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Antidiarrheal Activity of *Albizzia lebbeck* Leaves

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Abstract

Worldwide, more than 5–8 million deaths are caused each year by diarrhea. At present, used anti-diarrheal drugs show adverse effects in some form. Hence, there is a need to explore natural drugs as alternative therapy. Preliminary phytochemical screening, Acute oral toxicity and antidiarrheal activity of 70% ethanolic extract of the *Albizzia lebbeck* leaves (ALL) were performed. ALL were assessed for antidiarrheal activity using castor oil, Prostaglandin-E_2 (PGE_2) and intestinal motility induced diarrhea in rats. On the basis of acute oral toxicity, 100 and 200 mg/kg body weight doses were selected for antidiarrheal activity. Preliminary phytochemical screenings revealed presence of flavonoids tannins and saponins in extract. ALL showed significant inhibitory activity against castor oil, PGE_2 induced diarrhea and intestinal motility. On the basis of the result, it can be concluded that the ALL possess significant antidiarrheal activity.

Keywords: *Albizzia lebbeck*, Antidiarrheal activity, Castor oil, Charcoal, Ethanolic extract, Prostaglandin-E_2

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Introduction

Albizzia lebbeck benth. (Mimosaceae) is a large, erect, unarmed and deciduous tree. On literature review it was found that, the leaves are used in ophthalmia.[1] The bark is used in bronchial asthma and other allergic disorders.[2] The flowers are useful in chronic cough and bronchitis.[3] The seeds are aphrodisiac,[1] useful in inflammation, scrofula, skin disease, leprosy, leukoderma, chronic catarrh, seminal weakness, ophthalmopathy, and poisoning.[2] The leaves of the plant *A. lebbeck* are rich in flavon., echinocystic acid, β-sitosterol and vicenin II, etc.[2] A native practitioner in this area had claimed that the leaves are highly useful in treating diarrhea. The modern literature revealed that the plant is reported to possess hepatoprotective,[4] nootropic,[5,6] anxiolytic,[6] anticonvulsant,[7,8] antifertility,[9] antidiarrheal (seed),[10] anti-inflammatory,[11,12] and Antiulcer activity.[13] There is no data reference available in the literature regarding the antidiarrheal activity of *A. lebbeck* leaves (ALL) either in humans or in any animal model. Hence, the present study was undertaken with the aim to assess the antidiarrheal activity of ALL so as to verify the claim of the native practitioner.

Materials and Methods

Plant Material

The leaves of plant *A. lebbeck* were collected from fields of Anand, Gujarat. It was identified and authenticated by Prof. G.C. Jadeja, Dept of Agricultural Botany, Anand Agricultural University. The leaves were pulverized after shade dried at room temperature. The 70% ethanolic extract was prepared using 70% ethanol in a soxhlet apparatus after de-fatting with petroleum ether. Preliminary phytochemical investigation showed the presence of flavonoid and tannin in ALL. Hence, ALL was selected for the present activity.

Animals

Wistar albino rats (150–220 g) and mice (18–25 g) of either sex were used for the study. As per the Indian CPCSEA guidelines approved was obtained (1554/PO/a/11/CPCSEA).

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Acute Toxicity Studies

As per fixed dose method of OECD Guide line no 420 given by CPCSEA, acute toxicity test was performed. Groups of 6 mice were administered test drug by oral route in the range of 2000–300 mg/kg and mortality was observed after 24 h.

Anti-diarrheal Activity

Castor oil-induced diarrhea[14]

Animals were divided into four groups of six animals each and fasted for 18 h before the test with free access to water. The animals were treated as follows:

Group I - Control (0.5 ml, 2% Gum acacia)
Group II - Standard (loperamide 1 mg/kg i.p.)
Group III - ALL (100 mg/kg p.o.)
Group IV - ALL (200 mg/kg p.o.)

Each rat received 1 ml of castor-oil orally after 30 min treatment and housed separately in perforated cage over a clean filter paper. During 4 h, diarrheal episodes were observed, that is, onset of diarrhea, number of wet feces, number and mean weight of stool.

Gastro-intestinal motility test[15]

The animals were divided into four groups of six rats each which were fasted for 24 h. The animals were treated as follows:

Group I - Control
Group II - Standard (Atropine sulfate 5 mg/kg i.m.)
Group III - ALL (100 mg/kg p.o.)
Group IV - ALL (200 mg/kg p.o.)

Each rat was received 1 ml of charcoal meal through oral route after 30 min of the above treatment. Thirty minutes after charcoal meal treatment, each rat was sacrificed and intestinal distance moved by the charcoal meal from pylorus to cecum was measured and expressed as a percentage of distance travelled from pylorus to cecum. The mean percentage movement of charcoal meal in ratio to the intestinal length, and percentage of inhibition was calculated by following the formula:

$$\%\,Travelled = \frac{Distance\ travelled\ by\ the\ charcoal\ meal}{Total\ length\ of\ small\ intestine} \times 100$$

$$\%\,of\ Inhibition = \frac{Total\ length\ of\ the - Distance\ travelled\ by\ small\ intestine\ the\ charcoal\ meal}{Total\ length\ of\ small\ intestine} \times 100$$

Prostaglandin-E_2 (PGE_2) induced enteropooling model[14]

The animals were divided into four groups of six animals each. These animals were placed in perforated cages and fasted for 18 h prior to the experiment and water ad *libitum*.

Treatment

Group I - Positive control (PGE_2 100 µg/kg in 5% v/v ethanol in normal saline p.o.)
Group II - Standard (Loperamide 5 mg/kg i.p.)
Group III - ALL (100 mg/kg p.o.)
Group IV - ALL (200 mg/kg p.o.)

Group I was treated with PGE_2 and served as PGE_2 control. Immediately after the standard and extract treatment, each rat was administered PGE_2 in the Group II, III and IV. All the rats were killed after 30 min and the whole length of the intestine from the pylorus to the cecum was dissected out and its contents were weight and collected to measure its volume.

Statistical Analysis

The results were expressed as ±SEM. The statistical analysis was carried out by One-way Analysis of Variance followed by student "*t*" test.

Results

In the castor oil induced diarrhea, the total number of feces, number of wet feces, and total weight of feces were reduced by test extract (ALL). Similarly, the time elapsed between the administration of the castor-oil and the excretion of the first diarrheic feces is increased, by 70% ALL. The test extract has shown dose dependent antidiarrheal activity against castor oil induced diarrhea in rats (Table 1). In the gastro intestinal motility model, the 70% ALL caused a decrease in propulsion of the charcoal meal through the gastrointestinal tract when compared to the control group. However, it was comparable to that of the standard drug (Table 2). In the PGE_2 induced enterpooling model, the test extract has shown

Table 1: Effect of 70% ethanolic extract of leaves of ALL on castor oil induced diarrhea in rats.

Gr. No.	*Treatment*	*Onset of diarrhea (min)*	*Total number of feces*	*Number of wet feces*	*% of inhibition*	*Total wt. of feces (gm)*	*% of inhibition*
I	Control	51.5±3.845	21.83±1.376	17.66±1.33	-	3.81±0.152	-
II	Loperamide 1 mg/kg	135.83±7.15***	5.83±0.60***	1.5±0.42***	91.50	0.77±0.42***	79.79
III	ALL 100 mg/kg	84.0±7.9^{ns}	11.33±1.05***	6.83±0.87**	61.32	1.85±0.14***	51.44
IV	ALL 200 mg/kg	103.83±11.39**	9.0±0.81***	3.83±0.60***	78.31	1.235±0.10***	67.58

Values are Mean±SEM., *n*=6. Significance ***P<0.001, **P<0.01, *P<0.05, ^{ns}P>0.05 (vs. Control) respectively. ALL: *Albizzia lebbeck* leaves

Table 2: Effect of 70% ethanolic extract of leaves of ALL on gastrointestinal motility in rats

Gr. No.	*Treatment*	*Mean Length of intestine (cms)*	*Mean distance travelled by charcoal (cms)*	*Mean percentage movement of charcoal (cms)*	*Percentage of inhibition (%)*
I	Control	109.83±3.17	92.0±3.633	83.76	--
II	Atropine sulphate 5 mg/kg	110.36±3.56	47.28±3.48***	42.84	57.15
III	ALL 100 mg/kg. p.o.	113.33±3.48	78.26±2.07*	69.05	31.07
IV	ALL 200 mg/kg p.o.	116.83±4.88	70.86±1.40***	60.65	39.35

Values are Mean±SEM., *n*=6. Significance ***P<0.001, **P<0.01, *P<0.05, ^{ns}P>0.05 vs. Control. ALL: *Albizzia lebbeck* leaves

Table 3: Effect of 70% ethanolic extract of leaves of ALL on PGE_2 induced enteropooling in rats

Gr. No.	*Treatment*	*Dose*	*Mean volume of intestinal contents (ml)±SEM*	*% of inhibition*	*Mean weight of intestinal contents (ml) ± SEM*	*% of inhibition*
I	Control	PGE_2 100µg/kg i.p.	3.05±0.260	--	3.77±0.22	--
II	Standard (loperamide)	5 mg/kg.p.o.	0.86±0.074***	71.86	1.69±0.15***	55.17
III	ALL	100 mg/kg. p.o.	2.11±0.113***	30.81	2.66±0.263***	29.44
IV	ALL	220 mg/kg.p.o.	1.48±0.197***	51.47	2.03±0.169***	46.56

ALL: *Albizzia lebbeck* leaves, PGE_2: Prostaglandin-E_2. Values are Mean±SEM., *n*=6. Significance ***P<0.001, **P<0.01, *P<0.05, ^{ns}P>0.05 (vs. control), respectively

a significant inhibition of volume and weight of intestinal content in rats which was comparable to standard group animals (Table 3).

Discussion

Castor oil contain ricinoleic acid cause permeability changes, irritation, and inflammation at intestinal mucosa[16,17] lead to prostaglandins release.[18,19] ALL delayed the onset of diarrhea in castor oil induced diarrhea. Anti-diarrheal activity of extracts may be due to inhibition of prostaglandins biosynthesis.

PGE_2 stimulate intestinal absorption of water and electrolytes.[20] ALL showed inhibition of diarrhea may be due to inhibition of prostaglandins biosynthesis or by decreasing peristaltic movement.

In gastro-intestinal motility test, charcoal prevent absorption thereby decrease peristalsis movement.[21] The inhibition of peristaltic movement with ALL may be due to antihistaminic and antianaphylactic activity.[21,22]

However, further studies are needed to establish exact mechanism of action.

Phytochemical screening revealed the presence of tannins and flavonoids. Earlier studies showed that antidysentric and antidiarrheal properties of medicinal plants were due to tannins, alkaloids, saponins, flavonoids, sterol and/or triterpenes and reducing sugars.[23] Hence, tannins and flavonoids may be responsible for the significant antidiarrheal activity in the present study. Further studies are required to identify and isolate the active principles responsible for the antidiarrheal activity to establish the exact mechanism of action of the test extract (ALL).

Conclusion

In conclusion, the present study demonstrates that ALL possesses antidiarrheal properties. In addition, the antidiarrheal activity may be attributed to the polyphenolic compounds of plant, namely tannins and flavonoids.

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