\pm$ 0.26**	27.50

* $P<0.05$, ** $P<0.001$, significant compared to control.

Table 3

Effect of *T. trilobatum* on latent period of diarrheal induction in castor oil-induced diarrheal mice (mean $\pm$ SEM, $n=5$).

Group	Mean latent period (h)	% of increase in latent period
Blank control (Castor oil 0.50 mL, <i>p.o.</i> + vehicle 10 mL/kg, <i>p.o.</i>)	0.75 $\pm$ 0.06	0.00
Positive control (Castor oil 0.50 mL, <i>p.o.</i> + loperamide 50 mg/kg, <i>p.o.</i>)	2.28 $\pm$ 0.19**	67.10
Test 1 (Castor oil 0.50 mL, <i>p.o.</i> + plant extract 250 mg/kg, <i>p.o.</i>)	1.22 $\pm$ 0.18*	30.71
Test 2 (Castor oil 0.50 mL, <i>p.o.</i> + plant extract 500 mg/kg, <i>p.o.</i>)	1.64 $\pm$ 0.16**	58.16

* $P<0.05$, ** $P<0.001$, significant compared to control.

Table 4

Effect of *T. trilobatum* on frequency of defecation in castor oil-induced diarrheal mice (mean $\pm$ SEM, $n=5$).

Group	Mean no. of stools	Inhibition rate
Blank control (Castor oil 0.50 mL, <i>p.o.</i> + vehicle 10 mL/kg, <i>p.o.</i>)	8.60 $\pm$ 1.07	0.00
Positive control (Castor oil 0.50 mL, <i>p.o.</i> + loperamide 50 mg/kg, <i>p.o.</i>)	2.40 $\pm$ 0.51**	72.09
Test 1 (Castor oil 0.50 mL, <i>p.o.</i> + plant extract 250 mg/kg, <i>p.o.</i>)	4.40 $\pm$ 0.24*	48.84
Test 2 (Castor oil 0.50 mL, <i>p.o.</i> + plant extract 500 mg/kg, <i>p.o.</i>)	3.20 $\pm$ 0.37*	62.79

* $P<0.05$, ** $P<0.001$, significant compared to control.

diarrhea at 250 & 500 mg/kg body weight doses in comparison with standard drug 67.10% ($P<0.001$) at the dose of 5 mg/kg body weight (Table 3). The extract also reduced the frequency of defecation by almost 48.84% and 62.79% ($P<0.01$) at the dose of 250 & 500 mg/kg body weight, respectively (Table 4).

3.5. Acute toxicity test

Although the mice were given 800 mg/kg of the plant extract, no mortality was observed during the assessment period (48 h). So we can draw a conclusion that the minimum lethal dose of the plant extract is more than 800 mg/kg.

4. Discussion

The ethanolic leaf extract of *T. trilobatum* showed significant analgesic action in comparison with control group when evaluated using acetic acid-induced writhing test in mice. Acetic acid causes pain and localized inflammation by the action of prostaglandins production [mainly, prostacyclines and prostaglandin-E (PG-E)] which have been reported to stimulate the A δ -fibres that cause a sensation of sharp well localized pain[20,21]. It has also been reported that acetic acid induces the increased level of PGE2 and PGF2 α in the peritoneal fluid which is responsible for pain production[22–27]. There are various peripherally acting analgesic drugs such as ibuprofen, aspirin, diclofenac sodium and indomethacin that have been reported to inhibit acid induced writhing by inhibition of prostaglandin synthesis[28]. Therefore it can be concluded that any agent that reduces the number of writhing will demonstrate analgesic effect by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition[29]. The result of the plant extract in acetic acid-induced writhing method suggests that the reduction of pain might be occurred due to the presence of analgesic properties in the extract via inhibition of prostaglandin synthesis.

Xylene is known to cause severe vasodilation and edematous changes of skin as signs of acute inflammation[30]. The increased thickness of the ear tissues is caused by these histopathological changes. In the present investigation; the plant extract significantly inhibited the xylene-induced increases in ear weight in a dose related manner. This inhibition capacity of the plant extract can be regarded as the evidence of anti-inflammatory efficacy through reducing vasodilation and so that improving edematous condition.

The preliminary phytochemical analysis of the plant extract showed the presence of reducing sugars, alkaloids, flavonoids, tannins, steroids, gums and glycosides. The previous scientific studies have been reported that alkaloids, flavonoids and tannins are known to inhibit prostaglandin synthetase that is responsible for its antinociceptive and anti-inflammatory effects[31–38]. Therefore antinociceptive and anti-inflammatory effect of the extract may be due to the presence of flavonoids, tannins, and alkaloids either singly or in combination. Presence of tannins, alkaloids,

saponins, flavonoids, sterols and/or triterpenes and reducing sugars in the medicinal plants have also been known to indicate antidiarrheal activity[39,40]. In general antidiarrheal activity of tannins and flavonoids has been recognized for the inhibition of intestinal motility, antimicrobial action and antisecretory effects[36]. In addition, the astringent properties of tannins are known to cause antinociceptive, antilamatory and antidiarrheal effects[31,32,41]. Therefore antinociceptive and anti-inflammatory effect of the extract may be due to the presence of flavonoids, tannins, alkaloid either singly or in combination. Besides alkaloids, flavonoids or tannins may also be responsible for anti-diarrheal potential of the plant extract.

According to the results of the present investigation, it can be concluded that the ethanolic leaf extract of *T. trilobatum* has significant anti-nociceptive, anti-inflammatory and anti-diarrheal effects that support to the traditional use of this plant for the treatment of related diseases. This study also suggests for the further detail investigation of mechanisms of the pharmacological effects and also to isolate the active compound(s) responsible for those properties.

Conflict of interest statement

We declare that we have no conflict of interest.

Acknowledgements

Authors sincerely thank to Biotechnology and Genetic Engineering Discipline, Khulna University, Bangladesh for providing adequate financial support to carry out the research work. We are also thankful to Pharmacy Discipline, Khulna University for providing laboratory facilities and necessary reagents during this study. We also express gratefulness to the ICDDR, Bangladesh for giving mice and Bangladesh National Herbarium, Mirpur for the identification of the plant.

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