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PharmTech

International Journal of PharmTech Research
CODEN(USA): IJPRIF ISSN : 0974-4304
Vol.1, No.3, pp 832-836, July-Sept 2009

Evaluation of antidiarrhoeal potentials of ethanolic extract of leaves of *Holoptelea integrifolia* in mice model

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Abstract: The ethanolic extract of leaves of *Holoptelea integrifolia* was studied for its antidiarrhoeal properties in experimental diarrhoea, induced by castor oil and magnesium sulphate in mice. At the doses of 250 and 500 mg/kg per oral, the ethanolic extract showed significant and dose-dependent antidiarrhoeal activity in both models. The extracts also significantly reduced the intestinal transit in charcoal meal test when compared to atropine sulphate (5 mg/kg; i.m.). The results showed that the ethanolic extract of leaves of *Holoptelea integrifolia* have a significant antidiarrhoeal activity and supports its traditional uses in herbal medicine.

Keywords: *Holoptelea integrifolia*; (Urticaceae); Antidiarrhoeal activity; Intestinal transit

Introduction

In developing countries, diarrhoea is a major cause of infant mortality and morbidity[1]. Despite the availability of vast spectrum of approaches for diarrhoeal management, a vast majority of the people in these developing countries rely on herbal drugs for the management of diarrhoea. WHO has encouraged studies for treatment and prevention of diarrhoeal diseases using traditional medical practices[2].

Holoptelea integrifolia belongs to the family of Urticaceae. It is an important pollen allergen of India and sensitizes almost 10% of the atopic population in Delhi[3]. Until today; some recent explorations have been reported on this plant in which antiviral activity[4], antioxidant, antimicrobial & wound healing activity[5] and antiemetic activity[6] is important. Ethnomedically, the leaves and stem bark of this plant were used by local people for skin diseases[7], obesity, cancer[8], and leaves decoction were used in the management of diarrhea[9].

Hence, the present study was under taken to evaluate the antidiarrhoeal activity of ethanolic extract of leaves of *Holoptelea integrifolia* in mice model subjected to its traditional claim.

Materials and Methods

Preparation of ethanolic extract of *H. integrifolia*, Planch. (HIE)

Plant leaves of H. integrifolia, Planch were obtained from the foothills of Yercaud (Tamil Nadu, India) in the month of November 2008 and were authenticated at Dept. of Botany, SRM University, Kattankulathur, Tamil Nadu. The dried and coarsely powdered leaves (400 g) extracted successively with 1.5 L each of petroleum ether (60–80^{0}C), ethanol and in a Soxhlet extractor for 72 h. The extracts were concentrated to dryness under reduced pressure and controlled temperature (40–50^{0}C). The petroleum ether extract yielded a yellowish green sticky semisolid, weighing 3 g (3%) The ethanol extracts yielded brown and semi-solid residues, weighing 7.0 g (7.0%).

Phytochemical screening

The presence of phytochemicals, alkaloids (Draggendorff's), flavonoids (Shibata's reaction), saponins (Frothing test), tannins (5% ferric chloride), terpenoids (2,4 dinitrophenylhydrazine), glycosides (fehling's solution), steroids (Liebermann's Burchard test) and anthraquinones (Borntrager's test) were evaluated.

Animals

Swiss albino mice (22–25 g) of either sex were housed in standard isolation cages (45×35×25 cm) under environmentally controlled conditions with 12-h light/12-h dark cycle. Mice were allowed free access to water and standard laboratory chow (Hindustan Liver Pvt. Ltd, Mumbai). The experimental protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the SRM University, Kattankulathur, Tamil Nadu, India.

Antidiarrhoeal activity study by castor oil-induced diarrhea

The method, described by Shoba and Thomas, was followed for this study[1]. The animals were all screened initially by giving 0.5 ml of castor oil and only those showing diarrhea were selected for the final experiment. The animals were divided into control, positive control and test groups containing five mice in each group. Control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. The positive control group received loperamide at the dose of 3 mg/kg orally, and test groups received the ethanolic extract of leaves of *Holoptelea integrifolia* at the doses of 250 and 500 mg/kg body weight orally. The dose of extract selected on the basis of our previous work[6]. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. The floor lining was changed every hour. Diarrhoea was induced by oral administration of 0.5 ml castor oil to each mouse, 30 min after the above treatments. During an observation period of 4 h, the total number of faecal output and the number of diarrhoeic faeces excreted by the animals were recorded.

Antidiarrhoeal activity study by magnesium sulphate-induced diarrhea

A similar protocol as for castor oil-induced diarrhoea was followed. Diarrhoea was induced by oral administration of magnesium sulphate at the dose of 2 g/kg to the animals 30 min after pre-treatment with vehicle (1% Tween 80 in water, 10 ml/kg, p.o.) to the control group, loperamide (3 mg/kg) to the positive control group, and the ethanolic extract of leaves of *Holoptelea integrifolia* at the doses of 250 and 500 mg/kg to the test groups. All the administrations were carried out through oral route[10].

Effect on gastrointestinal motility

The method, described by Abdullahi et al.[11] and Uddin et al[12], was adopted to study the effect of the ethanolic extract on the gastrointestinal transit in mice. The test animals were starved for 24 h prior to the experiment but were allowed free access to water. The animals were divided into control and test groups containing five mice in each group. Control group received vehicle (1% Tween 80 in water) at a dose of 10 ml/kg body weight orally. Positive control group received atropine sulphate at the dose of 0.1 mg/kg intraperitoneally, and test groups received the ethanolic extract of leaves of *Holoptelea integrifolia* at the doses of 250 and 500 mg/kg body weight orally. After 30 min, mice of each group were fed with 1ml of charcoal meal (3% suspension of deactivated charcoal in 0.5% ethanolic methyl cellulose). After 30 min of the administration of charcoal meal, the animals of each group were sacrificed and the length of the intestine (pyloric sphincter to caecum) as well as the distance traveled by charcoal as a fraction of that length was measured. The charcoal movement in the intestine was expressed as a percentage.

Statistical analysis

Results are expressed as mean ± S.E. Mean values were evaluated by One Way ANOVA followed by Dunnett test.. Statistical significance was accepted at $P < 0.05$.

Results

In the castor oil-induced diarrhoeal experiment in mice, the ethanolic extract of leaves of *Holoptelea integrifolia*, at the doses of 250 and 500 mg/kg, reduced the total number of faeces as well as the total number of diarrhoeic faeces in a dose dependent manner (Table 1). These results were shown to be statistically significant ($P < 0.01$).

In the magnesium sulphate-induced diarrhoeal model in mice, the ethanolic extract of leaves of *Holoptelea integrifolia* at the above dose levels significantly ($P < 0.01$) reduced the extent of diarrhoea in test animals (Table 2). Both the doses were shown to reduce the total number of faeces and wet faeces when compared to the control.

In the gastrointestinal motility test, ethanolic extract of leaves of *Holoptelea integrifolia*, at the doses of 250 and 500 mg/kg, retarded ($P < 0.01$) the intestinal transit of charcoal meal in mice when compared to the control (Table 3).

Discussion

Several mechanisms have been previously proposed to induce the diarrhoeal effect of castor oil[13]. These include inhibition of intestinal Na^+,K^+-ATPase activity to reduce normal fluid absorption[14], activation of adenylate cyclase or mucosal cAMP mediated active secretion[15,16], stimulation of prostaglandin formation[17], platelet activating factor[18] and most recently nitric oxide has been claimed to contribute to the diarrhoeal effect of castor oil[19]. It is well documented that castor oil produces diarrhoea due to its most active component recinoleic acid by a hypersecretory response[20]. Since the ethanolic extract of leaves of *Holoptelea integrifolia* successfully inhibited the castor oil-induced diarrhoea, the extract might have exerted its antidiarrhoeal action by antisecretory mechanism. This was also evident from the reduction of total number of wet faeces in the test groups in the experiment.

On the other hand, magnesium sulphate has been reported to induce diarrhoea by increasing the volume of intestinal content through prevention of reabsorption of water. It has also been demonstrated that it promotes the

liberation of cholecystokinin from the duodenal mucosa, which increases the secretion and motility of small intestine and thereby prevents the reabsorption of sodium chloride and water[21]. The ethanolic extract of leaves of *Holoptelea integrifolia* was found to alleviate the diarrhoeic condition in this model. The extract may have increased the absorption of water and electrolyte from the gastrointestinal tract, since it delayed the gastrointestinal transit in mice as compared to the control. The delay in the gastrointestinal transit prompted by the extract might have contributed, at least to some extent, to their antidiarrhoeal activity by allowing a greater time for absorption. Traditionally to treat diarrhoea the leaves is used as water decoction for overnight. Water is a strong polar solvent considered to extract most plant secondary constituents. Previously anthraquinones, flavonoids, tannins and saponins have been reported from the methanol extract[5]. Though several constituents were present in the extract, the compound responsible for the observed actions is unknown. Flavonoids possess a wide range of activities *in vitro* including antidiarrhoeal activity[11,21,22] may have contributed to this activity, but further studies are required.

The ethanolic extract of leaves of *Holoptelea integrifolia* showed antidiarrhoeal activity in a number of models of diarrhoeic conditions in test animals. The obtained results thus give the experimental basis to understand the use of *Holoptelea integrifolia* in traditional medicine, as an antidiarrhoeal agent. However, further bioassay guided phytochemical and pharmacological studies are required to identify the active principle(s) and exact mechanism(s) of action.

Table 1. Effect of *Holoptelea integrifolia* leaves on castor oil-induced diarrhoea in mice.

Treatment	Dose (mg/kg, p.o.)	Total number of faeces in 4 h	Total number of wet faeces in 4 h
Control	–	15.40 ± 0.51	11.0 ± 1.00
Loperamide	3	2.20 ± 0.37**	1.20 ± 0.20**
Ethanolic extract	250	7.20 ± 1.06**	4.00 ± 0.44**
Ethanolic extract	500	4.0 ± 0.31**	2.60 ± 0.40**

Values are mean±S.E. (n =5). **P < 0.01 vs. control, One way ANOVA followed by Dunnett test.

Table 2. Effect of *Holoptelea integrifolia* leaves on magnesium sulphate-induced diarrhea in mice

Treatment	Dose (mg/kg, p.o.)	Total number of faeces in 4 h	Total number of wet faeces in 4 h
Control	–	12.60 ± 0.81	9.00 ± 1.41
Loperamide	3	31.80 ± 0.37**	0.80 ± 0.37**
Ethanolic extract	250	6.20 ± 1.35**	4.40 ± 0.87**
Ethanolic extract	500	4.40 ± 0.67**	2.80 ± 0.58**

Values are mean±S.E. (n =5). **P < 0.01 vs. control, One way ANOVA followed by Dunnett test.

Table 3. Effect of *Holoptelea integrifolia* leaves on the intestinal transit of charcoal meal in mice.

Treatment	Dose (mg/kg, p.o.)	% traversed by charcoal meal
Control	–	75.50 ± 2.11
Atropine sulphate	0.1 mg/kg i.p.	35.47 ± 2.50**
Ethanolic extract	250	56.81 ± 1.97**
Ethanolic extract	500	44.65 ± 1.87**

Values are mean±S.E. (n =5). **P < 0.01 vs. control, One way ANOVA followed by Dunnett test.

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