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# ANTIDIARRHEAL ACTIVITY OF THE METHANOL EXTRACT OF *LUDWIGIA HYSSOPIFOLIA* LINN.

**MOHAMMAD SHAPHIULLAH, SITESH CHANDRA BACHAR[+], JOYDEB KUMAR KUNDU, FARIDA BEGUM, M. AFTAB UDDIN*, SUVASH CHANDRA ROY** AND MOHAMMAD TAREQ HOSSAIN KHAN*****

*Department of Pharmacy, University of Dhaka, Dhaka-1000, Bangladesh*
*[*]Department of Biochemistry and Molecular Biology, University of Dhaka, Dhaka-1000*
***Department of Pharmacy, University of Science and Technology, Chittagong*
****HEJ Research Institute of Chemistry, International Centre for Chemical Sciences, University of Karachi, Karachi 75270, Pakistan*

## ABSTRACT

Dried whole plant parts of *Ludwigia hyssopifolia* were subjected to successive cold extraction with *n*-hexane, ethylacetate and methanol. The methanol extract (LHM), obtained as 1% yield, showed significant antidiarrheal property by reducing diarrheal episodes in castor oil and serotonin induced diarrhea in laboratory mice at a dose of higher than 100mg/kg body weight as compared to standard drug loperamide given at a dose of 66.67 µg/kg body weight. The percent reduction in diarrheal episode by 56.32 and 89.66 after castor oil challenge and 59.09 and 86.36 in serotonin induced diarrhea was observed at doses of 200mg/kg and 400mg/kg body weight of the extract respectively. The extract LHM was also found to reduce the gastrointestinal motility by 53.8% at a dose of 100mg/kg body weight as compared to control, while no remarkable inhibition of gastrointestinal motility was seen at a dose of 50mg/kg body weight of the extract.

## INTRODUCTION

Diarrhoeal disease is a leading cause of mortality and morbidity, especially in children in developing countries (Das *et al.,* 1999). A vast majority of the people of developing countries relies on herbal drugs for the management of diarrhoea. Considering this fact the World Health Organization has constituted a diarrhoeal disease control programme, which includes studies of traditional medicinal practices, together with the elevation of health education and prevention approaches (Das *et al.,* 1999).

In continuation of our effort to evaluate biological activities of different indigenous medicinal plants, attempt was made to investigate the antidiarrheal potential of *Ludwigia hyssopifolia* Linn. (synonym: *Jussiaea linifolia* Vahl., *Jussiaea hyssopifolia* Linn, Family – Onagraceae; Bengali name – Lalbun longa) (Huq, 1986). The plant is an annual herb that grows extensively in almost all parts of Bangladesh, India (except the Western desert region) and Ceylon (Hooker, 1973). The plant is considered as astringent, anthelmintic, carminative and diuretic. A decoction is used in diarrhoea and dysentery, flatulence, licorrhoea and spitting of blood. The leaves are used for poulticing in orchitis and glands in the neck (Ambasta, 1986). *In vitro* antibacterial and antitumor activity of the alcoholic extract of the plant was reported by Das *et al.* (2002).

[+]*Correspondence:*
E-mail scbachar@yahoo.com (Sitesh Chandra Bachar)

## MATERIALS AND METHODS

***Plant Material:***

Whole plant parts were collected at flowering stage from Dhaka and identified by Bangladesh National Herbarium, Dhaka, Bangladesh.

***Extraction and Phytochemical Screening:***

Dried coarse powder (2.0 kg) of the plant was successively extracted by cold extraction process with *n*-hexane, ethylacetate and methanol. Each of the extracts was filtered off and concentrated to dryness *in vaccuo* at 45$^{o}$C under reduced pressure.

***Preparation of Sample Solution:***

The methanol extract (100 mg) was dissolved in normal saline (4 ml) with the addition of DMSO (1 ml). The solution was then diluted with normal saline to obtain different concentrations of 10 mg/ml, *5* mg/ml and 2.5 mg/ml. Thus doses of 50, 100, 200 and 400mg/kg body weight was prepared and the standard drug loperamide was also prepared to obtain a dose of 66.67 µg/kg body weight.

***Study of Antidiarrheal Activity by Castor Oil Challenge:***

Six groups of Swiss albino mice, each group consisting of five animals, were taken for the study. Group I was kept as control providing only saline while group II, III, IV and V were considered as test groups giving the methanol extract of the plant at doses of 50, 100, 200 and 400 mg/kg body weight. Group VI was given the standard antidiarrheal drug loperamide orally at a dose of 66.67 µg/kg body weight. Extract pre-treatment was made orally 1 hour before the mice were administered with the standard dose of 1.0 ml of castor oil per oral. The animals were caged individually and examined for the occurrence of diarrhoea hourly for 5 hours after the castor oil challenge (Nwodo and Alumanah, 1991). Diarrhoea was defined as the presence of fluid material in the stool, which stained the absorbent paper placed beneath the cage. The number of diarrheal episodes in terms of drop count was measured and the cumulative number of diarrheal episode up to 5th hour of study was calculated.

***Study of the Effect of Methanol Extract of L. hyssopifolia on Serotonin (5-HT) Induced Diarrhoea*** (Yegnanarayan and Shrotri, 1982):

Swiss albino mice were screened initially by giving 600 µg/kg body weight serotonin (5-HT) sulfate orally and the animals showing diarrhoea were selected for this study. Three groups, each consisting of five animals, were taken for the study. Group I was kept as control while Group II and Group III were given the methanol extract of *L. hyssopifolia* at a dose of 200mg/kg and 400mg/kg body weight.

Extract pre-treatment was done orally 1 hour before the mice were administered with the standard dose of serotonin. The animals were caged individually and the number of diarrheal episode in terms of drop count was measured on an hourly basis. The cumulative number of diarrheal episode up to 4$^{th}$ hour of serotonin challenge was calculated.

***Gastrointestinal (GI) Motility Test with Barium Sulphate Milk:***

This experiment was carried out following the method of Chatterjee (1993) with minor modifications. Barium sulphate ($BaSO_4$ :15% in 0.5% Na-Carboxymethyl cellulose suspension) milk was given to a group of 15 mice (both treated and control) after 10 minutes of administration of the test extract intraperitoneally. The treated mice were divided into two groups and were sacrificed after 30 minutes of the administration of $BaSO_4$ milk. The distance traversed by $BaSO_4$

milk was measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileocecal junction). The movement of gastrointestinal contents i.e. percent travelled value was calculated and compared with that of the control.

## RESULTS AND DISCUSSION

The methanol extract (LHM) of *L. hyssopifolia* was obtained as 1% yield. Since the plant has folkloric use in the treatment of diarrhea, the methanol extract was evaluated for antidiarrheal activity. Phytochemical screening revealed the presence of terpenoid and alkaloid type of compounds in the extract, which was evident from the vanillin sulphuric acid treated and Dragendorff reagent treated thin layer chromatogram.

Antidiarrheal activity of LHM was investigated by castor oil challenge (1 ml/kg body weight) in the laboratory mice at a dose of 50mg/kg, 100mg/kg, 200mg/kg and 400mg/kg body weight. Result of the experiment (Table-1) showed that the extract LHM, at a dose of 50mg/kg body weight, did not exhibit any significant antidiarrheal activity. At a dose of 100mg/kg body weight, it showed almost similar effect as that of the standard drug loperamide in reducing the number of diarrheal episodes. The extract at the higher doses of 200mg/kg and 400mg/kg body weight reduced the number of stool more than that observed with the standard drug loperamide.

The appearance of fluid material in the feces was considered as diarrheal episode. The onset of diarrheal episode was rapid in control group (3.00 ± 0.32 min) and in the group treated with LHM at a dose of 50mg/kg body weight (4.20 ± 0.88 min). The onset of diarrheal episode was found to be delayed in the test groups giving the extract at a dose of 100mg/kg, 200mg/kg and 400mg/kg body weight as 73.0 ± 2.14, 104.20 ± 2.44, 140.80 ± 3.08 minutes respectively. The standard drug loperamide showed the onset of diarrheal episode (66.60 ± 2.94 min), which was less than that observed with 100mg/kg, 200mg/kg and 400mg/kg dose of the extract. The cumulative number of diarrheal episode after $5^{th}$ hour of study was found to be 17.40, 16.40, 13.20, 7.60, 1.80 and 11.00 for control, 50mg/kg, 100mg/kg, 200mg/kg, 400mg/kg dose of the extract and loperamide respectively. The percent inhibition of cumulative number of diarrheal stool was found to be 5.75, 24.14, 56.32, 89.66 and 36.78 by the extract at doses of 50mg/kg, 100mg/kg, 200mg/kg, 400mg/kg and the standard drug loperamide respectively. The result indicated that the methanol extract of *L. hyssopifolia* was highly effective in controlling castor oil induced diarrhea at a dose of 200mg/kg and 400mg/kg body weight, but was moderately effective at a dose of 100mg/kg body weight. The extract was found to be ineffective against castor oil induced diarrhea at a dose of 50mg/kg body weight.

The LHM was also evaluated for its antidiarrheal activity in serotonin-induced diarrhea in mice. The extract was given at a dose of 200mg/kg and 400mg/kg body weight. The extract, at both the dose levels, exhibited statistically significant ($p<0.001$) reduction in number of diarrheal episodes in comparison to control. The dropping counts at different hours of study indicated the good antidiarrheal activity of the extract at both the doses (Table-2). After the $4^{th}$ hour of study the cumulative number of diarrheal episodes were found to be 4.4, 1.8 and 0.6 for control, 200mg/kg dose and 400mg/kg dose of the extract respectively.

The effects of LHM on the gastrointestinal motility were then studied in laboratory mice. Result of this experiment (Fig. 1) showed that the barium sulphate milk traversed 75.54% of the intestinal lumen after 30 minute in the control group. The propagation of gastrointestinal contents was found to be 71.87%, 34.92% and 23.60% of the intestinal lumen after 30 minutes of the

administration of LHM at a dose of 50mg/kg, 100mg/kg body weight and the standard drug loperamide at a dose of 66.67 µg/kg body weight respectively. This result indicated that the extract at dose of 100mg/kg body weight reduced the gastrointestinal motility by 53.8% in comparison to control, which was comparable to that of the standard drug loperamide that caused a reduction in motility by 68.8% in comparison to control. However the extract at dose of 50mg/kg did not show any inhibitory effect on the propulsion of gastrointestinal contents.

Although the extract was found to reduce castor oil and serotonin induced diarrheal episodes but the mechanism of its antidiarrheal activity is uncertain. Since castor oil produces diarrhea by preventing fluid and electrolyte absorption and thus resulting in an increase in intestinal peristalsis (Goodman and Gillman, 1996), one of the probable mechanism of antidiarrheal activity of the test extract LHM may be its ability to enhance fluid and electrolyte absorption through the gastrointestinal tract. As cholinergic stimulation often cause diarrhea by increasing GI motility (Mycek *et al.,* 1997), the significant inhibition of GI motility by the extract LHM suggested its probable mode of action to be the prevention of cholinergic transmission or its anticholinergic effect on gastric mucosa. Besides these possibilities, the extract under investigation may contain certain components having affinity to µ (mu) receptor, which is an opioid receptor located on the GI mucosa and relieves diarrhea when activated by an agonist (Goodman and Gillman, 1996). Thus further phytochemical studies are required to isolate antidiarrheal component(s) from the extract to establish its exact mode of antidiarrheal activity.

**Table 1**
Effect of methanol extract (LHM) of L. *hyssopifolia* on castor oil induced diarrhea after 5 hr of administration

| Animal Group | Dose (per kg body weight) | Onset of diarrhea Mean ± SE (min) | Cumulative number of diarrheal episode Mean ± SE | % Inhibition of diarrheal episode |
|---|---|---|---|---|
| Control | Saline | 3.00 ± 0.32 | 17.40 ± 2.43 | -- |
| LHM | 50mg | 4.20 ± 0.88 | 16.40 ± 1.15 | 5.75 |
| LHM | 100mg | 73.0 ± 2.14 | 13.20 ± 0.87 | 24.14 |
| LHM | 200mg | 104.20 ± 2.44 | 7.60 ± 1.80* | 56.32 |
| LHM | 400mg | 140.80 ± 3.08 | 1.80 ± 0.72* | 89.66 |
| Loperamide | 66.67 µg | 66.60 ± 2.94 | 11.00 ± 0.94* | 36.78 |

*P<0.001

**Table 2**
Effect of methanol extract (LHM) of *L. hyssopifolia* on serotonin induced diarrhea after 4 hr of administration

| Animal Group | Dose (mg/kg body weight) | Cumulative number of diarrheal episode Mean ± SE | % Inhibition of diarrheal episode |
|---|---|---|---|
| Control | Saline | 4.40 ± 0.46 | -- |
| LHM | 200mg | 1.80 ± 0.59* | 59.09 |
| LHM | 400mg | 0.60 ± 0.22* | 86.36 |

*P<0.001

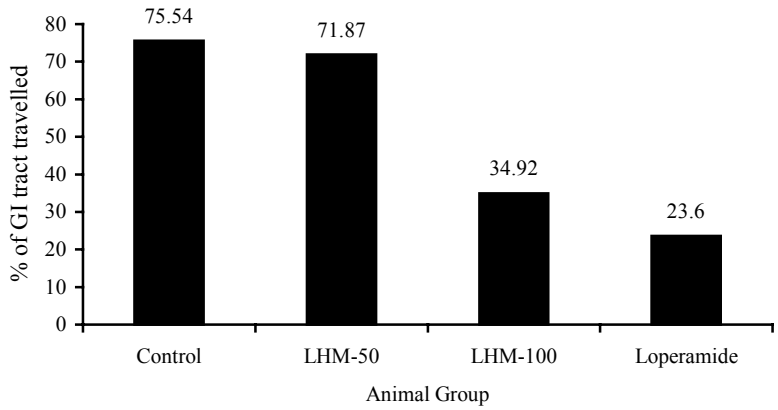


Fig. 1: Effect of methanol extract of *L. hyssopifolia* on gastrointestinal motility.

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