Original Article

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

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Abstract

The present study was performed to substantiate the traditional claim of the antidiarrheal activity of stem bark extract of Albizia 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: Albizia lebbeck, diarrhea, castor oil, charcoal meal, enteropooling

1. Introduction

Albizia 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 dysentry, 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.

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 Kashiwal Brothers, Indore, India.

2.2 Preparation of the extract from the stem bark of *Albizia 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 aluminium 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% aluminium 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% aluminium 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 cecum 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).

\[
\%\text{ Inhibition} = \frac{Mc – Md}{Mc} \times 100
\]

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 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. Pre-treatment 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 antimitotility 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 dysentry. 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
cells and changes in electrolyte transport, which results in a hyper-secretory 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 1. Effect of Albizzia lebbeck bark extract on castor oil induced diarrhea

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

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.

*p<0.05= compared to control group, *p<0.05= compared to standard group (loperamide 3 mg/kg).

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>% Intestinal Transit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>80.5 ± 7.9</td>
</tr>
<tr>
<td>Atropine Sulphate</td>
<td>3</td>
<td>29.4 ± 0.9*</td>
</tr>
<tr>
<td>ALEE</td>
<td>500</td>
<td>48.0 ± 6.2ab</td>
</tr>
</tbody>
</table>

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.

*p<0.05= compared to control group, *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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Volume of fluid (ml)</th>
<th>Weight of intestinal contents (g)</th>
<th>Percentage inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>1.8 ± 0.2</td>
<td>2.3 ± 0.5</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide</td>
<td>3</td>
<td>0.63 ± 0.50a</td>
<td>0.76 ± 0.3a</td>
<td>67</td>
</tr>
<tr>
<td>ALEE</td>
<td>500</td>
<td>1.40 ± 0.02ab</td>
<td>1.01 ± 0.45ab</td>
<td>56</td>
</tr>
</tbody>
</table>

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.

*p<0.05= compared to control group, *p<0.05= compared to standard group (loperamide 3 mg/kg).
Intestinal fluid secretion 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 anti-diarrheal potential of ethanolic extract of *A. lebbeck*, and supports its traditional use as anti-diarrheal medicine.

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

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose (mg/kg)</th>
<th>Volume of fluid (ml)</th>
<th>Weight of intestinal content (g)</th>
<th>Percentage inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>-</td>
<td>5.5 ± 0.27</td>
<td>11.13 ± 0.36</td>
<td>-</td>
</tr>
<tr>
<td>Loperamide</td>
<td>3</td>
<td>2.8 ± 0.25</td>
<td>7.27 ± 0.26</td>
<td>34.6</td>
</tr>
<tr>
<td>ALEE</td>
<td>500</td>
<td>3.8 ± 0.26</td>
<td>9.47 ± 0.41</td>
<td>14.9</td>
</tr>
</tbody>
</table>

ALEE = *Albizizia 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.

*p<0.05* compared to control group, *p<0.05* compared to standard group (loperamide 3 mg/kg).

The phytochemical analysis of the extract showed the presence of flavonoids and terpenes. These constituents may responsible for the anti-diarrheal activity of *A. lebbeck* ethanolic extract. The anti-diarrheal 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 anti-diarrheal potential of ethanolic extract of *A. lebbeck*, and supports its traditional use as anti-diarrheal medicine.

References


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