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STUDY OF ANTIDIARRHOEAL ACTIVITY OF TWO MEDICINAL PLANTS OF BANGLADESH IN CASTOR-OIL INDUCED DIARRHOEA

Pritesh Ranjan Dash [1], Mahmuda Nasrin [2], Sheikh Zahir Raihan [3] and Mohammad Shawkat Ali [* 1]

Department of Pharmacy [1], BRAC University, Dhaka, Bangladesh.
Department of Pharmacy [2], Jahangirnagar University, Savar, Dhaka, Bangladesh.
Institute of Molecular [3], Cell and System Biology, University of Glasgow, Scotland, United Kingdom.

Keywords:

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Correspondence to Author:
Mohammad Shawkat Ali

Chairperson and Professor,
Department of Pharmacy,
BRAC University, 41, Pacific Tower,
Mohakhali, Dhaka, Bangladesh.

E-mail: shawkat.ali@bracu.ac.bd

ABSTRACT: *Kaempferia galanga* (Family: Zingiberaceae) has immense importance in the traditional health care system as a carminative, cholera, anti-inflammatory, abdominal pain, dyspepsia, and stomachic as well as in the diseases of coughs, pectoral affections, and stoppage of the nasal blocks. *Grewia paniculata* (Family: Malvaceae) has been used in traditional medicine for the treatment of indigestion, eczema, itch, small-pox, typhoid fever, dysentery and syphilitic ulceration of the mouth. Leaves of this plant used along with turmeric and shell of snail for the treatment of jaundice. This study was aimed to investigate the antidiarrhoeal activity of the acetonic extract of *Kaempferia galanga* and ethanolic extract of *Grewia paniculata*. The acetone extract of rhizome (ACR), as well acetone extract of the leaf (ACL), ethanol extract of bark (EEB) and ethanol extract of the leaf (EEL) were subjected to antidiarrhoeal activity test. The antidiarrhoeal activity was performed by castor oil-induced diarrhea in mice. In this study, both plant extracts exhibited significant inhibition ($p < 0.05$-0.001) and a dose-dependent decrease in the total number of faecal dropping in castor oil induced diarrhea in mice. Maximum 80.00% and 77.36% inhibition of defecation were observed with ACL (200 mg/kg) and EEL (500 mg/kg) where the standard drug Loperamide (3mg/kg) showed 54.64% inhibition of defecation. The results suggest that both the plant extracts possess pronounced antidiarrhoeal activity. This study validates the use of these plants in traditional medicine.

INTRODUCTION: Diarrhoea is the passage of stools more than three times in an hour. It occurs due to an imbalance in the absorption and secretory mechanisms in the intestinal mucosa, which results in an increase in fluid and electrolyte loss into the gut lumen, leading to the production of unformed liquid faeces.

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However, malnourished individual's diarrhea can lead to severe dehydration and can become life-threatening if not treated. Three major diarrhoea syndromes exist: they are acute watery diarrhea, which results in varying degrees of dehydration; persistent diarrhoea, which lasts 14 days or longer, manifested by malabsorption, nutrient losses, and wasting; and bloody diarrhoea, which is a sign of the intestinal damage caused by an infectious agent, drugs, poisons (including bacterial toxins) or acute inflammatory reactions [1].

All three are physiologically different and require specific management.

Diarrhea is one of the leading causes of mortality and morbidity in developing countries, especially in children under five years. It is most commonly caused by gastrointestinal infections, which kill around 1.8 million people globally each year [2]. Medicinal plants have been used as traditional treatments for numerous human diseases for thousands of years in developing countries; the majority of people almost exclusively use traditional medicines in treating all sorts of diseases, including diarrhea. Antibiotics are the major remedy of infectious diseases, including diarrhea; however, a significant increase in antibiotics resistance has been observed in common human pathogens worldwide. Similarly, oral rehydration therapy (ORT) has been widely identified as a key factor in the decline of child mortality due to diarrhea. However, the attack rate of the disease has remained unchanged, and this treatment often fails in the high stool output state. For this reason, WHO has encouraged scientific studies for the treatment and prevention of diarrhoeal diseases based on traditional medical practices [1, 2].

Kaempferia galanga (Chandramulika in Bengali) belonging to the family Zingiberaceae is an aromatic perennial herb with tuberous rootstocks. This herb is possibly native only to India, where it is widespread. It is cultivated throughout Southeast Asia, including Southern China, Taiwan, Cambodia, in Malaysia east to the Moluccas, possibly also introduced in Northern Australia. In Bangladesh, the plant is specially grown in the forest floor of the Chittagong Hill Tracts and Sal forest of Dhaka and Sherpur and grown in limited scale at different parts of Mymensingh [3]. The rhizomes of this plant contain volatile oil and other important compounds of enormous medicinal values; they are very demanding to the traditional health care practitioner. The major chemical constituents of the volatile oil from the dried rhizome of *Kaempferia galanga* are ethyl-*p*-methoxycinnamate (31.77%), methyl cinnamate (23.23%), carvone (11.13%), eucalyptol (9.59%) and pentadecane (6.41%), respectively. Other constituents of the rhizome include cineol, borneol, 3-carene, camphene, kaempferol, cinnamaldehyde, *p*-methoxycinnamic acid, and ethyl cinnamate. A methanolic extract of the rhizome contains ethyl *p*-methoxy-*trans*-cinnamate, which is highly cytotoxic to HeLa cells [4]. The rhizome has immense importance in the traditional health care system as a carminative, cholera, anti-inflammatory, abdominal pain, dyspepsia, and stomachic as well as in the diseases of coughs, pectoral affections, and stoppage of the nasal blocks. Its rhizome juice is applied by the chakma of Rangamati district for toothache [3]. The plant has anti-inflammatory, analgesic, antioxidant, nematicidal, mosquito repellent, larvicidal, antiallergic, sedative, wound healing, vasorelaxant, antineoplastic and cytotoxic properties. It also has antimicrobial activities [5].

Grewia paniculata Linn. (Kathgua and Fattashi in Bengali) belonging to the family of Malvaceae is a large shrub or small tree, with ovate-lanceolate leaves, yellowish-white flowers, and purplish-red fruits. It is widely distributed and naturally grown throughout Bangladesh. It is also native and distributed more or less throughout India, Sri Lanka, China, Cambodia, Myanmar, Thailand, Vietnam, Indonesia, and Malaysia. Guangdong, Guangxi, Hainan, Laos, Philippines. In traditional medicine, the plant is used in the treatment of indigestion, eczema, itch, small-pox, typhoid fever, dysentery and syphilitic ulceration of the mouth. Tripura in the Chittagong Hill Tracts used leaves of this plant along with turmeric and shell of snail for the treatment of jaundice. Pharmacological properties such as analgesic and cytotoxic activities of ethanolic leaf extract, antidiarrhoeal activities of leaves have been reported. The stem bark contained a new alkaloid, N-Methyl- 6 beta-(deca-1', 3', 5'-trienyl)- 3beta- methoxy- 2 beta-methylpiperidine, which showed good insecticidal activity against *Aedes aegypti* second instar larvae [6]. Another study claims that two new piperidine alkaloids, micro cosamines A (1) and B (2), were isolated from the leaves. Their structures were elucidated by spectroscopic analysis. Both new compounds showed significant larvicidal activity against *Culex quinquefasciatus* [7]. Given this, there is a need to search for these plants with antidiarrhoeal activity.

MATERIALS AND METHOD:

Chemicals and Drugs: Castor oil and Loperamide were purchased from local manufacturers.

Collection of the Plant: The plant of *Kaempferia galanga* was collected from the local area of

Mauoa, Dhaka during December 2011 and *Grewia paniculata* was collected from Gazipur, Dhaka during September 2011. Dust, dirt, and undesirable materials were then separated manually. The collected plants were then identified by the taxonomist of Bangladesh National Herbarium, Mirpur, Dhaka, and a voucher specimen has been deposited (DACB: 36,064, *Kaempferia galanga* and DACB: 35,942, *Grewia paniculata*) for further reference.

Extraction of the Plant Material: The plant parts were extracted by a cold extraction method. The rhizome (900 g) and leaf (200 g) of *Kaempferia galanga* powder were taken and soaked with 2700 ml and 600 ml of acetone for 3 consecutive days at 25°C. The extracts were filtered and evaporated on a rotary evaporator under reduced pressure. The recovered solvent was again used for percolation for another 3 days. The process was repeated three times to obtained 58 g rhizome (yield 6.45%) and 4.14 g leaf (yield 2.07%) extract of *Kaempferia galanga*. In the same way, the powder of *Grewia paniculata* bark (1000 g) and leaf (400 g) was soaked with 3000 ml and 900 ml of ethanol to obtained 132 g bark (yield 13.2%) and 16.94 g leaf (yield 4.24%) extract.

Animals: For the experiment *Swiss albino* mice of either sex, 4-5 weeks of age, weighing between 25-30 gm, were collected from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). Animals were maintained under standard environmental conditions (temperature: (24.0 ± 1.0º), relative humidity: 55-65% and 12 h light/12 hr dark cycle) and had free access to feed and water *ad libitum*. The animals were acclimatized to laboratory condition for two weeks before experimentation [8]. The University Animal Research Ethical Committee approved the experimental protocol.

Experimental Groups: The animals were divided into control, standard, and test groups containing six mice of each.

Antidiarrhoeal Activity:

Castor Oil Induced Diarrhea: Experimental animals were randomly selected and divided into four groups denoted as control, standard, and test samples (group-I and group-II) and consisting of 6 mice in each group. This experiment described by Shoba and Thomas [9, 10]. Mice fasted for 18h before the test with free access to water. Control (water 5ml/kg), standard (Loperamide 3mg/kg) and test samples such as *Kaempferia galanga* (100 and 200 mg/kg) and *Grewia paniculata* (250 and 500 mg/kg) were administered orally. Then 1 h later, 0.3ml castor oil was administered orally to each mouse to induce diarrhea. Each animal was placed in an individual cage, the floor of which was lined with white blotting paper. The papers were changed every hour. The total numbers of both dry and wet faeces excreted by the animals were counted every hour for a period of 4 h. The total number of diarrhoeal faeces of the control group was considered 100%. Percentage of inhibition of defecation was calculated using the following formula:

Inhibition of defecation (%) = Mean No. of defecation by castor oil - Mean No. of defecation by drug or extract × 100 / Mean No. of defecation caused by castor oil

Statistical Analysis: The statistical analysis for the animal experiment was carried out using one-way ANOVA followed by Dunnett's multiple comparisons. The results obtained were compared with the control group. P<0.05- 0.001 were considered to be statistically significant.

RESULTS: In the castor oil-induced diarrhea experiment, the mice group that did not receive the plant extracts showed typical diarrhoeal signs and symptoms such as watery and frequent defecation. Both the effects of *Kaempferia galanga* and *Grewia paniculata* were found to be statistically significant (p<0.05-0.001) **Table 1, 2** and **Fig. 1, 2**. Maximum 80.00% and 73.36% inhibition of defecation were exhibited with the ACL and ACR extracts (200 mg/kg) respectively. On the other hand, ACL and ACR extracts (100 mg/kg) were produced 74.64% and 62.64% inhibition of defecation, respectively. The effects of the ethanol extracts of *Grewia paniculata* on castor oil induced diarrhea **Table 2** revealed that the extracts also decreased the number of faecal matter pass by the animals. The extracts (250 and 500 mg/kg) showed a significant (P<0.05- 0.001) reduction in diarrhoea representing 69.36%, 74.64% and 69.36%, 77.36% inhibition respectively. In this study, the standard drug Loperamide (3 mg/kg) produced 54.64% inhibition of defecation.

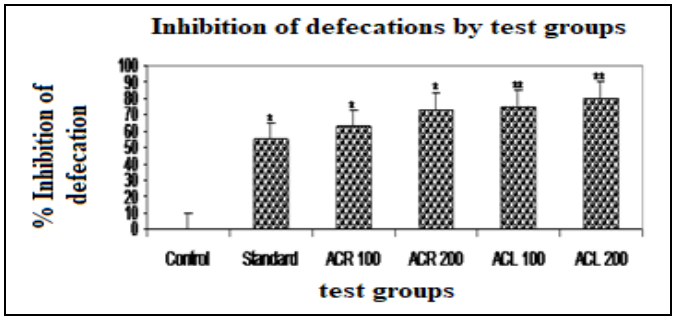

FIG. 1: EFFECTS OF THE RHIZOME AND LEAF EXTRACTS OF *KAEMPFERIA GALANGA* ON CASTOR OIL-INDUCED DIARRHOEA IN MICE. Control group received water 5 ml/kg (p.o.), standard group received Loperamide 3mg/kg body weight (p.o.), test groups ACR and ACL were treated with 100 and 200 mg/kg body weight of the extracts (p.o.) respectively. Values are mean ±SEM, (n=6); * $p < 0.05$, ** $p < 0.001$, *Dunnett t-test* as compared to control. ACR = Acetone extract of rhizome and ACL = Acetone extract of leaf

TABLE 1: EFFECTS OF THE RHIZOME AND LEAF EXTRACTS OF *KAEMPFERIA GALANGA* ON CASTOR OIL-INDUCED DIARRHOEA IN MICE

Group	Dose (mg/kg)	No. of faeces in 4 h	% Inhibition of defecation
Control	5 ml/kg	12.5 ± 1.56	---
Standard	3	5.67 ± 1.40*	54.64
ACR	100	4.67 ± 1.40*	62.64
	200	3.33 ± 2.18*	73.36
ACL	100	3.17 ± 0.79**	74.64
	200	2.5 ± 1.71**	80.00

Control group received water 5 ml/kg (p.o.), standard group received Loperamide 3mg/kg body weight (p.o.), test groups ACR and ACL were treated with 100 and 200 mg/kg body weight of the extracts (p.o.) respectively. Values are mean ±SEM, (n=6); * $p<0.05$, ** $p<0.001$, *Dunnett t-test* as compared to control. ACR=Acetone extract of rhizome and ACL=Acetone extract of leaf.

TABLE 2: EFFECTS OF THE BARK AND LEAF EXTRACTS OF *GREWIA PANICULATA* ON CASTOR OIL-INDUCED DIARRHOEA IN MICE

Group	Dose (mg/kg)	No. of faeces in 4 h	% Inhibition of defecation
Control	5 ml/kg	12.5 ± 1.56	---
Standard	3	5.67 ± 1.40*	54.64
EEB	250	3.83 ± 1.64*	69.36
	500	3.17 ± 1.90*	74.64
EEL	250	3.83 ± 1.04**	69.36
	500	2.83 ± 1.27**	77.36

Control group received water 5 ml/kg (p.o.), the standard group received Loperamide 3mg/kg body weight (p.o.), test groups EEB and EEL were treated with 250 and 500 mg/kg body weight of the extracts (p.o.) respectively. Values are mean ±SEM, (n=6); * $p<0.05$, ** $p<0.001$, *Dunnett t-test* as compared to control. EEB=Ethanol extract of bark and EEL= Ethanol extract of the leaf.

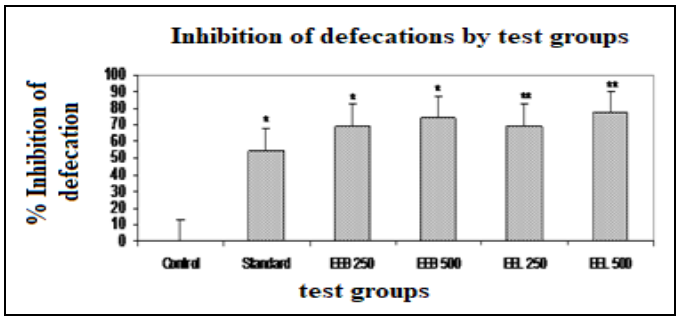

FIG. 2: EFFECTS OF THE BARK AND LEAF EXTRACTS OF *GREWIA PANICULATA* ON CASTOR OIL-INDUCED DIARRHOEA IN MICE. Control group received water 5 ml/kg (p.o.), the standard group received Loperamide 3mg/kg body weight (p.o.), test groups EEB and EEL were treated with 250 and 500 mg/kg body weight of the extracts (p.o.) respectively. Values are mean ±SEM, (n=6); * $p<0.05$, ** $p<0.001$, *Dunnett t-test* as compared to control. EEB = Ethanol extract of bark and EEL = Ethanol extract of the leaf.

Maximum 80.00% and 73.36% inhibition of defecation were exhibited with the ACL and ACR extracts (200 mg/kg) respectively. On the other hand, ACL and ACR extracts (100 mg/kg) were produced 74.64% and 62.64% inhibition of defecation, respectively. The effects of the ethanol extracts of *Grewia paniculata* on castor oil induced diarrhea **Table 2** revealed that the extracts also decreased the number of faecal matter pass by the animals. The extracts (250 and 500 mg/kg) showed a significant (P<0.05- 0.001) reduction in diarrhoea representing 69.36%, 74.64% and 69.36%, 77.36% inhibition respectively.

In this study, the standard drug Loperamide (3 mg/kg) produced 54.64% inhibition of defecation.

DISCUSSION: Castor oil induced diarrhea model is widely used for the evaluation of anti-diarrhoeal property of drugs. The most active component of the oil is ricinoleic acid. Ricinoleic acid causes irritation and inflammation of the intestinal mucosa. The irritation stimulates the peristaltic activity of the small intestine, causing changes in the electrolytic permeability of the intestinal mucosa. This sequence of events leads to the release of prostaglandins, which stimulates motility and secretion, thereby decreases the absorption of sodium and potassium ions [1]. In this study, both the extracts of *Kaempferia galanga* and *Grewia paniculata* were found to inhibit the severity of diarrhoea induced by castor oil **Table 1, 2** and **Fig. 1, 2**. It is possible that the extracts were able to inhibit electrolyte permeability to the intestine due to castor oil and through the inhibition of prostaglandin release. Suppression of the intestinal fluid accumulation by the extracts might also suggest inhibition of gastrointestinal functions [1]. Membrane bound enzyme Na^+ and K^+ ATP_{ase} has been related to sodium and potassium transport in the intestine. When a decrease in Na^+ and K^+ ATP_{ase} in diarrhoeal conditions relates to an interruption in the normal water and electrolyte absorption, diarrhoea results. Therefore the decrease of water together with Na^+ accumulation might affect the activity of Na^+ and K^+ ATP_{ase}. The stimulated fluid, Na^+ and K^+ secretion induced by the castor oil were inhibited by the extracts in a dose dependent manner. Previous studies showed that antidysenteric and antidiarrhoeal properties of medicinal plants were mostly due to tannins, alkaloids, saponins, flavonoids, sterol and triterpenes [1]. The antidiarrhoeal property of the extract of *Kaempferia galanga* and *Grewia paniculata* found in the present study could be owing to the presence of tannins, alkaloids, steroids, resins, proteins in this plant.

CONCLUSION: In the present study, both *Kaempferia galanga* and *Grewia paniculata* extracts possessed significant antidiarrhoeal properties, thus supports the traditional use of *Kaempferia galanga* and *Grewia paniculata* in the treatment of diarrhea. Concerning the castor oil–induced diarrhea model, the results revealed that the *Kaempferia galanga* extract showed slightly better protection from diarrhea in the animals as compared with *Grewia paniculata* extract. Further studies are required to identify and isolate the active principles to establish the exact mechanism of action of the test extract.

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CONFLICT OF INTEREST: Nil

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