Analgesic and Anti-diarrhoeal Activities of *Lagerstroemia speciosa* Roots in Experimental Animal Model

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ABSTRACT: The methanolic crude extract of *Lagerstroemia speciosa* roots was investigated for its possible analgesic and anti-diarrhoeal activities in experimental animal model. Phytochemical screening of the methanolic extract of *L. speciosa* roots showed the presence of alkaloids, flavonoids, saponins, tannins and reducing sugar. In acute toxicity study, no mortality or toxic reaction was recorded in animal model after administration of the *L. speciosa* roots extract. Analgesic activity was evaluated by using acetic acid induced writhing inhibition method in Swiss albino mice. In peripheral method of anti-nociception, the methanolic crude extract of *L. speciosa* roots showed significant analgesic activity. At the dose of 200 and 400 mg/kg body weight, the extract produced 35.38% and 53.85% ($P<0.001$) of writhing inhibition, respectively compared to standard diclofenac sodium (70.77% inhibition). The extract had also anti-diarrhoeal activity in castor oil induced method and inhibited the mean number of defecation by 32.75% ($P<0.01$) and 51.72 % ($P<0.001$) at the dose of 200 and 400 mg/kg body weight, respectively. The latent period for the extract treated group also increased significantly.

Key words: *Lagerstroemia speciosa*, analgesic, writhing, anti-diarrhoeal, castor oil, Swiss albino mice

INTRODUCTION

Pain is an important symptom that brings the patient to physician. Analgesics relieve pain as a symptom, without affecting its cause.[1] Diarrhoeal disease is a leading cause of mortality and morbidity, especially in children in developing countries.[2] Majority of the people of developing countries rely on herbal drugs for the management of diarrhea. Considering the fact, the World Health Organization has constituted a diarrhoeal disease control programme, which includes studies of practices of traditional medicine, together with the elevation of health education and prevention approaches.[2]

Lagerstroemia speciosa (L.) Pers. (Bengali name: Jarul or Banaba; Family: Lythraceae) is a deciduous or semi-deciduous small to medium sized or rarely large tree up to 40-45 meter tall. It is found all over the tropical and subtropical regions including Bangladesh, India, Malaysia, Thailand, Philippines, Indonesia and Japan.[3] Traditionally the plant is called natural plant insulin due to its remarkable anti-diabetic effects without any noticeable side effect.[3] Almost every part of the plant such as (bark, flower, fruit, leaf, root) has several important biological properties. Leaf decoction or infusion is used for bladder and kidney inflammation, dysuria and other urinary dysfunctions.[4] Leaves are also used to make slimming tea and for cholesterol deduction, hypertension and diabetes.[4] The bark part of the plant *L. speciosa* has local use for the treatment of diarrhea.[4] Root of this plant has been used for a variety of stomach ailments. Some chemical constituents like gallic acid, 4-hydroxybenzoic acid, 3-*O*-methylprotocatechuic acid, caffeic acid, *p*-coumaric acid, kaempferol, quercetin, and isoquercitrin, have been isolated from *L. speciosa* roots. Six pentacyclic triterpene acids (oleanolic acid,

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arjunolic acid, asiatic acid, maslinic acid, corosolic acid and 23-hydroxyursolic acid) were isolated from *L. speciosa* leaves.[5] The plant also contains flavonoids and saponins in a remarkable amount among other phytoconstituents which can act against pain and diarrhea. As the roots of the plant are widely available and only a limited study was conducted on roots of this plant, our present work was undertaken to validate the analgesic and anti-diarrhoeal potential of the methanolic crude extracts of *L. speciosa* using acetic acid- induced writhing inhibition and castor oil- induced diarrhea method, respectively in swiss albino mice for the first time.

MATERIALS AND METHODS

Collection and preparation of plant material. Fresh roots of the plant *Lagerstroemia speciosa* were collected from Kalia, Narail, Bangladesh in September, 2011. The roots of *L. speciosa* were identified and authenticated by taxonomist in the National Herbarium, Mirpur, Dhaka where a voucher specimen (accession no: 38212) has been deposited for future reference. Roots of *L. speciosa* were washed properly, cut into small pieces and then air dried for several days. The pieces were oven dried for 24 hours at considerably low temperature to facilitate grinding. The pieces were then ground into coarse powder by using a high capacity grinding machine.

Extraction of the plant material. About 700 g of the powdered material was taken in a clean, round bottomed flask (5 liters) and soaked in 2.5 liter of methanol. The container with its content was sealed with aluminium foil and kept for 7 days accompanying occasional shaking and stirring. The whole mixtures were then filtered through a fresh cotton plug and finally with a Whatman Number 1 filter paper. The volume of the filtrate was then reduced with a Büchi rotary evaporator at low temperature and pressure. The weight of the crude extract was 23 g.

Drugs and reagents. Methanol, acetic acid, tween 80 (Sigma chemicals, USA), diclofenac sodium (ACI Pharmaceuticals Ltd, Dhaka), loperamide (Square Pharmaceuticals Ltd, Dhaka), normal saline (Opsonin Pharaceutical Ltd, Dhaka) were collected from the mentioned sources. Highly pure castor oil was collected from local market. All the chemicals and solvents were of analytical grade.

Experimental animals. Swiss albino mice (25-30 g) of either sex, aged 4-5 weeks and Wistar rats (125-150 g) were obtained from Department of Pharmacy, Jahangirnagar University. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 2ºC; relative humidity 60-70%) in a 12 hour light-dark cycle in the animal house of Institution of Nutrition and Food Science, University of Dhaka and fed with ICDDR,B formulated rodent food and water *ad libitum.* As these animals are very sensitive to environmental changes, they were kept before the test for 3-4 days in the environment where the experiment was conducted.

Phytochemical screening. The freshly prepared crude methanolic extracts of roots of *L. speciosa* were qualitatively tested for the presence of alkaloids, phenols, tannins, reducing sugar, flavonoids, steroids and saponins by using standard procedures. [6, 7]

Acute toxicity test. The acute toxicity for methanolic extract of the roots of *L. speciosa* was determined in rats according to the method of Hilaly[8] with slight modifications. Rats fasted for 16 hour were randomly divided into groups of five rats per group. Graded doses of the extract (200, 400, 800, 1600 and 3200 mg/kg p.o.) were separately administered to the rats in each of the group by means of bulbed steel needle. All rats were then allowed free access to food and water and observed over a period of 48 hour for signs of acute toxicity. The number of deaths within this period was recorded.

Analgesic activity study

Peripheral analgesic activity study. The peripheral analgesic activity of the crude methanolic extracts of *L. speciosa* was determined by the acetic acid- induced writhing inhibition method according to procedure described by Koster and Turner.[9,10]

Twenty Swiss albino mice were divided into four groups consisting of five animals in each. Each group received particular treatment as shown in Table 2. Then the response of the extract and diclofenac sodium treated groups was compared with those of the animals in the control group. Percentage inhibition of writhing in comparison to control group was taken as an index of analgesia and was calculated using the following formula:

Inhibition (%) = [(Wc-Wt) × 100]/Wc

Where Wc is the average number of writhing reflex in the control group and Wt is the average number of writhing reflex in the test group.

Anti-diarrhoeal activity study. The method, described by Shoba and Thomas[11] was followed for this study with slight modification. 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 10 ml/kg b.w. orally. The positive control group received loperamide at the dose of 50 mg/kg b.w. orally; test groups received the methanol extract 200 and 400 mg/kg b.w. Thirty minutes intervals were given to ensure proper absorption of the administered substances. Then 1 ml of castor oil was administered to each mouse for inducing diarrhea. Each animal was placed in an individual cage, the floor of which was lined with blotting paper. Each of the mice was observed for four hours. The floor lining was changed every hour. Each time a mouse had given stool was recorded. The average of total number of stool given by the test group and the average of total number of stool given by the control group was compared. Percent inhibition of defecation in mice was calculated by using the following equation:

% inhibition = {(Mo–M)/Mo} × 100; where, Mo = Mean defecation of control and M = Mean defecation of test sample.

Statistical analysis. All values were expressed as the mean ± standard error of the mean (SEM) and the results were analyzed statistically by one way analysis of variance (ANOVA) followed by Dunnett's't'-test by using SPSS ver.19. $P < 0.05$ was considered to be statistically significant.

RESULTS AND DISCUSSION

Phytochemical screening. Chemical group test results of the methanolic extract of *L. speciosa* roots showed the presence of alkaloids, flavonoids, tannins, saponins and reducing sugars (Table 1).

Acute toxicity test. In acute toxicity study, oral administration of graded doses (200, 400, 800, 1600 and 3200 mg/kg, b.w., p.o.) of the methanol extract of *L. speciosa* to rats showed no significant changes in behavior, breathing, cutaneous effects, sensory nervous system responses or gastrointestinal effects during the observation period. No mortality or any toxic reaction was recorded in any group within 48 hour after administration. Thus *L. speciosa* was safe up to a dose level of 3200 mg/kg of body weight.

Table 1. Results of different chemical group tests of the methanolic extract of *L. speciosa* roots.

Plant extract	Alkaloid	Reducing sugar	Tannins (Phenolic compounds)	Flavonoids	Saponins	Glycoside
MCE	+	+	+	+	+	-

MCE: Methanolic crude extract of *L. speciosa*; +: positive result; - : negative result

Peripheral anti-nociceptive activity study. The peripheral anti-nociceptive effects of methanolic crude extract of the roots of *L. speciosa* in acetic acid- induced writhing inhibition method are shown in Table 2. The extracts effectively reduced the number of abdominal muscle contractions induced by 0.6% acetic acid solution in a dose dependent manner. The methanolic crude extract at the dose of 200 and 400 mg/kg b.w. showed significant anti-nociceptive activity having 35.38% and 53.85% ($P<0.001$) of writhing inhibition, respectively compared to that exhibited by standard diclofenac (70.77% inhibition).

Anti-diarrheal activity study. The result of methanolic extract of *L. speciosa* roots on castor oil-induced diarrhea is shown in Table 3. Here, the methanolic extract at the dose of 200 and 400mg/kg b.w. exhibited reduction of diarrhoeal feces by 32.75% and 51.72% (P<0.001), respectively compared to the reduction obtained by the standard drug loperamide (58.62% feces reduction).

In this study, anti-nociceptive activity was evaluated peripherally by acetic acid-induced writhing inhibition method. This method is a simple and reliable method and is widely used to screen and study compounds for peripheral type analgesic action.[12,13] In this experiment, the crude methanolic extract of *L. speciosa* roots significantly (P<0.001) lowered the number of writhing compared to standard diclofenac sodium.

Table 2. Peripheral anti-nociceptive activity of methanolic crude extract of *L. speciosa* roots.

Animal Group	Writhing count					Number of writhing (Mean ± SEM)	% of Inhibition of writhing
	M_1	M_2	M_3	M_4	M_5		
NC	27	25	28	24	26	26 ± 0.7	-
PC_{50}	7	8	7	10	6	7.6 ± 0.67	70.77***
MCE_{200}	17	19	17	15	16	16.8 ± 0.66	35.38***
MCE_{400}	14	12	10	13	11	12 ± 0.70	53.85***

Values expressed as Mean ± SEM (n=5). ***P<.001, **P<.01, *P<.05 significant compared to control. NC = negative control, PC = positive control (Diclofenac sodium), MCE_{200} = Methanolic crude extract of *L. speciosa* roots at 200 mg/kg body weight, MCE_{400} = Methanolic crude extract of *L. speciosa* roots at 400 mg/kg body weight. M_1, M_2, M_3, M_4 and M_5 = Mice 1, Mice 2, Mice 3, Mice 4 and Mice 5.

Table 3. Effect of methanol extract of *L. speciosa* roots on castor oil (1ml/mice) induced diarrhea in mice.

Treatment	Dose	Number of diarrheal feces (Mean ± SEM)	% Reduction of diarrhea
Control (Saline)	10 ml/kg b.w.	11.6 ± 0.50	---
Standard (Loperamide)	50 mg/kg b.w.	4.8 ± 0.37	58.62***
MCE_{200}	200 mg/kg b.w.	7.8 ± 0.58	32.75**
MCE_{400}	400 mg/kg b.w.	5.6 ± 0.50	51.72***

Values are expressed as Mean ± SEM (n=5). ***P<0.001, **P< 0.01, *P< 0.05 compared to control (One way ANOVA followed by Dunnett's test). Standard = (Loperamide) 50mg/kg, MCE_{200} = Methanolic crude extract of *L. speciosa* roots at 200 mg/kg body weight, MCE_{400} = Methanolic crude extract of *L. speciosa* roots at 400 mg/kg body weight.

In evaluation of anti-diarrhoeal activity by castor oil method, methanolic crude extract dose-dependently reduced diarrhoeal feces which was comparable to that obtained by the standard loperamide. Castor oil causes diarrhea due to its active metabolite, ricinolic acid, which stimulates peristaltic activity in the small intestine, leading to changes in electrolyte permeability of the intestinal mucosa.[14,15] The liberated ricinoleic acid also causes irritation and inflammation of the intestinal mucosa leading to the release of endogenous prostaglandins.[16] Several other mechanism have been reported to cause diarrhea by castor oil inducing inhibition of intestinal Na^+/K^+-ATPase activity, activation of adenylate cyclase or mucosal cAMP- mediated active secretion and platelet activating factor.[17] Diarrhea also results from an active intestinal secretion driven predominantly by net secretion of sodium and potassium. Therefore, the decrease in the wetness of feces and the frequency of defecation observed with the extract prove the potent anti-diarrhoeal activity of *L. speciosa* roots and this effect might be due to inhibition of prostaglandins biosynthesis.

The analgesic and anti-diarrheal activities of many plants have been attributed to their saponin,[18] terpenoids, flavonoids and steroids contents.[19] Moreover, the flavoloids, triterpenoids are known to inhibit prostaglandin systhesis.[20] The phytochemical investigation revealed that *L. speciosa* possessed the maximum phytochemicals like alkaloids, flavonoids,

saponins, reducing sugars, tannins and phenolic compounds. It is not unreasonable, therefore to speculate that the flavonoids, saponins, tannins present in the plant extract may be responsible for the observed analgesic and anti-diarrhoeal effects.

CONCLUSION

In conclusion, the overall results of the present study indicate the analgesic and anti-diarrhoeal activities of the roots of *L. speciosa* which deserves further investigation to isolate the active constituents responsible for these activities and to establish the mechanisms of action. The results of the present study provided a scientific support for the use of *L. speciosa* in the treatment of pain and diarrhea related disorders in ethnomedicine.

Declaration of interest. The authors declare no conflict of interest.

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