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Original Article

Evaluation of antidiarrheal activity of ethanolic stem bark extract of *Albizzia lebbeck* Linn. in rats

Neelam Balekar*, Dinesh Kumar Jain, Pankaj Dixit, and Veena Nair

College of Pharmacy, IPS Academy, Rajendra Nagar, A.B. Road, Indore- 452012, India.

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Abstract

The present study was performed to substantiate the traditional claim of the antidiarrheal activity of stem bark extract of *Albizzia lebbeck* Linn. in rats. The effects of ethanolic extract of the stem bark of *A. lebbeck* on castor oil-induced diarrhea, castor oil magnesium sulphate-induced enteropooling, and gastrointestinal motility test using charcoal meal method were examined. The extract was initially assayed for its effects in castor oil-induced diarrhea at different doses (250, 500, and 1000 mg/kg, p.o.) in which significant activity ($p<0.05$) was observed at a dose level of 500 mg/kg. Hence, this dose level was then used in other models. The extract was found to inhibit peristaltic movements in charcoal meal test, and intestinal fluid secretions in castor oil as well as magnesium sulphate induced enteropooling, confirming its antidiarrheal activity, which might be due to its high flavonoid content. The results provide evidence that the ethanolic extract of *A. lebbeck* stem bark possesses antidiarrheal activity.

Keywords: *Albizzia lebbeck*, diarrhea, castor oil, charcoal meal, enteropooling

1. Introduction

Albizzia lebbeck Linn. (Mimosaceae) is deciduous tree available throughout India. The tree has a wide variety of uses in the traditional medicinal system of India (Kritikar and Basu, 1985). Though the stem bark of *A. lebbeck* is used in Indo-China for the treatment of piles, diarrhea and dysentery, no systematic studies have been carried out to confirm these activities in experimental animals. In preliminary studies it was found that the ethanolic extract of stem bark of *A. lebbeck* was devoid of any excitatory activity in isolated smooth muscle preparations and the extract delayed gastric transit in rats (Kritikar and Basu, 1985). Hence it was felt worthwhile to study the antidiarrheal activity of the stem bark of *A. lebbeck* employing conventional animal models of diarrhea.

* Corresponding author.
Email address: neelambalekar@gmail.com

This study aimed to investigate antidiarrheal activity of the ethanol extract from the stem bark of *A. lebbeck*, initially on castor oil-induced diarrhea (250, 500, and 1000 mg/kg) and subsequently confirming its action in castor oil-induced enteropooling, magnesium sulphate induced enteropooling, and gastrointestinal motility test using the charcoal meal method in rats.

2. Materials and Methods

2.1 Plant materials and chemicals

The fresh stem bark of *A. lebbeck was* collected in January 2010 from local gardens of Indore, Madhya Pradesh. The voucher specimen (number: VENAAL3) was kept at the Herbarium of the Dept. of Botany, Botanical Survey of India, Pune. The following drugs and AR grade of chemicals were used: atropine sulphate and loperamide (standard reference antidiarrheal drugs), castor oil (laxative agents), normal saline solution (0.9% NaCl), charcoal meal (10% activated charcoal

in 0.5% w/v sodium carboxy methyl cellulose) and vehicle (0.5% w/v NaCMC) were used. All the treatments were given in a dose volume of 10 ml/kg, p.o. All the other chemicals and reagent used were of analytical grade, obtained from Kasliwal Brothers, Indore, India.

2.2 Preparation of the extract from the stem bark of *Albizzia lebbeck*

The shade-dried stem bark material was powdered. The coarse powder was subjected to successive extraction with alcohol in soxhlet apparatus at (60-80°C) and the marc obtained after ethanolic extraction was macerated with water to obtain an aqueous extract. The concentrated extract was stored in amber bottles and refrigerated. The drug extract was suspended in sodium carboxymethyl cellulose (NaCMC 0.5%, w/v).

2.3 Phytochemical investigation

The total flavonoid content was determined by aluminum chloride colorimetric method (Chang *et al.*, 2002 and Pourmorad *et al.*, 2006). In brief, 0.5 ml of ethanolic extract was mixed with 1.5 ml of methanol, 0.1 ml of 10% aluminum chloride, 0.1 ml of 1 M potassium acetate and 2.8 ml of distilled water. After incubation at room temperature for 30 min, the absorbance of the reaction mixture was measured at 415 nm with a UV spectrophotometer (Shimadzu UV-1601, Japan). The amount of 10% aluminum chloride was substituted by the same amount of distilled water in the blank. Total flavonoid contents were calculated as quercetin equivalent from calibration curve prepared using quercetin.

2.4 Animals

Wistar rats of either sex weighing 120-200 g were used. Animals were housed under standard condition of temperature (25±2°C), 12 h/12 h light/dark cycle and fed with standard pellet diet and water ad libitum. The animals were allowed to acclimatize for one week before the experiments. All experimental protocols were approved by the Institutional Animal Ethics Committee (IAEC).

2.5 Antidiarrheal activities

2.5.1 Castor oil induced diarrhea

The antidiarrheal activity of ethanolic extract was evaluated according to the method described by Teke *et al.*, 2007. Rats were fasted for 18 hours and divided into five groups of five animals each group. Castor oil at a dose of 1 ml was given orally to all groups of animals for the induction of diarrhea. One hour prior to castor oil administration various treatments were given, Group I (control) and given 0.5 % sodium carboxymethyl cellulose (Na CMC), Group II (standard) was treated with loperamide (3 mg/kg p.o.), a positive control. Group III-V were administered ethanolic extract ALEE (250, 500, and 1000 mg/kg) respectively by oral route. Animals were placed separately in individual cages lined with filter paper. The filter papers were changed every hour and the severity of diarrhea was assessed hourly for 4 hours.

2.5.2 Gastrointestinal motility test

Wistar rats were fasted for 18 hours and divided into three groups of five animals each, group I animals served as control and was treated orally with 0.5% w/v Sodium CMC in distilled water. Group II were treated orally with atropine 5 mg/kg, a positive control. Group III received orally 500 mg/kg extract of *A. lebbeck*. After 1 h, each animal received charcoal meal 0.25 ml (10% charcoal in 0.5%w/v Sodium CMC) administered orally. Thirty minutes later, the animals were sacrificed. Total small intestine from pylorus to caceum was isolated and the total length and the length traveled by the charcoal meal were measured. This distance was expressed as a percentage of the length of the small intestine (Teke *et al*., 2007).

$$\% \text{ Inhibition} = Mc - Md/Mc \times 100$$

Mc: mean distance travelled by charcoal meal; Md: mean distance travelled by drug or extract.

2.5.3 Castor oil-induced enteropooling

Rats were fasted for 18 hours and divided into three groups of five animals each. Group I which received normal saline (2 ml p.o.), served as the control group. Group II received loperamide (3 mg/kg p.o.). Group III received *A. lebbeck* extract of 500 mg/kg p. o., one hour before the oral administration of castor oil (2 ml v/v). One hour later, the rats were sacrificed, and the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder and its volume measured. The intestine was reweighed and the difference between the full and empty weights calculated (Inayathulla *et al*., 2005).

2.5.4 Magnesium sulphate-induced enteropooling

Rats were fasted for 18 hours and divided into three groups of five animals per group. Solutions of magnesium sulphate were made in the 10% w/v aqueous solution. Group I which received normal saline (2 ml p.o.) served as the control group. Group II received loperamide (3 mg/kg p.o.). Group III received *A. lebbeck* extract of 500 mg/kg p. o. Immediately after the extract administration, magnesium sulphate (10 % w/v) was administered. After 30 minutes following administration of magnesium sulphate the rats were sacrificed, the small intestine was removed after tying the ends with threads and weighed. The intestinal content was collected into a graduated cylinder and its volume measured.

The intestine was reweighed and the difference between the full and empty weights calculated (Sunilson *et al*., 2009).

2.6 Statistical analysis

Data were expressed in as the mean ± standard error of mean (S.E.M.) and statistical analysis was carried out employing one way analysis of variance (ANOVA) followed by Tukey-Kramer multiple comparison test at $P < 0.05$ significance level using "Graphpad Instat" version 3.00 for Windows 95, Graphpad Software, San Diego, California, USA (www.graphpad.com).

3. Results

3.1 Phytochemical investigation

The percentage yields of the ethanolic extracts were found to be 12.36% w/w. The aqueous and ethanolic extract showed the presence of flavonoid, tannin, saponin, and protein. The total flavonoid content in ethanolic was found to be 12.15 mg quercetin equivalents/g.

3.2 Effects of the ethanolic extract of *A. lebbeck* on castor oil-induced diarrhea

One hour after castor oil administration, all the rats in the control group of animals produced copious diarrhea. Pretreatment of rats with ethanolic extract of *A. lebbeck* (ALEE 250, 500, and 1000 mg/kg, p.o.) dose dependently and significantly ($p<0.05$) delayed the onset of diarrhea, reduced the frequency of defecation and the wetness of the fecal droppings (reduction in the no. of wet stool and the general diarrheal scores including the hard and copious stool (Table 1). The standard antidiarrheal drug loperamide (3 mg/kg p.o.) produced a significantly greater ($p< 0.05$) inhibitory effect in all the diarrheal parameters.

3.3 Effects of the ethanolic extract of *A. lebbeck* on gastrointestinal motility test

Compared with the control group, (ALEE 500 mg/kg p.o.) significantly ($p<0.05$) decreased the propulsive movement and transit of charcoal meal to the gastrointestinal tract. The standard antidiarrheal drug, atropine (5 mg/kg p.o.) produced greater antimotility effect than the higher dose of (ALEE 500 mg/kg p.o.) (Table 2).

3.4 Effects of the ethanolic extract of *A. lebbeck* on castor oil-induced enteropooling

Oral administration of castor oil (2 ml p.o.) produced a marked and significant ($p<0.05$) increase in the intestinal fluid volume of castor oil-treated groups of rats compared to control group of animals treated with normal saline (2 ml p.o.) only. Compared with the control group of rats, pretreatment of the 'test' group of rats with ALEE (500 mg/kg p.o.) dose dependently and significantly ($p<0.05$) inhibited castor oil-induced enteropooling in rats (Table 3). The standard drug, loperamide produced a marked and significantly greater ($p<0.05$) inhibitory effect on castor oil-induced fluid accumulation than the higher dose of ALEE (500 mg/kg p.o.) (Table 3).

3.5 Effects of the ethanolic extract of *A. lebbeck on* magnesium sulphate-induced enteropooling

The extract reduced the intestinal fluid secretion induced by magnesium sulphate, in a dose dependent fashion (Table 4). The standard antidiarrheal drug, loperamide (3 mg/kg, p.o.), produced a more marked and significantly greater ($p<0.05$) inhibitory effect on magnesium sulphate-induced fluid accumulation than the extract (Table 4).

4. Discussion

Diarrhea is a very common ailment and national problem in many tropical countries and the cause of 4-5 million deaths throughout the world annually (Abdullahi *et al*., 2001). Apart from modern medical therapy, the use of herbal drugs in the treatment of diarrheal diseases is a common practice in many countries of Asia including India and Bangladesh. A number of medicinal plants have been reported to be effective against diarrhea and dysentry, as they are used in traditional herbal practice. Many plants conveniently available in India are used in traditional folklore medicine for the treatment of diarrhea and dysentery. Indigenous plants used for this purpose are: *Andrographis paniculata, Asparagus racemosus, Butea monosperma, Cassia auriculata*, and others (Sunilson *et al.,* 2009). The present study was undertaken to substantiate out the scientific rationale behind the local use of *A. lebbeck* stem bark in diarrhea.

The antidiarheal activity of the alcoholic extract of the stem bark of *A. lebbeck* was evaluated by employing castor oil-induced diarrhea, gastrointestinal motility test, castor oil and magnesium sulphate-induced enteropooling methods. The results of the present study showed that the ethanolic extract of *A. lebbeck* stem bark in castor oil-induced diarrhea at 250, 500 and 1000 mg/kg body weight doses significantly lowered several typical parameters of diarrhea, producing a statistically significant reduction in the severity and frequency of diarrhea produced by castor oil. Furthermore, our preliminary investigations have revealed that the extract was safe up to 5 g/kg dose level in acute oral toxicity studies (data not shown in the manuscript), and there are no reports about any specific toxicity of the plant in literature.

It is known that the active component of castor oil is ricinoleic acid which is liberated from the action of lipases on castor oil. The ricinoleic acid produces irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins (Yoshio *et al*., 1999). This condition induces an increase in the permeability of the mucosal

Table 1. Effect of *Albizzia lebbeck* bark extract on castor oil induced diarrhea

Treatment	Dose (mg/kg) p.o.	No. of rats with diarrhea out of n=5	Mean defecation in 4 h (g)	Percentage inhibition of defecation (%)
Control	-	05	0.55 ± 0.03	-
Loperamide	03	00	0.001 ± 0.004[a]	99.8
ALEE	250	05	0.067 ± 0.031[a]	87.8
ALEE	500	05	0.024 ± 0.025[a]	95.6
ALEE	1000	05	0.023 ± 0.020[a]	95.1

ALEE = *Albizzia lebbeck* ethanolic extract ; LOP = Loperamide
Results are expressed as mean ± SEM; n=5 in each group comparison made with control (0.5% NaCMC) group and with standard (loperamide 3 mg/kg) group. Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test.
[a]$p<0.05$= compared to control group, [b]$p<0.05$= compared to standard group (loperamide 3 mg/kg).

Table 2. Effect of *Albizzia lebbeck* bark extract on gastrointestinal motility test

Treatment	Dose (mg/kg)	% Intestinal Transit
Control	-	80.5 ± 7.9
Atropine Sulphate	3	29.4 ± 0.9[a]
ALEE	500	48.0 ± 6.2[a,b]

ALEE = *Albizzia lebbeck* ethanolic extract
Results are expressed as mean ± SEM; n=5 in each group comparison made with control (0.5% NaCMC) group and with standard (atropine sulphate 5 mg/kg) group. Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test.
[a]$p<0.05$= compared to control group, [b]$p<0.05$= compared to standard group (atropine sulphate 5 mg/kg).

Table 3. Effect of *Albizzia lebbeck* bark extract on castor oil-induced enteropooling

Treatment	Dose (mg/kg)	Volume of fluid (ml)	Weight of intestinal contents (g)	Percentage inhibition (%)
Control	-	1.8 ± 0.2	2.3± 0.5	-
Loperamide	3	0.63 ± 0.50[a]	0.76 ± 0.3[a]	67
ALEE	500	1.40 ± 0.02[a,b]	1.01 ± 0.45[a,b]	56

ALEE = *Albizzia lebbeck* ethanolic extract
Results are expressed as mean ± SEM; n=5 in each group comparison made with control (2 ml normal saline) group and with standard (loperamide 3 mg/kg) group. Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test.
[a]$p<0.05$= compared to control group, [b]$p<0.05$= compared to standard group (loperamide 3 mg/kg).

cells and changes in electrolyte transport, which results in a hyper-secratory response (decreasing Na+ and K+ absorption), stimulating peristaltic activity and diarrhea (Zavala *et al.,* 1998). Inhibitors of prostaglandin synthesis are known to delay diarrhea induced with castor oil (Sunilson *et al.,* 2009). These observations suggest that the antidiarrheal effect of alcoholic extract may be due to the inhibition of prostaglandin biosynthesis.

In the evaluation of intestinal transit, atropine sulphate was used as the standard drug. Atropine is known to inhibit intestinal transit probably due to its anticholinergic effect (Izzo *et al*., 1999). The ethanolic extract of *Albizzia lebbeck*

Table 4. Effect of *Albizzia lebbeck* bark extract on magnesium sulphate-induced enteropooling

Treatment	Dose (mg/kg)	Volume of fluid (ml)	Weight of intestinal content (g)	Percentage inhibition (%)
Control	-	5.5 ± 0.27	11.13 ± 0.36[b]	-
Loperamide	3	2.8 ± 0.25[a]	7.27 ± 0.26[a]	34.6
ALEE	500	3.8 ± 0.26[a,b]	9.47 ± 0.41[a,b]	14.9

ALEE = *Albizzia lebbeck* ethanolic extract
Results are expressed as mean ± SEM; n=5 in each group comparison made with control (2 ml normal saline) group and with standard (loperamide 3 mg/kg) group. Data was analyzed by one way ANOVA followed by Tukey Kramer multiple comparison test.
[a]$p<0.05$= compared to control group, [b]$p<0.05$= compared to standard group (loperamide 3 mg/kg).

stem bark also appeared to act on all parts of the intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model at a dose level of 500 mg/kg of body weight, and a transit period for sixty minutes, though this was not comparable to the effect of atropine sulphate. Nevertheless, this is but logical since atropine sulphate is pure compared to the extract which is mixture of many compounds. Studies made on activated charcoal showed that it prevents the absorption of drugs and chemicals into the system by adsorbing them on the surfaces of the charcoal particles. Activated charcoal was used in the gastrointestinal motility test to find out the effects of these extracts on the peristaltic movement. The results show that these extracts suppressed the propulsion of charcoal meal (probably in the same way as atropine sulphate) and thereby increased the time for absorption of water and electrolytes. Further, the experiments carried out on gastrointestinal tract motility after charcoal meal administration also showed a reduction in the propulsive movement of the small intestine after pre-treatment with the extract of *A. lebbeck.*

In the enteropooling study, the ALEE (500 mg/kg) significantly reduced the intestinal content of the rat. The intraluminal fluid accumulation was blocked significantly by the ethanolic extract of *A. lebbeck*. The intestinal fluid secretion induced by castor oil was blocked by the test extract in a dose-related manner. Further, the experiments carried out on the gastrointestinal tract motility after charcoal meal administration also showed a reduction in the propulsive movement of the small intestine after pre-treatement with the extract of *A. lebbeck*. Intestinal fluid secretion has been analyzed by enteropooling assay in rat, evoked by magnesium sulphate (a standard laxative agent). It is known that castor oil induces alteration in intestinal electrolyte transport. Our results suggest that the effects of *A. lebbeck* extract may be due to an increase in the absorption of electrolytes and/or inhibition of the hypermotility of the intestine, thereby increasing its capacity to retain fluids, an action similar to that of loperamide.

The phytochemical analysis of the extract showed the presence of flavonoids and terpenes. These constituents may responsible for the antidiarrheal activity of *A. lebbeck* ethanolic extract. The antidiarrheal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydro-electrolytic secretion, which are known to be altered in this intestinal condition (Venkatesan *et al.*, 2005).

Thus, the present study systematically investigated the antidiarrheal potential of ethanolic extract of *A. lebbeck*, and supports its traditional use as antidiarrheal medicine.

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