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In vivo antidiarrheal study of ethanolic extracts of *Mikania cordata* and *Litsea monopetala* leaves

Fatema Nasrin and Md. Lukman Hakim

Department of Pharmacy, Southeast University, Banani, Dhaka, Bangladesh.

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Abstract

In this study the antidiarrheal activity of ethanolic extracts of the leaves of *Mikania cordata* and *Litsea monopetala* was evaluated. Diarrhea was induced in mice by oral administration of castor oil (0.5 mL) 30 min after the administration of the extracts. During a 4 hour study the number of diarrheal feces and percentage inhibition of the extracts (200 and 400 mg/kg body weight) was determined. Loperamide (3 mg/kg body weight) served as standard and belonged to the positive control group. The extracts exhibited potent antidiarrheal activity as well as achieved statistically significant p value ($p<0.01$ and $p<0.05$) compared to control group. Among the extracts the highest percentage inhibition of defecation (60%) was recorded for leaf extract (400 mg/kg body weight) of *L. monopetala*. So, the study corroborates the significant antidiarrheal activity of *M. cordata* and *L. monopetala* leaf extracts and raises the demand of further sophisticated investigation.

Introduction

Diarrhea is a familiar disease of the world, especially in tropical countries. According to World Health Organization (WHO) diarrhea is the passage of three or more loose or liquid stools per day, or more frequently than normal for the individual. Children are more vulnerable against diarrhea and it is estimated that 17% of all deaths in children up to five years are due to diarrhea, correspond to 1.8 million annual deaths (Wangensteen et al., 2013). At 1998, estimated by WHO, about 7.1 million deaths were caused by diarrhea (Choudhary, 2012). Though the mortality rate of diarrhea has decreased in recent years, but still it is a leading cause of malnutrition and fatal childhood disease in developing countries. Besides the synthetic drugs, traditional medicines are also used to treat diarrhea. Rural peoples largely depend on folklore medicines to combat various diseases including diarrhea. The present global market of plant derived drugs is worth more than 20 billion and the market is expanding continuously (Lin et al., 2013). About 80% of people from developing countries use traditional medicines (Akuodor et al., 2011). Moreover, World Health Organization (WHO) encourages the inclusion of effective, safe herbal medicines in the health care delivery system of developing countries (Magaji et al., 2008).

Mikania cordata (Family: Asteraceae) is a fast growing, creeping or twinning, perennial vine, from 3 to 6 meters long. Roots may form stem nodes and even on small stem fragments with a single node. Leaves are opposite, heart shaped and contains a wide range of phytochemicals like alkaloids, steroids, tannins, glycosides, flavonoids, saponins (Barua et al., 2014; Chowdhury et al., 2011). The leaf exhibits antimicrobial (Nogodula et al., 2012), cytotoxic (Ali et al., 2011), antiulcer (Alam et al., 2013), antinociceptive (Nayeem et al., 2011) characteristics besides the presence of essential oils (Bedi et al., 2003). Roots have anti-inflammatory (Bhattacharya et al., 1992), CNS depressant (Bhattacharya et al., 1988), antiulcer (Pal et al., 1988) properties along with tissue repair quality (Mandal et al., 1993). The whole plant is reported to posses anthelmintic and antiemetic (Bulbul et al., 2013) properties, whereas the aerial parts have antimicrobial (Chowdhury et al., 2011) potency.

Litsea monopetala (Family: Lauraceae) is an evergreen medium sized tree, leaves are broadly elliptic, 9-24 cm, by 5-11 cm, apex blunt or with a small point. Leaves are reported to exhibit antioxidant (Choudhury et al., 2013), antihyperglycemic (Hasan et al., 2014), antimicrobial, clot lysis, anti-inflammatory (Ahmad et al., 2012) properties, whereas barks possess antioxidant (Arfan et al., 2008) and analgesic (Ghosh and Sinha, 2010) activities. The leaves are potent sources of alkaloids, tannins, steroids, flavonoids, reducing sugars (Ahmad et al., 2012).

To search new potent natural drugs plant origin is an effective source. Both the plants of *M. cordata* and *L. monopetala* are traditionally used to treat diarrhea. Besides this, World Health Organization (WHO) has inaugurated a diarrhea disease control program to study traditional medicine practices and related aspects along with the evaluation of health education and prevention approaches (Nogodula et al., 2012). Therefore the present study was dedicated to finding out the antidiarrheal potency of ethanolic extracts of the leaf of *M. cordata* and *L. monopetal* to justify their folklore uses.

Materials and Methods

Collection of plant materials

M. cordata was collected from Sher-E-Bangla Agricultural University campus, Dhaka whereas *L. monopetala* was obtained from Natore district, Bangladesh. The plants were identified by the taxonomists of Bangladesh National Herbarium, Mirpur, Dhaka where voucher specimen No. 40255 and 41343 were deposited for *M. cordata* and *L. monopetala* respectively.

Extraction of plant materials

The leaves of the plants were washed to remove dirts, cut into small pieces and sun dried for 3 days. Then the leaves were separately ground into coarse powders. The powders (200 g) were extracted separately with 1 L of ethanol in two flat bottom glass containers through occasional shaking and stirring for 7 days. The extracts were individually filtered through cotton by decantation and finally through Whatman No. 1 filter paper. Then the filtrates were concentrated at 40°C by a rotary evaporator. The extracts were air dried to solid residues and preserved at 5°C. For *in vivo* antidiarrheal experiment, the air dried extracts were dissolved in 2% tween solution.

Experimental animals

Young Swiss-albino mice of either sex aged 7-8 weeks, average weight 30-35 g, were used for the experiment. The mice were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease Research, Bangladesh (ICDDR,B). Mice were kept in standard environmental conditions (temperature: 25°C, humidity: 55-65% and 12 hours dark/ 12 hours light cycle) and fed ICDDR,B formulated rodent food and water.

Chemicals and drugs

Loperamide was obtained from Square Pharmaceuticals Ltd, Bangladesh and Caster oil, Tween-80 were purchased from BDH Chemicals, UK. All chemicals were of analytical grade.

Ethical approval

Animal experiment was done by following instructions followed by the ICDDR,B that were approved by the Institutional Animal Ethical Committee. The Southeast University Ethical Committee observed and approved the experiment.

Acute toxicity study

The acute toxicity study of the extracts was performed by following OECD-423 guidelines (acute toxic class method) (Acute toxic class method, 2001). Only female mice were treated with the respective extract at a dose of 5, 50, 300 and 2000 mg/kg body weight. Before the administration of extracts mice were fasted for 3-4 hours, but only water was supplied and after the administration, food was withheld for 1-2 hours. Mice were monitored next 24 hours for any behavioral, neurological and autonomic profiles and 14 days for mortality.

Antidiarrheal test

The method was followed as described elsewhere (Shoba and Thomas, 2001). Mice were divided into the following groups, each comprised of six mice: Control group: Treated with vehicle (1% Tween 80 in water) at a dose of 10 mL/kg body weight orally; Positive control group: Treated with loperamide at a dose of 3 mg/kg body weight orally; Test group 1 (MCL 200): Treated with ethanolic extract of *M. cordata* leaf at a dose of 200 mg/kg body weight orally; Test group 2 (LML 200): Treated with ethanolic extract of *L. monopotala* leaf at a dose of 200 mg/kg body weight orally; Test group 3 (MCL 400): Treated with ethanolic extract of *M. cordata* leaf at a dose of 400 mg/kg body weight orally; Test group 4 (LML 400): Treated with ethanolic extract of *L. monopotala* leaf at a dose of 400 mg/kg body weight orally.

Thirty minutes after the above treatment diarrhea was induced in each mouse by oral administration of 0.5 mL castor oil. Each mouse was placed in individual cage and the floor was lined with white blotting paper. In every hour the blotting papers were changed and the experiment was performed 4 hours. During the study period the total number of diarrheal feces (watery unformed stool) was recorded. The total number of

diarrheal feces of control group was considered as 100%. Percentage of inhibition of defecation was determined by using the following formula.

% Inhibition of defection = [(A-B)/A] × 100

A= Mean number of defecation by castor oil; B= Mean number of defecation by extract.

Statistical analysis

Results were expressed as mean ± S.E.M. To determine the statistical significance One way ANOVA followed by Dunnett's multiple comparisons was performed.

Results

Acute toxicity study

During the observation period, no behavioral change symptoms nor any mortality was recorded, so the extracts were considered safe (Acute toxic class method, 2001).

Antidiarrheal study

As expected the highest number of feces (12.5 ± 1.1) was obtained in the control group, whereas the lowest number of feces (2.8 ± 0.4) was recorded for standard loperamide (3 mg/kg body weight) (Table I). The positive control group exhibited 77.6% of inhibition of defecation. All the extracts achieved antidiarrheal potency by means of reduced number of diarrheal feces compared to control in a 4 hour period of study. The percentage inhibition of defecation of the extracts was optimistic too.

Discussion

To perform *in vivo* antidiarrheal experiment castor oil induced animal model is adopted. Castor oil contains 90% ricinoleate that is metabolized to ricinoleic acid playing the vital role to induce diarrhea (Magaji et al., 2008). Ricinoleic acid causes the irritation and inflammation of the intestinal mucosa, that leads to the stimulation of peristalic activity of the small intestine, altering the electrolytic permeability of the intestinal mucosal membrane. These events in turns stimulate the release of endogenous prostaglandins that actuate the motility and secretion thereby reduced sodium and potassium ion absorption occurs (Dash et al., 2014). Besides the calculation of percent inhibition of defecation, total number of defecation in a period of 4 hours was recorded. All the extracts yielded antidiarrheal activity by means of reduced diarrheal feces compared to control. Comparatively the plant *L. monopetala* found more potent than *M. cordata*. The inhibition of defecation of *M. cordata* was recorded 42.4% and 48.0% for 200 mg/kg and 400 mg/kg body weight respectively; whereas for the plant *L. monopetal* it was 52.0% (200 mg/kg) and 60.0% (400 mg/kg). Among the test extracts the highest percent of inhibition (60.0%) was obtained from the leaf extract of *L. monopetala* whereas the positive control group (loperamide at 3 mg/kg body weight) exhibited 77.6% inhibition of defecation. All the extracts exhibited significant p value ($p<0.01$ and $p<0.05$) and thus proved their statistical significance.

Previous phytochemical researches revealed that the antidiarrheal activity of medicinal plants was responsible for the presence of tannins, alkaloids, flavonoids, saponins, sterol and triterpenes (Dash et al., 2014). Tanins denature proteins in the intestinal mucosa by forming protein tanuates that make the intestinal mucosa more resistant to chemical alteration and decrease secretion (Begum et al., 2013). Flavonoids are reported to inhibit release of autocoids and prostaglandins, thus may inhibit motility and secretion induced by castor oil (Choudhary et al., 2012). The experimental plants in the study are rich sources of tannins, alkaloids, phenols, flavonoids, steroids and may other phytochemicals (Chowdhury et al., 2011; Ahmad et al., 2012). So, it may be attributed that the potent antidiarrheal

Table I

Antidiarrhea activity of *Mikania cordata* and *Litsea monopetala* leaves

Group	Dose	Number of diarrheal faces in 4 hours	% Inhibition of defecation
Control	10 mL/kg	12.5 ± 1.1	-
Positive control	3 mg/kg	2.8 ± 0.4[b]	77.6
Leaf extract of *Mikania cordata*	200 mg/kg	7.2 ± 0.6[a]	42.4
Leaf extract of *Litsea monopetala*	200 mg/kg	6.0 ± 0.6[b]	52.0
Leaf extract of *Mikania cordata*	400 mg/kg	6.5 ± 0.6[a]	48.0
Leaf extract of *Litsea monopetala*	400 mg/kg	5.0 ± 0.5[b]	60.0

Results are expressed as mean ± SEM; [a]$p<0.05$ and [b]$p<0.01$ when compared to control group; One way ANOVA followed by Dunnett´s test was performed in Graphpad Prism version 6.05

activity of the plants due to the presence of these valuable phytochemicals.

By precisely studying the whole experiment, it is approbated that the leaves of both *M. cordata* and *L. monopetala* posses potent antidiarrheal activity. Moreover, the natural abundance and safety of the plants may turn these into useful antidiarrheal drugs.

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Author Info
Md. Lukman Hakim (Principal contact)
e-mail: mlhshohag1221@gmail.com; cell: +8801682539776