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Evaluation of Antidiarrheal Activity of *Leea aequata* L. (Family: Vitaceae) in Mice Models

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Abstract

Diarrhea is one of the most common reasons for why thousands of people die every year particularly in the underdeveloped and developing country. Furthermore, the identification of new antidiarrheal medication sources has become one of the most prominent areas of contemporary study. In addition, the plants of the *Leea* species are used traditionally for the treatment of diarrhea in different cultures. Considering this background, present study was to look into the antidiarrheal effects of an ethanolic extract of *Leea aequata* L. leaves in mice. The castor oil induced diarrhea and the small intestinal transit models were utilized in this study to examine the extract's potential antidiarrheal efficacy. The extract was given to the test groups in various quantities (100, 200 mg/kg), whereas positive controls got loperamide (3 mg/kg) and negative controls received distilled water (10 ml/kg). In a model of diarrhea caused by castor oil, 200 mg/kg of the extract significantly ($p<0.01$) decreased the total number of bowel movements. The extracts also demonstrate a substantial ($p<0.05$) reduction in the frequency of wet defecation at all doses. The onset of diarrhea was extended with dose of the extracts which is statistically significant ($p<0.001$). At higher doses, the extract caused a significant ($p<0.05$) reduction in the weight and volume of wet feces. Furthermore, in the charcoal meal test, increasing doses of the extract caused a substantial ($p<0.01$) reduction in gastrointestinal motility. Phytochemical screening of the extract revealed the presence of flavonoids, alkaloids, tannins, and phytosterols that may play a key role in its antidiarrheal activity. The results of our study indicate that bioactive compounds are present in ethanolic leaf extract of our plant including significant antidiarrheal activity and thus provide the scientific basis for the traditional uses of this plant as a treatment for diarrhea.

Key words: Castor oil, antidiarrheal, motility, loperamide.

Introduction

Citizens in third-world countries are particularly vulnerable to a variety of diseases, particularly diarrhea, as a result of their unsanitary living conditions. Diarrhea affects between 1.7 and 5 billion people each year (Abdelmalak and Doyle, 2012; Vos *et al*., 2015). It is particularly frequent in developing nations, where infants develop diarrhea three times each year on average. As a result of diarrhea, 1.26 million individuals died in 2013, down from 2.58 million in 1990 (Abubakar *et al*., 2015). For children under the age of five, this was the greatest cause of mortality in 2012 (0.76 million or 11 percent). Recurrent attacks of diarrhea are also a major cause of malnutrition, with diarrhea being the most common cause in children of below five. Long-term repercussions include stifled growth and impeded intellectual development (Liu *et al*., 2012). Based on the most recent WHO data from 2020, diarrheal disease deaths in Bangladesh accounted for 36,111 deaths or 5.05 percent of all deaths. For every 100,000 inhabitants in Bangladesh, there are 29.61 deaths. The country's death rate is 52nd in the world (World Health Rankings, 2020). The statistical data presented above is shocking and concerning.

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As a result, there is a constant need for novel alternative medications to successfully combat the threat of diarrhea. Herbal remedies have been used to treat diarrheal disorders, and up to 80% of the population in impoverished countries relies on traditional medicines for primary healthcare (Balemba *et al*., 2010). There are a large variety of herbal medications that are reported to be effective in treating diarrhea all over the world (Hossain *et al*., 2021). New antidiarrheal medications could come from medicinal plants. As a result, the WHO has urged researchers to conduct studies on the treatment as well as prevention of diarrheal disorders in accordance with conventional medical practice (Shanta *et al*., 2018). WHO has authorized the use of herbal formulas to treat diarrhea (Liu *et al*., 2012). Adverse effects and contraindications have been connected to currently available medications (FDA, 2016). When it comes to antibiotics used to treat diarrhea, drug resistance is another issue to consider (Alam and Bhatnagar, 2006). Because of the high prevalence of diarrhea in developing nations, as well as the limits of currently available antidiarrheal pharmaceuticals and inadequate healthcare coverage, traditional remedies may prove to be effective alternative agents for the management of diarrhea in these countries. Fever, diarrhea, dysentery, joint pain, rheumatism, diabetes, bone fracture, body discomfort, wound, sexual abnormalities and so on are just a few of the ailments that *Leea* plants have been utilized for in the past (Kekuda *et al*., 2018)

Tetanus leaves (*Leea aequata* L.) are a medicinal plant used as an antitetanus remedy and to heal wounds. Astringent, anthelmintic, dyspepsia, jaundice, chronic fever and malaria are all treated with the stems and roots. The leaves and twigs are used to cure wounds and as an antibacterial. Alkaloids, glycosides, steroids/terpenoids, flavonoids and tannins are among the secondary metabolites found in tetanus leaf. Flavonoids have been reported to give biological and pharmacological activities in vitro, including antiallergic, anti-inflammatory, antioxidant, antibacterial, cancer-preventive, and antidiarrheal properties (Ginting *et al*., 2018). Several investigations have been conducted on *L. aequata* and have reported on its anticonvulsant (Meliala *et al*., 2021; Suwarso *et al*., 2018), anti-inflammatory, antidepressant, anxiolytic (Chowdhury *et al*., 2020) and antiplatelet (Fakhrudin *et al*., 2021) effects.

Researchers discovered that two glycosides, 7-O-methylmearnsitrin and roseoside A, were proven to exhibit anticancer action after being isolated from the leaves of this plant (Rahim *et al*., 2021). The leaves of *L. aequata* have also been shown to exhibit relaxing (Ginting *et al*., 2018), antiproliferative, antinociceptive and anthelmintic properties (Halder *et al*., 2018) in addition to their other properties. Seed, stem and roots of *L. aequata* was found to be active against *Bacillus anthracis* (+), *Bacillus pumilis* (+), *Salmonella paratyphi* (1st strain) (-), *Salmonellla paratyphi* (II strain) (-), *Staphylococus albus* (+), *X. compestria* (-), *X. malvacearum* (1st strain) (-), *X. malvacearum* (II strain) (+) (Jain *et al*., 2010). It was also reported to be active against *Vibrio cholera* (Chander and Vijayachari, 2017) which is responsible for diarrhea.

There are a wide variety of bacteria, viruses and parasites that can cause diarrhoea in the digestive tract (National Institutes of Health, 2016). According to the findings of ethnopharmacological research, *L. aequata* has been shown to have therapeutic potential against the microorganisms that cause diarrhea (Halder *et al*., 2018; Jain *et al*., 2010; Chander and Vijayachari, 2017). In addition, an *in vitro* investigation conducted on excised guinea pig trachea revealed that this herb exhibited antispasmodic efficacy by inhibiting the effects of acetylcholine (Ginting *et al*., 2018). It has a high likelihood of antidiarrheal activity because it may relieve gastrointestinal muscular spasms as well.

Since *Leea* species traditionally used in diarrhea or dysentery (Hossain *et al*., 2021; Kekuda *et al*., 2018) and there is no scientific evidence reported till now on antidiarrheal activity of *L. aequata*, we decided to investigate possible antidiarrheal activity of ethanolic extracts of leaves of the plant using castor oil-induced diarrhea and gastrointestinal motility models in mice to assist in the long-run to the betterment of health care coverage.

Methods and Materials

Plant material collection and processing: In September 2016, the leaves of *L. aequata* (Family: Vitaceae) were taken from the Sylhet Hill Track in Bangladesh and identified by an experienced taxonomist from the National Herbarium of Bangladesh. The accession number was 43474. After that, the plant was thoroughly washed to eliminate any dirt and shade dried for several days, with some sun drying thrown in for good measure. A low-temperature oven was then used to dry it for 24 hours, allowing for a more efficient grind. A blender pulverized the dried plant into a coarse powder.

Experimental animal's selection and handling: The experiment employed healthy young female wistar albino mice weighing 25-30 g and aged 4-5 weeks. Mohakhali, Dhaka, Bangladesh-based ICDDR'B's Animal Resource Branch provided the mice. Following their purchase, they were kept in a regular habitat for one week to acclimate and were fed ICDDR'B designed rodent food and water. At a constant ambient temperature of 25–30°C and on a 12-hour light–dark cycle, they were housed in individual cages. The excreta were taken daily out of the cages and cleaned. The tests took place in a quiet environment. In a room with adequate ventilation, the mice were kept in cages of a standard size, each holding eight mice. Care and treatment of the animals were carried out in accordance with rules that are recognized on a global scale (National Research Council, 2011) and were given the green light by the animal ethics committee of the Pharmacy Department at Southeast University.

Animal grouping and dosing: There were four groups (negative control, positive control and two test groups) with four animals each in each group. In all models, the vehicle (10ml/kg, distilled water) was administered to the negative controls, whereas the loperamide (3mg/kg) was administered to the positive controls. Both the acute oral toxicity test and the pilot study were used to identify the appropriate doses of EELAL to administer orally to the test groups (group 3, 4). These doses ranged from 100 to 200 mg/kg, depending on the group.

Castor oil-induced diarrhea: As indicated by the published method (Awouters *et al*., 1978), this investigation was conducted using the approach described. The mice were allowed full access to water but fasted for 18 hours before being grouped and treated according to the procedures detailed in the section on grouping and dosage. Oral castor oil (0.5 ml) was supplied to each animal one hour following treatment with the medication and extract. The floor of the cage was lined with white paper, and the animals were kept in a separate, transparent cage. Over the course of four hours, the paper was replaced every hour. The commencement of diarrhea, the quantity and weight of wet stools, and the overall number and weight of fecal output were also documented during these monitoring intervals.

% of fecal output = (Mean fecal weight of each treatment group/ Mean fecal weight of control group) ×100

% inhibition of defecation = $\{(M_0 - M)/ M_0\}\times100$

Where, Mo: Mean defecation of control, M: Mean defecation of test sample/standard drug.

Gastrointestinal motility test: This investigation looked at the effect of an ethanolic extract of *L. aequata* on normal gastrointestinal transit. Mice went without food for 18 hours but had free access to water. They were then put into groups and treated as described in the section on grouping and dosing. Following the administration of the drugs and extracts for an hour, mice from each group were then given 0.5 ml of castor oil to consume. All mice were given 1 cc of 5% activated charcoal suspension an hour after receiving castor oil. The animals were sacrificed after 30 minutes of charcoal meal administration, the small intestine was removed and the distance (pylorus to caecum) traveled by charcoal meal through the organ, as well as the total length of the small intestine, were measured. In order to compute the peristaltic index and the percent of inhibition, we used the following formula (Than *et al*., 1989).

Peristaltic index = (Distance travelled by charcoal meal/ Length of small intestine) × 100

% inhibition = $\{(D_0 - D)/ D_0\}\times100$

Where, D_0: Mean distance travelled by the control, D: Mean distance travelled by the test group.

Phytochemical screening: It was determined that EELAL contained numerous secondary metabolites by conducting a qualitative phytochemical investigation utilizing conventional procedures (Kalita *et al.*, 2011; Sasidharan *et al.*, 2011).

Statistical analysis: The findings were analyzed utilizing the SPSS 16.0 statistical analysis program. There was a one-way analysis of variance followed by a Dunnett's test to compare results with controls and between groups, with the results expressed as a mean ± standard error of the mean (SEM). In every instance, a value of $p<0.05$ was chosen to denote statistical significance.

Results

Acute oral toxicity test: There were no significant toxic effects or deaths in the animals after a single oral dose of 2000 mg/kg body weight for all plant extracts. Throughout this observation period, the animals' consumption of water and food was normal. Therefore, it may be deduced that EELAL 1 and EELAL 2 have a larger margin of safety and that their LD_{50} value will be greater than 2,000 mg/kg.

Castor oil induced diarrhea test: Diarrhoeal induction was considerably ($p<0.001$) delayed in this experiment when *L. aequata* leaf ethanolic extracts were used. Total stooling frequency, fecal weight, and water content of the feces were all reduced. When compared to the control group, there was a significant reduction ($p<0.05$, $p<0.01$) in total number of feces, moist feces and feces weight. The percentage of fecal output for conventional loperamide and ethanolic leaf extracts at 100 mg/kg and 200 mg/kg were 44.49 percent, 81.5 percent and 60.80 percent respectively (Table 1).

Gastrointestinal motility test: These plant extracts lowered the gastrointestinal motility inducing castor oil to an extent that was substantial and concentration-dependent in this experiment. There were 26.67% and 40.61% inhibition rates for ethanolic leaf extracts in the 100 and 200 mg/kg doses, whereas 38.18% inhibition was seen in the body weight-based standard dose of loperamide (3 mg/kg). At 100 mg/kg and 200 mg/kg ethanolic leaf extract, the peristaltic index was lowered by 52.61% and 46.01%, respectively, compared to the control (Table 2).

Table 1. Effects of ethanolic extracts (mg/kg bw) of *L. aequata* leaves on castor oil-induced diarrhea in mice.

Treatment groups	Onset of diarrhea (Minute)	Dose (mg/kg bw)	Total number of feces	Total number of wet feces	% Inhibition of wet defecation	Total weight of feces (g)	% Inhibition of total weight of defecation	% of fecal output
Control	33.25 ± 0.48	-	7 ± 0.82	3.5 ± 0.65	-	0.13 ± 0.01	-	-
Loperamide	50.36 ± 0.69***	3	2.75 ± 0.85**	0.75 ± 0.48**	78.57	0.06 ± 0.02**	55.51	44.49
EELAL_1	45.25 ± 1.65***	100	5 ± 0.41	1.25 ± 0.48*	64.29	0.10 ± 0.002	18.50	81.50
EELAL_2	53.25 ± 1.11***	200	3 ± 0.71**	1.2 5 ± 0.48*	64.29	0.08 ± 0.02*	39.17	60.83

All values are stated as mean ± SEM (n=5; One way ANOVA followed by Dunnett's test was carried out. Here, *p<0.05, **p<0.01, ***p<0.001 significant when compared with control.

Phytochemical screening: Carbohydrates, flavonoids, phenols, alkaloids, tannins, glycosides, phytosterols, and saponin were identified as the principal ingredients after a phytochemical analysis of an ethanolic extract of *L. aequata* leaves.

Table 2. Effects of ethanolic extracts (mg/kg bw) of *L. aequata* leaves on gastrointestinal motility in mice.

Treatment groups	Dose (mg/kg bw)	Total length (cm) of small intestine	Distance (cm) traveled by the charcoal meal	% of inhibition	Peristaltic index (%)
Control	-	60.75 ± 2.69	41.25 ± 2.69	-	67.90%
Loperamide	3	59.75 ± 2.02	25.5 ± 1.26**	38.18%	42.68%
EELAL_1	100	57.5 ± 3.01	30.25 ± 4.75	26.67%	52.61%
EELAL_2	200	53.25 ± 0.63	24.5 ± 1.94**	40.61%	46.01%

All values are stated as mean ± SEM (n=5; One way ANOVA followed by Dunnett's test was carried out. Here, *p<0.05, **p<0.01 significant when compared with control.

Table 3. Phytochemical screening of ethanolic extracts of *L. aequata* leaves.

Sl. No.	Name of the test		Remark
1	Carbohydrate	Molish	++
		Benedict	+
		Fehling A & B	-
2	Alkaloid	Mayer	+
		Wagners	+
3	Glycoside		+
4	Phytosterol		+
5	Phenolic		++
6	Flavonoids		++
7	Protein		-
8	Saponin		+
9	Tannin		+

(+) = Present, (++) = Rapidly present, (-) = Absent

Discussion

The goal of our study was to assess the antidiarrheal activity of the ethanolic leaves extract of *L. aequata* in mice utilizing several diarrhoea models. Each mouse was given castor oil to cause diarrhoea in all models. Ricinoleic acid which is metabolized from castor oil and released in intestine can upset GIT (Kulkarni and Pandit, 2005). It works by binding to EP3 prostanoid receptors on smooth muscle cells (Tunaru *et al*., 2012), allowing fluid to accumulate in the intestine by limiting absorption and increasing fluid and electrolyte output (Racusen and Binder, 1979). The motility of GI smooth muscles is similarly affected by this metabolite (Mathias *et al*., 1978). There was a substantial effect of the castor oil extract across all examinations conducted: diarrhoea onset, number and weight of wet and total feces.

A prior study found that methanolic extracts of *L. aequata* leaves have anti-inflammatory properties when compared to diclofenac sodium as a control (Chowdhury *et al.*, 2020). Diclofenac sodium is a nonsteroidal anti-inflammatory medication (NSAID) that works by inhibiting prostaglandin production (Batislam *et al*., 1995). As a result, the extract's antidiarrheal effect could be linked to its inhibition of prostaglandin synthesis. It is supported by the fact that castor oil-induced diarrhoea is linked to prostaglandin biosynthesis provocation (Robert *et al.*, 1976).

The extract's phytochemical examination confirmed the presence of several bioactive compounds (Table 3). Flavonoids and phytosterols can alter the synthesis of cyclooxygenase 1 and 2 and lipooxygenase (LOX), hence reducing prostaglandin formation (Awad *et* al., 2004; Hamalainen *et al.*, 2011).

As the extracts shows the presence of tannin, it has the ability to precipitate the proteins in intestinal mucosa to protect it from chemical modification (Ashok and Upadhyaya, 2012). These protein tannates inhibit peristaltic movements and intestinal secretion. The inclusion of flavonoids, alkaloids, tannins and phytosterols in the crude extract may explain the anti-diarrheal action of EELAL demonstrated in this study.

Antidiarrheal medications work by lowering the propulsive activity of GI smooth muscles also. As a result, gastrointestinal motility tests were performed

on the extract. In this test we observed the reduction of gastrointestinal motility with the increase of given dose. The maximum dose of plant extract is more inhibiting than loperamide. Antidiarrheal medications can reduce gastrointestinal motility, that's why the charcoal meal approach was utilized to track gastrointestinal content during the study (Ezekwesili *et* al., 2010; Qnais *et* al., 2005). The retention of substances in the intestine is increased when the motility of gut muscles is reduced. This provides for increased absorption time (Islam *et al*., 2013). Due to their capacity to reduce intestinal motility, secondary metabolites such as flavonoids (Di Carlo *et* al., 1993) and tannins (Ashok and Upadhyaya, 2012) have been found to have anti-diarrheal action. It is possible that this synergistic inhibition by flavonoids and tannins on intestinal motility is responsible for the anti-movement action of the extract. This may cause the peristaltic movement of the colon to be slowed down, which in turn may lengthen the time it takes for the body to absorb water and electrolytes.

Conclusion

The ethanolic leaf extract of *L. aequata* was found to have strong anti-diarrheal activity in this investigation. It decreased the number of times that one needed to defecate and also had an inhibiting effect on the propulsion of the digestive tract. Phytochemicals as tannins, alkaloids, saponins, flavonoids and phytosterols may be responsible for the antidiarrheal properties of the extract. These data confirm the traditional use of *Leea* species as a diarrhoea treatment.

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