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Antidiarrheal Activity of *Lithocarpus dealbata* and *Urena lobata* Extracts: Therapeutic Implications

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Abstract

This study reports the antidiarrheal potential of *Lithocarpus dealbata* (Miq.) Rehder (Fagaceae) and *Urena lobata* L. (Malvaceae) used in the traditional medicine system of the Naga tribes of in India. The antidiarrheal efficacies of the seed extract of *L. dealbata* and leaf extract of *U. lobata* were evaluated in murine models by three different approaches: (i) castor oil–induced diarrhea, (ii) PGE_2-induced enteropooling, and (iii) small intestinal transit. The 800 mg/kg dose of methanol extracts of both plants showed significant ($p < 0.001$) inhibitor activity against castor oil–induced diarrhea and PGE_2-induced intrafluid accumulation. Both extracts also showed a significant ($p < 0.001$) reduction in the gastrointestinal motility in charcoal meal test. Acute toxicity tests did not reveal any sign of toxicity in the animals. The observed results could explain their use as antidiarrheal agents in traditional medicine.

Keywords: Antidiarrheal activity, folklore medicines, India, *Lithocarpus dealbata*, Naga tribe, *Urena lobata.*

Introduction

In the northeastern region of India, the Naga tribes inhabiting in the state of Nagaland have a rich knowledge of folk medicines. They often use plant-based medicines to combat various disorders such as diarrhea (Changkija, 2000). During our earlier studies on the ethnopharmacology of Nagas (Tangpu et al., 2004, 2006), we found that *Rhus javanica* L. (Anacardiaceae), *Swertia augustifolia* Buch.-Ham. ex D. Don (Gentianaceae), *Lithocarpus dealbata* (Miq.) Rehder (Fagaceae), and *Urena lobata* L. (Malvaceae) are the medicinal plants that are very frequently used for treating diarrheal diseases. In our earlier study, we reported antidiarrheal activity of *R. javanica* and *S. augustifolia* (Tangpu & Yadav, 2004, 2005). This article examines the antidiarrheal activity of *L. dealbata* and *U. lobata*.

L. dealbata (Miq.) Rehder (Fagaceae) is a lofty evergreen tree distributed in the eastern Himalayas, southwestern China, Bhutan, Myanmar, Thailand, and Vietnam. The seeds and bark of this plant are reported to be used in the treatment of hemorrhages, chronic diarrhea and dysentery by the Apatani and Naga tribes (Manandhar, 2002; Kala, 2005). The seeds contain bitter-tasting tannins, though they also possess edible value after they are leached and are consumed either as porridge or mixed with cereal flours in baking bread. A review of the literature reveals that no study has experimentally validated the acclaimed uses of *L. dealbata*. However, studies on related species under the genus reveal that they possess antioxidant, antimicrobial, and antiprotozoal activities (Khan et al., 2001; Yang et al., 2004; Subeki et al., 2005).

Urena lobata L. (Malvaceae) is native to China, but it is cultivated in many tropical countries, including India, South America, Africa, Australia, and the United States (Florida). It is a shrub, 0.6–3 m high, with pink flowers. *U. lobata* finds use in traditional medicine to cure diarrhea, colic, skin diseases, boils, pneumonia, rheumatism, cough, and diabetes in India, Indonesia, Vietnam, Malaysia, Philippines, Cuba, and Nepal (Van Duong, 1993; Mazumder et al., 2001; Manandhar, 2002; Ahmad & Holdsworth, 2003; Pinto et al., 2005). The methanol extract of *U. lobata* roots shows a broad-spectrum antibacterial activity against Gram-positive and Gram-negative microorganisms (Mazumder et al., 2001), and aerial parts contain two major chemical constituents: mangiferin and quercetin (Ghosh, 2004). In addition, Choi and Hwang (2005) have reported that

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U. lobata also reveals antioxidant activity and inhibitory action against NO release from macrophages.

This study reports the antidiarrheal effects of seed extract of *L. dealbata* and leaf extract of *U. lobata*, using experimental diarrheal models in albino mice.

Materials and Methods

Preparation of plant extracts

Acorns of *L. dealbata* and leaves of *U. lobata* were collected from Nagaland in July 2004 and duly identified by a plant taxonomist. Voucher specimens are kept under reference AKY-205 and AKY-218 at the Department of Zoology, NEHU, Shillong, India, respectively. The seed coats of *L. dealbata* were removed by grinding in a trunk mortar with wooden pestle. The bare seeds of *L. dealbata* and leaves of *U. lobata* were air-dried under shade. The powdered seed and leaf materials were extracted with MeOH in a Soxhlet apparatus. The final recovery of extracts was made with the help of a rotatory evaporator. The final yields (w/w) of the extracts was 1.72% in *L. dealbata* and 3.30% in *U. lobata*.

Experimental animals

Experiments were performed on 6- to 8-week-old albino mice (20–30 g). They were housed in polyacrylic cages and fed with standard rodent pellet diet and given water *ad libitum*. All the animal experiments were performed in accordance with the rules and regulations approved by the institutional animal care and use committee.

Acute toxicity

The mice were divided into 14 groups of 6 animals each. For *L. dealbata*, group I served as control (0.5 mL of 2% gum acacia suspension, p.o.) while groups II–VII were given 100, 200, 400, 800, 1600, and 3200 mg/kg dose of extracts. In case of *U. lobata*, group VIII served as control and received 2% gum acacia; groups IX–XIV received the similar doses of extracts. The animals were observed for mortality and general signs of toxicity pertaining to food and water intake for 72 h. The serum biochemical profile of animals was studied 24 h post-administration of 800 mg/kg dose of extracts (the dose that showed the maximum antidiarrheal activity). The levels of serum glutamic-oxaloacetic transaminase (SGOT; EC 2.6.1.1), serum glutamic-pyruvic transaminase (SGPT; EC 2.6.1.2), cholesterol and total protein were estimated as per the methods of Allain et al. (1974) and Henry et al. (1974) using a semiautomated biochemical analyzer (Bayer).

Castor oil–induced diarrhea

The methods of Robert et al. (1976) were followed; overnight-fasted mice were divided into 10 groups of 6 animals each, and diarrhea was induced by administering 0.5 mL of castor oil (S. D. Fine Chem., India) orally to mice. Group I served as control (0.5 mL of 2% gum acacia suspension, p.o.); groups II–IX received 100, 200, 400, and 800 mg/kg p.o. doses of *L. dealbata* and *U. lobata* extracts, respectively (the extract dosages were selected to match with the doses actually used in practice by people); group X received the standard drug, loperamide (Lopax, Agar Pharmaceuticals, India) (0.5 mL of 5 mg/kg, p.o.). After 1 h of treatment, all the animals were challenged with 0.5 mL/mouse, p.o., dose of castor oil and placed separately over clean filter papers inside cages. The filter papers were inspected for the presence of diarrheal droppings at hourly intervals for a period of 4 h. The total number of diarrheal episodes was counted group-wise. At hour 4, its absence was considered as a protection from diarrhea.

PGE_2-induced enteropooling

The method of Robert et al. (1976) was applied. Overnight fasted mice were divided into 11 groups of 6 animals each. Group 1 was given 2% gum acacia and kept as a control; group II served as a vehicle control and received 2% gum acacia $+PGE_2$ (0.5 mL of 100 μg/kg, i.p.); groups III–X received 100, 200, 400, and 800 mg/kg p.o. of *L. dealbata* and *U. lobata* extracts, respectively; group XI received loperamide. Immediately afterwards, diarrhea was induced by 0.5 mL of 100 μg/kg, i.p., dose of PGE_2 (Sigma Aldrich, USA). After 30 min, the animals were sacrificed, small intestine was removed, and intestinal contents were collected and measured in a syringe. The percentage inhibition in intestinal fluid was determined by comparing the values with vehicle control.

Small intestine transit

The mice were fasted for 16 h and allowed free access to water. They were divided randomly into groups of six mice each. Group I received 2% gum acacia to serve as control; groups II–IX were given p.o. the different doses of both plant extracts; group X received loperamide (5 mg/kg, p.o.). The animals were given 0.5 mL of marker (3% charcoal suspension in 2% gum acacia) 5 min after the treatment. The animals were sacrificed 30 min later and the abdomen was opened. The distance traveled by charcoal meal from the pylorus was measured and expressed as percentage of the total length of the intestine from pylorus to caecum (Akah & Offiah, 1992).

Statistical analysis

The results are represented as mean ± SE (standard error of the mean). The significance of difference between the means was determined by the Student's *t*-test, and $p < 0.05$ was accepted as significant.

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Table 1. Effect of the methanol extracts of *L. dealbata* seed and *U. lobata* leaf on castor oil–induced diarrhea in mice.*

Treatment (mg/kg, p.o.)	No. of wet feces 0–4 h	% Reduction	No. of animals devoid of wet feces at 4 h (n = 6)	% Protection
Control (2% gum acacia)	7.50 ± 0.89	0.00	0/6	0.00
L. dealbata extract				
100	3.83 ± 0.91[a]	48.93	1/6	16.67
200	2.67 ± 0.95[b]	64.40	2/6	33.33
400	1.83 ± 0.87[b]	75.60	3/6	50.00
800	1.67 ± 1.09[c]	77.73	4/6	66.67
U. lobata extract				
100	5.00 ± 0.37[a]	33.33	0/6	0.00
200	4.00 ± 0.26[b]	46.67	0/6	0.00
400	1.83 ± 0.60[c]	75.60	2/6	33.33
800	1.50 ± 0.72[c]	80.00	3/6	50.00
Loperamide				
5	0.67 ± 0.67[c]	83.33	5/6	83.33

*Data represent mean ± SEM (n = 6).
[a,b,c] $p < 0.05$, $p < 0.01$, and $p < 0.001$, respectively, versus. control by Student's *t*-test.

Results

Castor oil–induced diarrhea

The extracts significantly reduced the number of diarrheal episodes in a dose-dependent manner when compared with the untreated controls. At 800 mg/kg dose, *L. dealbata* showed 77.73% and *U. lobata* 80.00% reduction in the number of fecal episodes, whereas loperamide (5 mg/kg) offered 83.33% protection. In terms of protection from diarrhea at 4 h, the 800 mg/kg dose of *L. dealbata* extract protected 4 out of 6 animals, and *U. lobata* protected 3 out of 6 animals. Loperamide (5 mg/kg) protected 5 out of 6 mice from diarrhea (Table 1).

PGE_2-induced enteropooling

The plant extracts reduced the intestinal fluid accumulation induced by PGE_2 in a dose-dependant manner Table 2). At 800 mg/kg dose, *L. dealbata* showed a greater reduction (25.60%) than *U. lobata* extract (18.43%) compared with the vehicle control. The reduction in the intestinal fluid accumulation by loperamide (5 mg/kg) was 29.35%.

Small intestinal transit

The distance traveled by the marker in the treated groups showed significant difference compared with control ($p < 0.01$ to $p < 0.001$). The intestinal transit of charcoal meal was 76.46% in the control group, but at 800 mg/kg dose was 46.41% in *L. dealbata* and 32.16% in *U. lobata*. The percent transit of marker was 32.50% in the case of 5 mg/kg dose of loperamide (Table 3).

Acute toxicity

The oral administration of extracts of both plants up to a dose of 3200 mg/kg neither showed any mortality nor any visible signs of food and water uptake in the animals.

Table 2. Effect of the methanol extracts of *L. dealbata* seed and *U. lobata* leaf on PGE_2-induced enteropooling in mice.

Treatment (mg/kg, p.o.)	PGE_2 induced enteropooling: Volume (mL) of the small intestine fluid per 100 g mouse*	% Reduction
Normal control (2% gum acacia)	1.42 ± 0.12	–
Vehicle control (2% gum acacia + PGE_2)	2.93 ± 0.10	0.00
L. dealbata extract		
100	2.58 ± 0.10[a]	11.95
200	2.41 ± 0.12[b]	17.75
400	2.22 ± 0.07[d]	24.23
800	2.18 ± 0.18[c]	25.60
U. lobata extract		
100	2.81 ± 0.13	4.10
200	2.73 ± 0.10	6.83
400	2.46 ± 0.12[b]	16.04
800	2.39 ± 0.16[b]	18.43
Loperamide		
5	2.07 ± 0.05[d]	29.35

*Data represent mean ± SEM, n = 6.
[a,b,c,d] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, respectively, versus. vehicle control by Student's *t*-test.

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Table 3. Effect of the methanol extracts of *L. dealbata* seed and *U. lobata* leaf on gastrointestinal transit in mice.

Treatment (mg/kg, p.o.)	Distance traveled by charcoal marker as % of total length of small intestine*	% inhibition
Control (2% gum acacia)	76.46 ± 2.46	0.00
L. dealbata extract		
100	71.63 ± 2.07	6.32
200	67.00 ± 3.60	12.37
400	64.76 ± 2.67[b]	15.30
800	46.41 ± 2.95[c]	39.30
U. lobata extract		
100	66.51 ± 2.03[a]	13.01
200	54.56 ± 3.21[c]	28.64
400	47.98 ± 4.59[c]	37.25
800	32.16 ± 2.10[c]	57.94
Loperamide		
5	32.50 ± 2.59[c]	57.49

*Data represent mean ± SEM, n = 6.
[a,b,c] $p < 0.02$, $p < 0.01$, and $p < 0.001$, respectively, versus. control by Student's *t*-test.

The serum biochemical tests did not reveal any noticeable changes in SGOT, SGPT, cholesterol, and total protein levels in treated mice, except a significant rise ($p < 0.02$) was observed in SGPT levels in *L. dealbata* extract (800 mg/kg dose) in comparison with the control (Table 4).

Discussion

People customarily using the plant(s) or plant-derived preparations consider them to be efficacious against diarrheal disorders without any scientific basis to explain the action of such plants. The aim of this study was to experimentally evaluate the acclaimed uses of *L. dealbata* seeds and *U. lobata* leaves, which are regarded to confer protection in diarrhea among the Naga tribes of India.

The main feature of the small intestine is to absorb and secrete materials. An imbalance in the absorptive and secretory mechanisms in the intestinal tract accompanied by intestinal hurry results in frequent loose stools or diarrhea (Yegnanarayan & Shrotri, 1982). Clinically, in some cases of diarrhea, the secretory component predominates, whereas other diarrheas are characterized by hypermotility. Several studies have validated the use of antidiarrheal medicinal plants by investigating the biological activity of extracts of such plants, which have antispasmodic effects, delay intestinal transit, suppress gut motility, stimulate water adsorption, or reduce the intraluminal fluid accumulation (Almeida et al., 1995; Atta & Mouneir, 2005). These experimental models were therefore employed to judge the antidiarrheal efficacy of *L. dealbata* and *U. lobata* extracts in the current study.

It emerged from the study that both extracts of plant parts, viz., seeds of *L. dealbata* and leaves of *U. lobata*, had demonstrable antidiarrheal efficacy in one or the other experimental models, though their effects were more pronounced at their large doses (800 mg/kg). With respect to the castor oil–induced diarrhea model, the results revealed that the *L. dealbata* seed extract showed slightly better protection from diarrhea in the animals as compared with *U. lobata* leaf extract and so was the case in PGE_2-induced enteropooling. It is likely that the extracts bring out the aforementioned action either through their proabsorbtive property that promotes faster fluid absorption in the intestine or through an antisecretory mechanism. Our first speculation gains support from the fact that castor oil, which was used as a diarrhea-inducing agent in the experimental protocol, is known to induce diarrhea by increasing the volume of intestinal content by preventing water absorbtion. Therefore, any agent that allows or promotes water absorption in the intestine obviously would have an antidiarrheal potential (Katzung, 2004). It is widely known that the castor oil is metabolized into ricinoleic acid in the gut, which in turn irritates and causes inflammation in the intestinal mucosa, resulting in release of inflammatory mediators, such as prostaglandins, histamine, and so forth. (Luderer et al., 1980). The prostaglandins thus released promote

Table 4. Effect of methanol extracts of *L. dealbata* seed and *U. lobata* leaf on SGOT, SGPT, cholesterol, and total protein levels of experimental mice.*

Treatment group (mg/kg, p.o.)	SGOT (U/L)	SGPT (U/L)	Cholesterol (mg/dL)	Total protein (g/dL)
Control (2% gum acacia)	135.33 ± 14.39	118.00 ± 10.38	134.33 ± 9.92	6.73 ± 0.54
L. dealbata 800	159.00 ± 14.01	162.67 ± 9.79[b]	141.67 ± 9.54	7.53 ± 0.32
U. lobata 800	147.50 ± 9.36	132.50 ± 11.58	136.00 ± 5.14	7.02 ± 0.14
Loperamide 5	189.67 ± 14.90[a]	163.00 ± 9.12[c]	188.33 ± 6.00[d]	7.57 ± 0.18

*Data represent mean ± SEM, n = 6.
[a,b,c,d] $p < 0.05$, $p < 0.02$, $p < 0.01$, and $p < 0.001$, respectively, versus. control by Student's *t*-test.

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vasodilation, smooth muscle contraction, and mucus secretion in the small intestines (Pierce et al., 1971; Robert, 1973). The prostaglandins of the E series are considered to be good diarrheogenic agents in experimental animals as well as in human beings (Jaffe, 1979). The inhibitors of prostaglandins biosynthesis are therefore considered to delay the castor oil–induced diarrhea (Pierce et al., 1971).

Most plant species that have antidiarrheal potential contain tannins as one of the major constituents (Mukherjee et al., 1998; Agbor et al., 2004; Atta & Mouneir, 2005), and seeds of *L. dealbata* also contain tannins. These tannins precipitate proteins of enterocytes, which in turn reduce the peristaltic movements and intestinal secretion (Almeida et al., 1995). Studies on the functional role of tannins also reveal that they could also bring similar functions by reducing the intracellular Ca^{2+} inward current or by activation of the calcium pumping system (which induces the muscle relaxation) (Belemtougri et al., 2006). It seems that the antidiarrheal effect of *L. dealbata* may be the result of similar mechanisms. The phytochemical analysis of the aerial parts of *U. lobata* reveals that quercetin and mangiferin are two major chemical constituents of *U. lobata* (Ghosh, 2004). Plants that have quercetin, a common dietary flavonoid, in their composition can produce antidiarrheic effects mainly due to their antihistamine and anti-inflammatory activities (Galvez et al., 1993a; Izzo, 1994). Galvez et al. (1995) reported that rats treated with quercetin had less diarrheal output as compared with controls. Similarly, in another study, Lutterodt (1989) found that *Psidium guajava* L. (Myrtaceae) leaf extract has antidiarrheic action through quercetin, which according to him and other workers is mediated by an inhibition of gastrointestinal release of acetylcholine. The role of quercetin as a promising antidiarrheal molecule has also been verified by several other workers, Galvez (1993b), Galvez et al. (1995), and Perrucci et al. (2006), to name a few. Similarly, several studies in the past have revealed mangiferin acts against anti-inflammatory response by inhibiting the production and release of inflammatory molecules, such as prostaglandins, mast cells, and histamine (Garcia et al., 2003; Rivera et al., 2006). These anti-inflammatory molecules have the ability to relax smooth muscles and thereby relieve the gastrointestinal tract (Katzung, 2004). Recently, Choi and Hwang (2005) also found that *U. lobata* plays an inhibitory role in NO production in lipopolysaccharides-stimulated macrophages. Nitric oxide plays an important role in intestinal fluid and electrolyte secretion in the intestine (Izzo et al., 1998). Inhibitors of nitric oxide synthesis seem to block the laxative action of diarrheal agents (Izzo et al., 1994). Based on the above facts, it seems reasonable to suggest that the antidiarrheal effect of *U. lobata* may be due to any such mechanisms.

In the small intestinal transit test, both plant extracts suppressed the propulsion of charcoal marker in a dose-dependent manner. This finding suggests that the extracts act on all parts of the intestine. The percentage propulsion of charcoal marker by *U. lobata* extract was similar to that of loperamide. Loperamide is believed to a effect the circular and longitudinal muscles of the intestinal wall. A decrease in the motility of gut muscles increases the stay of substances in the intestine (Katzung, 2004). This allows better water absorbtion. It is therefore presumed that the reduction in the intestinal propulsive movement in the charcoal meal model may be due to antispasmodic propertiesof the extracts. Salah et al. (2002) has reported that quercetin inhibits the intestinal motility in experimental-induced diarrhea in rats.

At the acute toxicity level, the extracts did not cause any mortality or visible signs of toxicity or differences in food and water uptake in the animals up to 3200 mg/kg doses of extracts. Similarly, the mice treated with an 800 mg/kg dose of plant extracts did not show any significant change in SGOT, SGPT, cholesterol, and total proteins levels compared with control. The nontoxic effect of *U. lobata* leaf extract, in particular, further gains support from the findings of a similar study by Oladele and Abatan (2004), where the leaf extract of *U. lobata* was reported to be nontoxic on the basis of serum biochemical profile and histopathology of liver and kidneys in rats.

In conclusion, this experimental study validates the presence of antidiarrheal activity in *L. dealbata* seeds and *U. lobata* leaves, which are used for humans suffering from diarrheal disorders.

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