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Analgesic and Anti-Diarrhoeal Activities of *Trema orientalis* in Mice

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Abstract

***Trema orientalis* Linn. is commonly grown in many parts of Bangladesh. Its leaves have been used for analgesic and anti-diarrhoeal activity in traditional medicine. This study evaluates the potential analgesic and anti-diarrhoeal activity of methanolic and aqueous extracts of leaves in experimental acetic acid-induced writhing and castor oil-induced diarrhoea in mice. The aqueous extract of leaves showed significant (p <0.001) analgesic effect in acetic acid-induced writhing in mice at a dose of 500 mg/kg body weight. In castor oil-induced anti-diarrhoeal screening both extract increased latent period (p <0.025) and decrease the number of stool (p <0.025) at the dose of 500 mg/kg body weight comparable with that of the standard drug loperamide. The results provide a support for the use of this plant in traditional medicine and suggest its further investigation.**

Key words: Analgesic, anti-diarrhoea, *Trema orientalis* Linn.

Introduction

During the past decade, traditional systems of medicine have become increasingly important in view of their safety. Current estimates suggest that, in many developing countries, a large proportion of the population relies heavily on traditional practitioners and medicinal plants to meet primary health care needs. Although modern medicine may be available in these countries, herbal medicines (phytomedicines) have often maintained popularity for historical and cultural reasons. Concurrently, many people in developed countries have begun to turn to alternative or complementary therapies, including medicinal herbs[1].

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 of the plants, plant extracts and pure compounds isolated from natural sources provided the foundation to modern pharmaceutical compounds.

Trema orientalis (Bengali name: Jibon or Chikon) is a tree and belongs to the Ulmaceae 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. 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[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 coughs and sore throats and the bark is used to make cough syrups. Other reported uses include remedies for bronchitis, gonorrhoea, malaria, yellow fever, toothaches, and intestinal worms[4].

As part of our continuing efforts to study the chemical and pharmacological aspects of the medicinal plants of Bangladesh, *T. orientalis* was investigated. Methanolic and aqueous extracts were used for investigation of their analgesic and anti-diarrhoeal activities. Since there is a vast resource of *T. orientalis* in Bangladesh, the present study will provide some valuable information about the pharmacological properties of this plant.

Materials and Methods

Plant materials: Fresh leaves of *Trema orientalis* were collected from Khulna University Campus in Bangladesh. The plant was identified by the experts of Bangladesh National Herbarium, Mirpur, Dhaka (Accession No. 31,285) and a voucher specimen was also deposited there. The fresh leaves were cleaned, dried

and pulverized. About 400 g of powdered material was taken in a clean, flat bottomed glass container (4-l) and soaked in 1,300 ml of 80% of methanol. The container was sealed and kept for a period of 10 days with occasional shaking and stirring. Then it was filtered and concentrated by evaporation.

Experimental animals: Young Swiss-albino mice aged 4-5 weeks, average weight 20-25 g were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B), Dhaka. They were kept in standard environmental condition for one week for adaptation after their purchase and fed ICDDR,B formulated rodent feed and water.

Analgesic activity test: Analgesic activity of both methanol and aqueous extract was tested by acetic acid induced writhing model in mice[5]. Eighty four mice of either sex were grouped in six (n = 14 per group). Each group received a particular treatment, *i.e.*, group-I for control, group-II for positive control, test group-I for aqueous extract at the dose of 250 mg/kg, test group-II for aqueous extract at the dose of 500 mg/kg, test group-III for methanol extract at the dose of 250 mg/kg and test group-IV for methanol extract at the dose of 500 mg/kg. Tween-80 solution (1%) in deionized water at a dose of 10 ml/kg per oral was used as control, and Diclofenac-sodium was used at a dose of 25 mg/kg body weights per oral as a standard positive control. To prepare suspension of the test samples at required doses, the required amount of extract was triturated in unidirectional manner by the addition of small amount of Tween-80. After proper mixing of extract and Tween-80, the volume was adjusted with deionized water. The test consists of injecting 0.7% acetic acid solution intraperitoneally. Control and test samples were given orally 30 min prior to acetic acid injection. Each mouse of all groups was observed carefully for counting the number of writhing that developed in 15 min. Incomplete writhing was taken as half-writhing, so two half-writhing were taken as one full writhing.

Anti-diarrhoeal activity test: Anti-diarrhoeal activity was tested using the castor oil induced diarrhoea in mice[6]. Experimental animals were randomly selected and divided into four groups (n = 5 per group). Each group received a particular treatment, *i.e.*, group-I for control, group-II for positive control, test group-I for aqueous extract and test group-II for methanol at a dose of 500 mg/kg. Loperamid was used at a dose of 4 mg/ kg body weight as a standard. Control vehicle and the extract were administered orally, 1 h prior to the oral administration of castor oil at a dose of 0.5 ml per mice. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhoea every hour in five hour study after castor oil administered. Number of stool or any fluid that stained the adsorbent paper was counted at each successive hour during the experiment. The latent period of each mouse also counted.

Determination of cytotoxic activity: Cytotoxic activity test was performed on newborn brine shrimp (*Artemia salina*) as described by Meyer *et al.*[7].

Statistical analysis: Student's t-test was used to determine a significant difference between the control groups and experimental groups for both the experiment.

Results

The crude extract of aqueous and methanol extract of leaves of *Trema orientalis* was used for analgesic and anti-diarrhoeal activity in mice at different doses. Analgesic activity of aqueous and methanol extracts of *T. orientalis* leaves was tested by acetic acid-induced writhing in mice. The methanol extract showed 16.67 and 30.01% acetic acid induced writhing inhibition in mice at the doses of 250 mg/kg and 500 mg/kg body weight respectively (Table 1). But the aqueous extract showed 38.34 and 56.67% acetic acid-induced writhing inhibition in mice at the doses of 250 mg/kg and 500 mg/kg body weight respectively. This was comparable to Diclofenac-sodium where the inhibition was 48.34% at the dose of 25 mg/kg body weight.

Table 1: ***Effect of aqueous and methanolic extracts of leaves of Trema orientalis on acetic acid-induced writhing in mice (n = 4)***

Animal group/ Treatment	No. (%) of writhing	Percent inhibition
Group I – Control (1% Tween-80 solution in water, 10 ml/kg, per oral)	30 ± 1.07 (100.0)	0.0
Group II – Positive control (Diclofenac-sodium 25 mg/kg, per oral)	15.5 ± 0.64*(51.66)	48.34
Test group I – Aqueous extract (250 mg/kg, per oral)	18.5 ± 0.97*(61.66)	38.34
Test group II – Aqueous extract (500 mg/kg, per oral)	13 ± 0.70*(43.33)	56.67
Test group III – Methanolic extract (250 mg/kg, per oral)	25 ± 1.29*(83.33)	16.67
Test group IV – Methanolic extract (500 mg/kg, per oral)	21 ± 1.13*(69.99)	30.01

Values are expressed as mean ± SEM; *indicates p <0.025 *vs.* control.

To study anti-diarrhoeal activity, aqueous and methanolic extracts at the dose of 500 mg/kg body weight was used for the test group, 1% Tween-80 at the dose of 10 mg/kg body weight for the control group and Loperamide at the dose 4 mg/kg body weight for the standard group. It was found that both aqueous and methanolic extracts increased the latent period as compared to the control and the standard (Table 2). The aqueous and the methanolic extracts increased the latent period of 1.456 and 1.240 h respectively, while the control and the standard increased the latent period of only 1.096 and 1.284 h respectively. It was also observed that the frequency of defecation decreased in the test

group as compared to the control and the standard (Table 3). During the first hour, the defecation frequency was 6.6 and 6.2 for aqueous and methanolic extracts respectively, while the frequency was 7.8 in case of control and 7.2 for in case of standard. The defecation frequency follows similar trend with time; it decreases with the increase of time. The mean defecation frequency was 6.2, 4.4, 3.8, 1.2 and 0.6 for aqueous extract and 6.6, 3.8, 0.8, 0.8 and 0.2 for methanol extract at the 1st, 2nd, 3rd, 4th and 5th hour respectively.

Table 2: ***Effect of aqueous and methanolic extracts of leaves of Trema orientalis on castor oil-induced diarrhoea in mice (Latent period)***

Animal group/Treatment	Dose (mg/kg, per oral)	Latent period (h)
Group-I – Control (1% Tween-80)	10	1.096 ± 0.025
Group-II – Standard (Loperamide)	4	1.284 ± 0.053
Group-III – Test group (Aqueous extract)	500	1.456 ± 0.029*
Group-IV – Test group (Methanolic extract)	500	1.240 ± 0.058*

Values are expressed as mean ± SEM (n = 5); *indicates p <0.001 *vs.* control.

Table 3: ***Effect of aqueous and methanolic extracts of leaves of Trema orientalis on castor oil-induced diarrhoea in mice (Defecation frequency)***

Animal group/ Treatment	Dose (mg/kg, per oral)	Period of study (h)	Defecation frequency (No.)
Group-I – Control (1% Tween-80)	10	1	7.8 ± 0.73
		2	5.0 ± 0.59
		3	4.0 ± 0.63
		4	3.6 ± 0.60
		5	1.2 ± 0.48
Group-II – Standard (Loperamide)	4	1	7.2 ± 0.73
		2	4.6 ± 0.67
		3	3.2 ± 0.37
		4	1.28 ± 0.48
		5	0.6 ± 0.40
Group-III – Test group (Aqueous extract)	500	1	6.6 ± 0.6*
		2	3.8 ± 0.73*
		3	0.8 ± 0.48*
		4	0.8 ± 0.48*
		5	0.2 ± 0.2
Group-IV – Test group (Methanolic extract)	500	1	6.2 ± 0.86*
		2	4.4 ± 0.5
		3	3.8 ± 0.68
		4	1.2 ± 0.37*
		5	0.6 ± 0.39

Values are expressed as mean ± SEM; *indicates p <0.025 *vs.* control.

Cytotoxic activity or lethal concentration 50 (LC_{50}) of the leaf extracts *T. orientalis* was performed against brine shrimp (*Artemia salina*). The LD_{50} of the methanolic extract was found to be 120 mg/ml and the LC_{90} was 200 µg/ml.

Discussion

For the millions of rural populations in the countries of the developing world, diarrhoeal diseases continue to be the major cause of morbidity and mortality, with an estimated 1 billion episodes of illness and some 5 million or more deaths in children under 5 years[8]. In such populations, preparations from herbs and plants remain the most common forms of treatment for diarrhoeal disease.

Acetic acid-induced writhing model represents pain sensation by triggering localized inflammatory response. Acetic acid, which is used to induce writhing, causes algesia by liberation of endogenous substances, which in turn excite the pain nerve endings[9]. Acetic acid-induced writhing demonstrates a noxious stimulation in mice. The test consists of injecting 0.7% acetic acid solution intraperitoneally and then observed the animal for specific contraction of body referred as 'writhing'. A comparison of writhing was made between positive control (Diclofenac-sodium), control and test sample given orally 30 min prior to acetic acid injection. If the sample possesses analgesic activity, the animal that received the sample will give lower number of writhing than the control, *i.e.*, the sample having analgesic activity will inhibit writhing.

It was found that aqueous extract of leaves of *T. orientalis* at the doses of 500 mg/kg exhibited highly significant (p <0.001) inhibition of writhing reflex by 56.76%, while with the standard drug, Diclofenac-sodium (25 mg/kg body weight), the inhibition was found to be 48.34% (Table 1). It is, therefore, apparent that the aqueous extract of the leaf possess strong analgesic action.

Anti-diarrhoeal activity of the aqueous and methanolic extracts of leaves of *T. orientalis* was tested using the castor oil-induced diarrhoea model in mice. Castor oil, which is used to induce diarrhoea in mice, mixes with bile and pancreatic enzymes and liberates ricinoleic acid from the triglycerides upon oral administration. Most of the ricinoleic acid remains in the intestine and produces its anti-absorptive effect. The ricinoleic acid thus liberated readily forms ricinoleic salts with sodium and potassium in the lumen of the intestine. The salt formed as such behaves like a soap or surfactant within gut and at the mucosal surface. Most agreed view is that ricinoleate salt stimulates the intestinal epithelial cell's adenylate cyclase[10] or release prostaglandin[11].

The results of the present study demonstrated that both extracts increase the latent period and decrease the frequency of defecation, *i.e.*, delayed the onset of diarrhoeal episode, at the dose of 500 mg/kg body weight. If the sample possesses anti-diarrhoeal activity, the animal that received the sample will give longer latent period than the control, *i.e.*, the sample having anti-diarrhoeal activity will increase the latent period and defecation frequency will decrease.

The toxicity evaluation of plant extracts by brine shrimp lethality bioassay, aqueous extract did not show any cytotoxic effect on brine shrimp but methanol extract displayed toxicity. The methanolic extract showed cytotoxicity LC_{50} = 120 µg/ml and LC_{90} = 200 µg/ml. Therefore it can be concluded that the tested aqueous extract sample may be a good source of analgesic and anti-diarrhoeal medicine.

Conclusion

Bangladesh imports a large quantity of pharmaceutical raw materials including medicinal plants and semi-processed plant products to produce drugs and medicines. This huge foreign exchange can be saved if the indigenous medicinal plants or their semi-processed products are utilized by the manufacturers to satisfy their needs. From the present study, it can be certain that both aqueous and methanol extracts of *Trema orientalis* leaves posses significant analgesic and anti-diarrhoeal activity in mice. Therefore, further pharmacological and toxicological studies are required to establish the therapeutic uses of the plant.

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