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In vivo antidiarrheal activity of methanolic extract of *Trema oreintalis* leaves

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Abstract

The present investigation is to screen out the antidiarrheal potential of methanolic extract of *Trema orientalis* Blume (*T. orientalis*) in animal model. Castor oil and magnesium sulphate were used to induce diarrhea in swiss albino mice. The crude extract of *T. orientalis* was administered orally at the doses of 200 and 400 mg/kg/b.w. respectively. Loperamide was given to a group of mice as positive control at a dose of 3 mg/kg/b.w. The study shows that, in both magnesium sulphate and castor oil induced diarrheal condition, the frequency and the severity of diarrhea in tested animal were reduced throughout the study period at the doses of 200 and 400 mg/kg body weight. A statistically significant ($p<0.05$) result was found by this experiment. By the result of present study and considering the traditional uses of this plant, it can be preferred for further laboratory studies to scrutinize the active compund responsible for antidiarrhoeal activity and to use this plant as antidiarrheal agent medicinally.

Keywords: antidiarrheal, *Trema orientalis* Blume, swiss albino mice, castor oil, magnesium sulphate.

Introduction

In developing countries childhood diarrhoea is reported as the second leading reason for child death, although in recent decades there is remarkable improvement but still no significant statistical changes in morbidity [1]. In Bangladesh, since the population based survey system is in developed greatly, the true statistics of diarrhoeal incidences is unknown. [2]. However, the occurrences are devastating during flood especially before and after monsoon season in Bangladesh [3]. Traditionally a huge number of medicinal plants are being used to treat diarrheal condition. *Trema orientalis* [4] is one of the commonly used plants around the world in this purpose. *Trema orientalis* is a medicinal shrub or tree belonging to the family Cannabaceae, locally it is known as chikan, jibon (Bengali) [5], charcoal tree, pigeon wood, gunpowder [6] or Nalita (English) [7], Gio (Hindi). The name Trema is based on the Greek word for a hole and alludes to the pitted seeds. The specific name 'orientalis' derives from Latin word means eastern-'of the orient.'[8]. *Trema orientalis* is found in the lowland humid tropics. It is native in Angola, Australia, Bangladesh, Brunei, Cambodia, Cameroon, Central African Republic, China, Ethiopia, Hawaii, India, Indonesia, Japan, Kenya, Laos, Madagascar, Malaysia, Mali, Myanmar, Nepal, Niger, Nigeria, Philippines, Saudi Arabia, Senegal, Sierra Leone, South Africa, Sudan, Vietnam, Zimbabwe and such other countries [9]. Traditionally the plant has been being used for many years to treat several diseases. Coughs, sore throats, asthma, bronchitis, gonorrhea, yellow fever, toothache are being treated by using leaves and bark of *Trema orientalis*, and as an antidote to general poisoning [10,11]. To control dysentery a bark infusion is reportedly drunk and a leaf decoction is used to deworm dogs [10]. In recent pharmacological studies, an aqueous extract from the bark has been shown to reduce blood sugar levels in an experimental animal model of diabetes mellitus, and may be useful for treating this disease [12].

Materials and Methods

Plant Collection and Identification

The plant was collected from near GEC Circle of Chittagong, Bangladesh; in the month of October 2012. Then the plant has been identified by Dr. Shaikh Bokhtear Uddin, associate Professor, Department of Botany, University of Chittagong, Bangladesh.

Extraction of Crude Drug

The fresh leaves of *Trema orientalis* were cut into pieces, washed and air dried at room temperature (24°±2 ° C) for about 10 days. The dried leaves were ground to course powder with a mechanical grinder and powdered samples were kept in clean closed glass containers pending extraction. Then the dried powder was dissolved into methanol, shaken continuously for 10days, filtered, and then filtrate was dried at 55° C. finally the crude extract found was preserved in alpine tube.

IN VIVO ANTIDIARRHEAL STUDY

Animal Selection

For the present study, Swiss albino mice of either sex, 3-4 weeks of age, weighing between 20-25 gm, were collected from the animal research branch of the International Centre for Diarrheal 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*.

Castor Oil Induced Diarrhoea

The mice were fasted for 24 hours and then randomly differentiated into four groups of three animals each. They are weighed and properly labelled as Group I, II, III and IV respectively. Group I animals were classified as control group and Group IV as positive control group. 1% CMC (10 ml/kg) was given orally to the mice of Group I. Group III have received orally 200 mg/kg of the drug extract; Group IV animals were given orally 400 mg/kg of the drug extract. Group II animals were given Loperamide (3 mg/ kg) in suspension. After 60 min, each animal was given 0.5 ml of castor oil. Each animal was placed in an individual cage, the floor of which was lined with blotting paper which was changed every hour. The animals were observed for 4 hrs and the characteristic diarrheal droppings were recorded. The overall procedure has been earlier described by Shoba and Thomas (2001) [13].

Magnesium Sulphate Induced Diarrhoea

Mice fasted for 24 h were randomly allocated to four groups of three animals each. They were weighed and labelled as Group I (control), Group II (positive control), Group III and Group IV respectively. Magnesium sulphate was given orally at the dose of 2g/kg to the animals of all groups to induce diarrhoea. After 30 minutes, Group I animals were given vehicle (1% Tween 80 in water, 10 ml/kg) orally; Group III & Group IV animals were given orally 200 mg/kg & 400 mg/kg of the drug extract respectively. Group II animals were given Loperamide (3 mg/ kg) in suspension. Each animal was placed in an individual cage, the floor of was lined with blotting paper which was changed every hour. After 4 hours, the total diarrheal dropping was observed [14].

Results

Castor Oil Induced

With the increase of dose of methanolic extract of *Trema orienralis* leaves the number of faeces reduced in case castor oil induced diarrheal model. It follows a dose dependent manner (Table-1 & Fig-1).

Comparatively a larger inhibition of characteristic diarrheal faeces was observed at 400 mg/kg dose of the extract, which was found to be statistically significant ($p < 0.05$). That is, a dose of 400 mg/kg can reduce extended diarrheal condition in animal test model.

Magnesium Sulphate Induced Diarrhoea

The experiment also shows that, in case of magnesium sulphate induced diarrhoea a significant inhibition of defecation occurred at a dose of 400 mg/kg extract (Table-2 & Fig-2).

Discussion

Diarrhoea occurs due to the irritation within the lining of the small or large intestine, which leads decrease water absorption hence increase in water being passed with stools. Many factors such as food poisoning, infection (bacterial, viral, parasitic), food intolerance, malnutrition, intestinal diseases and sometimes medications can contribute to diarrhoea [15]. However, it is well evident that castor oil comprises its most active component recinoleic acid which causes irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which results in stimulation of secretion that produces diarrhoea [16]. Since the total number of faeces reduced after administration of methanolic extract of *Trema orientalis* on diarrheal mice, the extract might exhibit antisecretory mechanism to reduce diarrhoea. On the other side, magnesium sulphate induces diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been reported that it promotes the liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water [17,18]. Considering it traditional uses and current laboratory study, by far it can be said that *Trema orientalis* possesses a significant antidiarrheal property.

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Table-1: Effect of *Trema orientalis* leaf extract on castor oil induced diarrhoea in mice.

Groups	Treatment	Dose	No. of faeces in 4 hrs**	% inhibition in defaecation
Group I	1% Tween 80	0.4 ml/mouse	22.33±0.882	0
Group II	Loperamide	3 mg/kg	3.67±0.333	83.56
Group III	Crude extract	200 mg/kg	16.67±0.667	25.34
Group IV	Crude extract	400 mg/kg	12.33±0.882	44.78

**Mean±SEM, p<0.05 that means statistically significant.

Table-2: Effect of *Trema orientalis* leaf extract on MgSO4 induced diarrhoea in mice.

Groups	Treatment	Dose	No. of faeces in 4 hrs**	% inhibition in defaecation
Group I	1% Tween 80	0.4 ml/mouse	15.67±0.67	0
Group II	Loperamide	3 mg/kg	3.33±0.33	78.75
Group III	Crude extrect	200 mg/kg	8.33±0.88	46.84
Group IV	Crude extrect	400 mg/kg	6.33±0.33	59.60

** Mean±SEM, p<0.05 that means statistically significant.

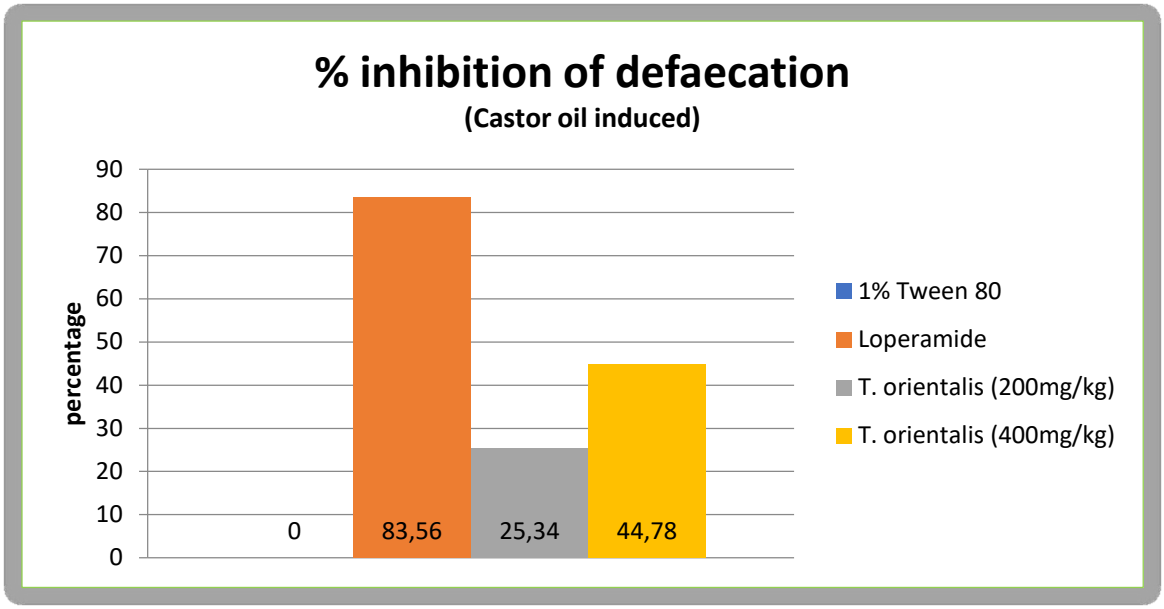

Fig-1: % inhibition defaecation in case of castor oil induced diarrhoea.

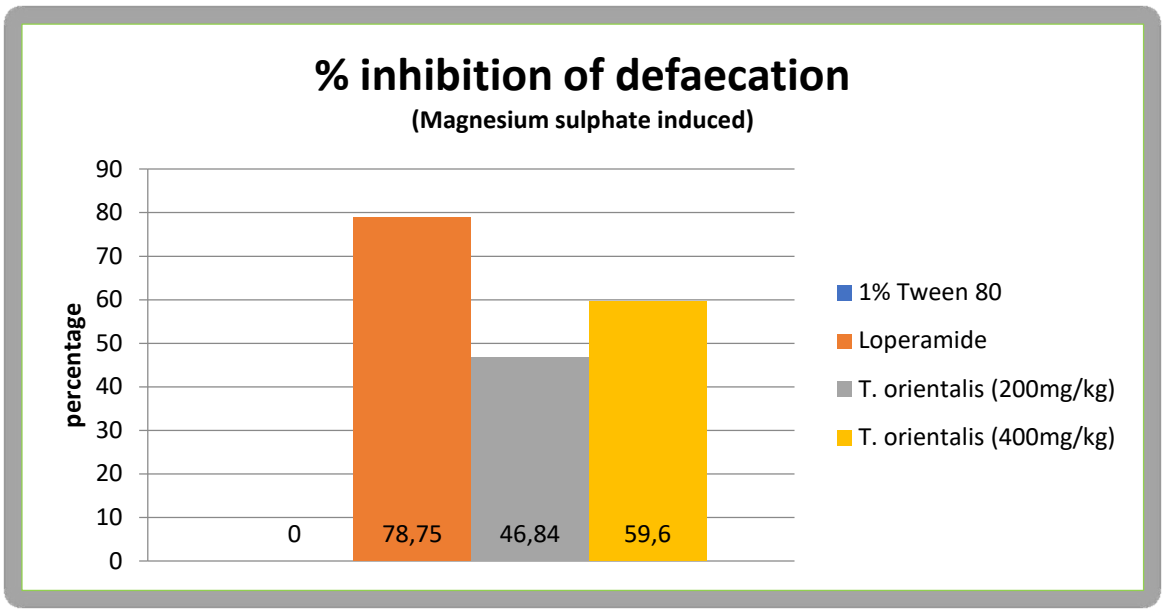

Fig-2: percent inhibition of defecation in case of magnesium sulphate induced diarrhoea.