



E-ISSN: 2278-4136

P-ISSN: 2349-8234

JPP 2018; 7(5): 1484-1492

Received: 05-07-2018

Accepted: 10-08-2018

SM Mushiur Rahman

Department of Pharmacy,
Faculty of Biological Science and
Technology, Jessore University
of Science and Technology,
Jessore-7408, Bangladesh

Trina Mony

Department of Pharmacy,
Faculty of Biological Science and
Technology, Jessore University
of Science and Technology,
Jessore-7408, Bangladesh

Koushik Ahammed

Department of Pharmacy,
Faculty of Biological Science and
Technology, Jessore University
of Science and Technology,
Jessore-7408, Bangladesh

Sharmin Naher

Department of Pharmacy,
Faculty of Biological Science and
Technology, Jessore University
of Science and Technology,
Jessore-7408, Bangladesh

Md. Rubel Haque

Department of Pharmacy,
Faculty of Biological Science and
Technology, Jessore University
of Science and Technology,
Jessore-7408, Bangladesh

Susmita Mistry Jui

Department of Pharmacy,
Faculty of Biological Science and
Technology, Jessore University
of Science and Technology,
Jessore-7408, Bangladesh

Correspondence**SM Mushiur Rahman**

Department of Pharmacy,
Faculty of Biological Science and
Technology, Jessore University
of Science and Technology,
Jessore-7408, Bangladesh

Qualitative phytochemical screening and evaluation of analgesic and antidiarrheal activity of ethanolic extract of *Leucas cephalotes* Leaves

SM Mushiur Rahman, Trina Mony, Koushik Ahammed, Sharmin Naher, Md. Rubel Haque and Susmita Mistry Jui

Abstract

Medicinal plants are widely used as alternative medicines for the treatment or prevention of many diseases. Traditionally, *Leucas cephalotes* is used for the treatment of asthma, pain, bronchitis, inflammation, dyspepsia, paralysis, jaundice, diarrhea, wounds and fever. To qualitatively evaluate the profile of phytochemical constituents as well as acute toxicity, analgesic and antidiarrheal activities of ethanol extract of *Leucas cephalotes* leaves, the present study was designed. Phytochemical constituents, acute toxicity, analgesic and antidiarrheal activities were determined and assessed by various tests such as Molisch's test, Fehling test, Mayer's test, frothing test, FeCl₃ test, alkali test, Salkowski's test, Kellerkilliani test and CuSO₄ test, OECD guidelines, formalin induced paw licking, acetic acid induced writhing, castor oil and MgSO₄ induced diarrheal test. This extract figured the presence of carbohydrates, flavonoids, tannins, glycosides, triterpenoids, fat and fixed oils. Behavioral changes, mortality or sign of any toxicity were not observed up to the dose as high as 4000mg/kg. The crude extract was found to have significant (p<0.05, vs. control) analgesic activity at the oral dose of 200 mg/kg and 400 mg/kg (b. wt.) in the tested animals. Moreover, both doses of (200 mg/kg and 400 mg/kg) ethanol extract significantly (p<0.05, vs. control) reduced the gastro-intestinal motility and inhibit the percentage of diarrhea in antidiarrheal models. But 400 mg/kg dose showed better analgesic and antidiarrheal activity than 200 mg/kg dose compared to control. The results indicate that *Leucas cephalotes* leaves may provide a potential source of analgesic and antidiarrheal activities.

Keywords: phytochemical screening, *Leucas cephalotes*, acute toxicity, acetic acid-induced writhing, antidiarrheal

1. Introduction

From the ancient period of human being development, various medicinal plants are used as traditional medicines for remedial purpose. Medicinal plants have the capability of producing biologically interesting and therapeutic chemical constituents [1].

Leucas cephalotes is a species of flowering herb in the Lamiaceae family (Synonym: *L. Capitata*). It is commonly known as dondokolos (In traditional medicine of Bangladesh), guma, dropushpi or drona puspi, Kubo or Kubi (In traditional medicine of Gujarat) [2]. It is a small erect, fast-growing, branched herb with attractive, lanceolate (shaped like a lance-head), ascending to spreading variegated leaves in shades of green and produces white flowers.

Medicinal herbs are the local heritage with global importance. Medicinal herbs have curative properties due to presence of various complex chemical substance of different composition, which are found as secondary plant metabolites in one or more parts of these plants. Phytochemical investigations show the presence of flavonoids, phenol, phytosterol, tannins, triterpenes, oleanolic acid [3, 4], Lauric acid, Tridecanoic acid, Adipic acid, Glutaric acid [5] in *Leucas cephalotes* leaves.

Generally, the plant is useful in pain, bronchitis, inflammation, asthma, dyspepsia, paralysis and leucoma. The leaves are useful in fever and urinary discharge [6]. The decoction of dried aerial parts of plant is used orally for diarrhoea, to reduce fever, as an appetizer, to treats coughs and colds [7, 8]. According to Ayurveda, the plant is mild stimulant and diaphoretic. The flowers and leaves are applied externally as poultice to treat headache [9]. It is valuable homoeopathic drug and as such is used for the treatment of chronic malaria and asthma [10]. The plant was evaluated for *in vitro* antifilarial activity [11] and antidiabetic activity [12]. Recently this plant is used in liver damage disease.

So, the present study was designed to identify phytoconstituents and to justify the analgesic and antidiarrheal activities of *Leucas cephalotes* leaves, and evaluate the traditional usage scientifically.

2. Materials and Methods

2.1 Collection and Identification of the Plant

Green and freshness leaves of *Leucas cephalotes* plant was collected to performed this study from Ambottola region of Jessore University of Science & Technology, Jessore, Bangladesh, in January, 2018. The collected leaves were identified and confirmed by National Herbarium, Bangladesh.

2.2 Extraction

For ethanolic extraction, 250 gm of powdered leaves were taken. First, the leaves of *Leucas cephalotes* were separated from plant and thoroughly washed with fresh water to remove all dirt and contaminants and dried in shade at room temperature ($25\pm 2^\circ\text{C}$) for 10-12 days. The materials were grinded into coarse powder and cold extraction method was used to extract the active components. The ground leaves (250 gm) were soaked in sufficient amount (approximately 1.6 L) of ethanol for 14 days at room temperature with periodical shaking and stirring. The whole mixture was primarily filtered through cotton and then through Whatman No.1 filters. The solvent was evaporated with a rotary evaporator under reduced pressure at 40°C temperature to yield semisolid crude extract. The percentage yield of the extract was 3.16 % (w/w). The extract was then preserved in a refrigerator till further use.

2.3 Experimental animals

One hundred Swiss albino mice of either sex, aged 4-5 weeks, weighing about 25-30 gm were collected in order to run the experiment of analgesic and antidiarrheal activity, from the Department of Pharmacy, Jahangirnagar University, Savar, Dhaka, Bangladesh. Before initiating the experiment, the animals were exposed to alternative 12:12 hours light and dark cycle at an ambient temperature of $26\pm 2^\circ\text{C}$. It was ensured the proper supplies of foods and water *ad libitum*. All protocols for animal experiment were approved by the Institutional Animal Ethical Committee of Jessore University of Science & Technology, Jessore, Bangladesh. Prior to the study, mice were acclimatized for 7 days in the laboratory environment, and maintained the constant environmental and adequate nutritional conditions throughout the period of the experiment.

2.4 Phytochemical Screening

Freshly prepared *Leucas cephalotes* leaves extract were subjected to different qualitative tests.

2.4.1 Molisch's test for carbohydrates

For this test, 500 mg of crude extract was dissolved in 5 mL of distilled water and later filtered. A few drops of Molisch's reagent (α -naphthol 10% (w/v) in 90% ethanol) were added to the filtrate. Then 1 mL of concentrated H_2SO_4 was poured carefully along the side of the test tube. Two minutes later, 5 mL of distilled water was added. A positive test, indicating the presence of carbohydrates, was confirmed with formation of dull violet or red color at the interphase of the two layers [13].

2.4.2 Fehling's test for reducing sugars

For this test, 2 mg plant extract was dissolved in 1 mL of distilled water and filtered. Then, 1 mL mixture of Fehling's solutions A and B (a ratio of 1:1) was added to the filtrate, which was heated in a water bath for a few minutes. Formation of brick-red precipitate confirmed the presence of reducing sugars [14].

2.4.3 Mayer's test for alkaloids

In Mayer's test, one or two drops of 0.35 mol/L Mayer's reagent (potassium- mercuric iodide solution, 1.36 g mercuric chloride and 5 g of potassium iodide, dissolved in 100 mL distilled H_2O) was added to 2 mL (50 mg extract dissolved in 5 mL of 1% aqueous HCl) filtrate along the side of the test tube. A positive test, demonstrating the presence of alkaloids, was indicated by a white creamy precipitate [15].

2.4.4 Frothing test for saponins

About 100 mg plant extract was dissolved in 10 mL of methanol for making stock solutions. These stock solutions were diluted to 0.5 mg/mL by the additions of 20 mL of distilled water. Test tube containing the dilution was then shaken for 15 min. Formation of foam on the top of the test tubes indicated the presence of saponin [14].

2.4.5 FeCl_3 test for tannins

50 mg plant extract was dissolved in 5 mL distilled water, followed by the addition of a few drops of 5% FeCl_3 . Tannin was confirmed by the development of a bluish- black color [16].

2.4.6 Alkali test for flavonoids

In Alkali test, a few drops of 5% NaOH solution were added to 1 mL of filtered stock solution (100 mg of extract dissolved in 10 mL of methanol), which produced a deep-yellow color. The color was lost in the presence of dilute HCl and confirmed flavonoids [16].

2.4.7 Salkowski's test for triterpenoids

For this screening, 2 mg plant extract was shaken in 1 mL of CHCl_3 . Then, a few drops of concentrated H_2SO_4 were added to the solution along the side of the test tube. Development of a red-brown color at the interface indicated the presence of triterpenoids [14].

2.4.8 Keller-killiani test for glycosides

For this screening, 1ml of extract, 1ml of Glacial acetic acid and few drops of 2% FeCl_3 were added and then 1ml of conc. H_2SO_4 is also added in the mixture. Appearance of Brown ring shows presence of glycosides [17].

2.4.9 CuSO_4 test for fat and fixed oils

In this test, 5 droops of extract solution (0.25g extract dissolved in 25 mL mother solvent) mixed with 1 mL of 1% CuSO_4 and then few drops of 10% NaOH was added. Appearance of clean blue solution shows presence of fat and fixed oils.

2.5 Acute Oral Toxicity Study

Adverse effects that result either from a single exposure or from multiple exposures over a short time (normally less than 24 h) are known as acute toxicity. According to Organization of Economic Cooperation and Development (OECD) guidelines, the acute toxicity study of *Leucas cephalotes* leaves was designed to estimate the half lethal dose (LD50) of the experimental samples [18]. Teen mice were divided into two groups: control group and test group (ELCL), with five animals per group. The experimental sample (ELCL) was administered orally at different concentrations (100, 250, 500, 1000, 2000, 3000 and 4000 mg/kg body weight). After that the animals were observed every 1 h for next 5-6 h for mortality, behavioral pattern changes such as salivation, weakness, aggressiveness, food or water refusal, diarrhea,

discharge from eyes and ears, noisy breathing, changes in locomotor activity, convulsion, coma, injury, pain or any sign of toxicity in each group of animals. A final evaluation at the end of a 2-week observation period was also conducted [18].

2.6 Antinociceptive Study

2.6.1 Formalin-Induced Paw Licking Test

For formalin-induced paw licking test, the slightly modified method of Hunskaar and Hole [19] was followed. Twenty Swiss albino mice were selected for this test and divided into four groups containing five mice in each group, and they were fasted for 16h with water *ad libitum*. Control group, standard group, and test groups were treated with distilled water (10mL/kg), diclofenac sodium (DS, 100 mg/kg), ELCL at 200 and 400mg/kg, respectively. All of the treatments were administered orally [per oral (p.o.) route]. After 1h of treatment, each mouse was injected with 20 μ L of 2.7% (v/v) formalin solution into the dorsal surface of left hind paw. Mice were observed for 5min after injection and the time spent in licking, biting, and shaking behaviors was measured in seconds, which was considered as acute phase (0-5 min). Again, they were monitored for 5min after 20min of injection which was defined as late phase (20-25 min).

The percentage of inhibition of licking was calculated by the following formula.

$$\text{Inhibition (\%)} = [1 - (\text{Licking time (standard or extracts)} / \text{Licking time (normal control)})] \times 100$$

2.6.2 Acetic Acid Induced Writhing Test

Analgesic activity was evaluated by the test of abdominal writhing induced by acetic acid in mice, the method of Koster *et al.* [20] was applied. Mice were kept unfed for 16h with water *ad libitum* prior to the experiment and pretreated with extracts as mentioned before. DS (100mg/kg) was used as standard or positive control and distilled water as normal control. After 45 min of respective treatment, each mouse was injected intraperitoneally with 0.7% (v/v) acetic acid at a dose of 10mL/kg body weight. The number of writhing responses of each mouse was counted for 5 min period, which began 15 min late of acetic acid administration.

To calculate the percentage of inhibition of writhing, the following formula was used.

$$\text{Inhibition (\%)} = [1 - (\text{No. of writhing (standard or extracts)} / \text{No. of writhing (normal control)})] \times 100$$

2.7 Antidiarrheal study

2.7.1 Castor oil induced antidiarrheal test

In castor oil induced antidiarrheal test in mice, the slight modified method of Shoba and Thomas [21] was followed. By administering 0.5mL of castor oil orally the preliminary screening of animals were performed, and those animals that started diarrhea were selected finally for the test. Twenty diarrheal screened mice were divided into control group (distilled water), positive control or standard group (Loperamide HCl, 3 mg/kg b.w.), and test groups (ELCL 200 mg/kg and 400 mg/kg b.w.), containing five mice in each group. Experimented animals were fasted for around 16 h with water *ad libitum*. Mice in the control group, standard group and test groups orally received one dose of distilled water, Loperamide HCl, ELCL 200 mg/kg and 400 mg/kg respectively. Then, each animal received 0.5mL of castor oil orally for initiating diarrhea after 30min of the above treatments. Observation for defecation continued up to 4h on

blotting paper lined individual cage was used for placing every animal. Blotting papers were replaced every hour. The number of diarrheal feces was count and recorded for a period of 4h and the percentage of inhibition of defecation was calculated for every group of animals.

2.7.2 MgSO₄ Induced Antidiarrheal Test

With slight modification of the method described by Doherty [22], MgSO₄ induced antidiarrheal test was performed. Here, a similar procedure as for castor oil induced diarrhea test was maintained for magnesium sulphate induced diarrheal model. Mice were screened for diarrhea was done by administering magnesium sulphate at a dose of 2g/kg orally. Experimented animals were fasted for 16 h with water *ad libitum*. Then, mice were grouped and treated as described before. Then, each animal received 2g/kg of magnesium sulphate orally for initiating diarrhea after 30min of the above treatments. Observation for defecation is same as for castor oil induced diarrhea test, and the antidiarrheal activity was expressed by comparing the percent of inhibition of defecation of different groups with control group.

2.8 Statistical Analysis

The experimental results were expressed as mean \pm SEM (Standard Error of mean). Statistical analyses for antinociceptive and antidiarrheal studies were evaluated by one-way ANOVA following Dunnett's test through the SPSS software (version 16; IBM Corporation, New York, USA). The obtained results were compared with the vehicle control group. The $p < 0.05$ was considered to be statistically significant.

3. Results

3.1 Phytochemical Screening

It is important to depict the chemical nature of plant materials after evaluation the pharmacological activities of plant extract. Phytochemical screening of the *Leucas cephalotes* leaf showed the presence of several primary and secondary metabolites, or phytoconstituents, which are summarized in table-1. In the phytochemical screening, ELCL showed the presence of almost all of the phytoconstituents like alkaloids, carbohydrates, flavonoids, tannins, phenols, glycosides, triterpenoids, fat and fixed oils that were tested here. However, some tests did not show consistent results such as carbohydrate content in ELCL was indicated by Molisch's test, but not by Fehling's test.

3.2 Acute Oral Toxicity Study

No mortality was viewed up to the dose as high as 4000 mg/kg for ELCL or control group in acute oral toxicity study. Any signs of toxicity or behavioral changes were not observed up to the dose as high as 4000 mg/kg for ELCL (test group) or control group, before or after their administration in any animal, which lived up to 14 days. This apparently indicated that the test group does not show acute oral toxicity.

3.3 Antinociceptive Study

3.3.1 Formalin-Induced Paw Licking Test

Of the ethanol extract of *Leucas cephalotes* leaves, both doses (200 mg/kg and 400 mg/kg) were showed significant reduction in duration of paw-licking as compared to the control group in the early phase. Mean paw licking time for 200 mg/kg and 400 mg/kg at the early phase were 68.04 \pm 7.43 and 52.50 \pm 4.46 sec respectively and percent inhibition were 48.78% and 60.48% respectively. In the late phase, the 200

mg/kg and 400 mg/kg dose were reduced paw licking time by 87.64% (6.77 ± 0.81 sec) and 93.66% (3.47 ± 0.28 sec) which were very significant compared to control. Increasing the dose also increasing the analgesic activity of that extract. Results are showed in table-2 and illustrated in figure 1.

3.3.2 Acetic acid induced writhing test

In this test, both doses of extract (200 mg/kg and 400 mg/kg) were highly inhibited the numbers of writhing. The extract was more effective at high dose (400 mg/kg) with a mean value of 12.80 ± 1.93 than the low dose (200 mg/kg) with a mean value of 18.80 ± 2.01 . But both of their result was very significant ($P = 0.000, 0.013$ in respectively). Percent protection offered by 200 and 400 mg/kg was 31.88% and 53.62% in respectively. Where the mean number of writhing's of standard drug was 11.60 ± 1.28 , which was very highly significant ($p = 0.000$) compared to that of the control (27.60 ± 2.93). Both doses of extract (200 mg/kg and 400 mg/kg) are dose dependent in manner. The effects are displayed in table-3 and illustrated in figure 2.

3.4 Antidiarrheal study

3.4.1 Castor oil induced antidiarrheal test

The experiment of ethanolic extract of *Leucas cephalotes* leaves showed significant inhibitor activity against castor oil induced diarrhea in mice at dose of 200 mg/kg and 400 mg/kg body weight. Here, the decrease of the total number of diarrheal feces is dose dependent in manner. Highest and most significant ($P < 0.05$, versus control) percentage of inhibition of diarrhea (55.00%) was revealed by ELCL 400 mg/kg and showed significant anti-motility activity like the standard drug Loperamide HCl. Castor oil induced antidiarrheal results are showed in table-4 and illustrated in figure 3.

3.4.2 MgSO₄ Induced Antidiarrheal Test

The results of this study show that there has been a statistically significant reduction in the incident and severity of diarrhoea with the ethanol extract of *Leucas cephalotes* leaves in experimental animal models. In the magnesium sulphate induced diarrheal mice, Loperamide HCl (3 mg/kg) and ethanol extract of *Leucas cephalotes* leaves at the doses of 200 mg/kg and 400 mg/kg significantly ($p < 0.05$, vs. control) reduced the total number diarrheal feces. Here, the decrease of the total number of diarrheal feces is dose dependent in manner. Highest and significant ($P < 0.05$, versus control) percentage of inhibition of diarrhea (56.25%) was revealed by ELCL 400 mg/kg. Magnesium sulphate induced antidiarrheal results are showed in table-5 and illustrated in figure 4.

4. Discussion

Phytochemical components are identified as bioactive compounds of plant extracts and may be responsible for the diverse activities when herbs are used medicinally [23]. From phytochemical analysis of the leaves of *Leucas cephalotes*, it revealed the presence of carbohydrates, tannins, flavonoids, glycosides and triterpenoids which initiates drug discovery and development.

Medicinal plants are used as a source of remedies by many people. However, severe toxicities can arise by using some of these plants. So, their toxicological studies must be performed [24]. The acute oral toxicity study is a vital factor for the investigation of therapeutic index of drugs and xenobiotics [25]. However, suitable range of doses of the materials for successive usage can be obtained by acute oral toxicity

studies [26]. As no mortality was observed up to the dose as high as 4000 mg/kg, LD₅₀ of *Leucas cephalotes* leaves extract could not be obtained. For this, the extract was found to be safe with a broad therapeutic range and two comparatively high doses (200 mg/kg and 400 mg/kg) of ELCL were used for in-vivo doses.

In formalin induced paw licking test, both peripheral and central activities of nociception are revealed. The response time of the animals spends in licking the injected paw were measured both acute and late phase during this test. Two different periods of licking activity, an early response (0-5 min after the formalin injection) and a late response (20-30 min after the formalin injection). Direct effect of formalin on nociceptors and prostaglandins was occurred in early phase which were not significantly in early phase (no inflammatory pain) where the late phase reflects pain from formalin-induced inflammation which can be inhibited by anti-inflammatory drugs. The late response is inhibited by peripheral analgesic only where both phases response are inhibited by the narcotic analgesic [27]. Ethanol extract of *Leucas cephalotes* leaves inhibited the percentage inhibition of licking at both phases.

In writhing response experiments, the pain sensation is arises through the activation of the localized inflammatory response by acetic acid. Free arachidonic acid from the tissue phospholipids is discharged by the pain stimulus [28]. It is a reliable and simple model to evaluate peripheral type of analgesic action of crude and other drugs. The abdominal constriction response induced by acetic acid is sensitive procedure to establish peripherally active analgesic [29], and it mediated by peritoneal mast cells [30], acid-sensing ion channels [31] and the PG pathways [32]. Here endogenous substances such as serotonin, bradykinin, histamin and prostaglandin are involved in pain generation [33]. Therefore, plant extract might be inhibiting the synthesis and /or release of these endogenous substances and thus reduced pain.

In many tropical countries diarrhoea is a very common ailment and national problem and the cause of 4-5 million deaths throughout the world annually [34]. Apart from modern medical therapy, the use of herbal drugs in the treatment of diarrhoeal diseases is a common practice in many countries of Asia including Bangladesh and India [35].

It is known that the active ingredient of castor oil is ricinolic acid. The castor oil induced diarrhoea demonstrates secretory diarrhoea. The ricinolic acid produce irritating and inflammatory actions on the intestinal mucosa leading to the release of prostaglandins [36]. The condition induces an increases the peristaltic activity and produces permeability changes in the intestinal mucosal membrane to electrolytes and water, which is associated with prostaglandin release [37]. As a result, absorption of sodium and potassium ions are reduced, which sequentially lessens the function of Na⁺, K⁺ - ATPase in colon plus small intestine [38]. In the study, both doses of ethanol extract (200 mg/kg and 400 mg/kg) of *Leucas cephalotes* leaves showed a significant inhibition ($P < 0.05$, versus control) of castor oil induced diarrhea in mice and may be due to the inhibition of electrolyte permeability of the intestine and prostaglandin release and it can be assumed that, the antidiarrheal action of plant extract was exerted by antisecretory mechanism.

In another experiment, after the oral administration of magnesium sulphate, results the gathering of fluid in the intestinal lumen and its movement from proximal to the distal intestine occurs. Magnesium sulfate induced diarrhea by promoting cholecystokinin release from the duodenal mucosa preventing the reabsorption of sodium chloride and water

from the lumen. Discharge of cholecystokinin and nitric oxide from duodenal mucosa occurs after its oral administration. Then two recurrently results come about and one is the rise of secretion and motility of small intestine. Another is the inhibition of reabsorption of NaCl and water that occurs from the previous case [39]. Ethanol extract of *Leucas cephalotes* leaves extract (200 mg/kg and 400 mg/kg) was effective in reducing diarrhea and that was expected due to increase in electrolyte and water reabsorption from the gastrointestinal tract and thus reduce diarrhea.

Above activities are seems to be due to the presence of tannins and flavonoids in the ethanol extract of *Leucas cephalotes* leaves. In fact tannins are responsible for the denaturation of proteins and form protein tannate, which reduces the intestinal mucosa permeability [40]. The ethanol extract of *Leucas cephalotes* leaves was administered at the dose of 200 mg/kg and 400 mg/kg showed 30.00% and 55.00% reduction of diarrhea in castor-oil induced diarrheal test and 40.62% and 56.25% reduction of diarrhea in MgSO₄

induced diarrheal test respectively.

So we can conclude that the present study seems to support the claims of a traditional medicine practitioner about the use of *Leucas cephalotes* in pain and diarrhea.

Table 1: Phytochemical screening of ethanol extract of *Leucas cephalotes* leaves.

Phytoconstituents	Test name	Observation
Carbohydrates	Molisch's test	+
	Fehling's test	-
Alkaloids	Mayer's test	-
Saponins	Frothing test	-
Tannins	FeCl ₃ test	+
Flavonoids	Alkali test	+
Triterpenoids	Salkowski's test	+
Glycosides	Keller-kiliani test	+
Fat and Fixed oils	CuSO ₄ test	+

'+' mean presence of specific phytoconstituents and '-' mean absence of specific phytoconstituents

Table 2: Effects of ethanol extracts of *Leucas cephalotes* leaves on formalin-induced paw licking test

Treatment Group	Dose	Acute phase		Late phase	
		Licking time (s)	% inhibition	Licking time (s)	% inhibition
Control	10 mL/kg	132.85±5.44	-	54.77±6.08	-
Diclofenac sodium	100 mg/kg	62.65±8.39	52.84	2.98±0.43	94.56
ELCL	200 mg/kg	68.04±7.43	48.78	6.77±0.81	87.64
ELCL	400 mg/kg	52.50±4.46	60.48	3.47±0.28	93.66

Numbers of licking time inhibition are presented as (mean ± standard error of mean). P<0.05, vs. control; (Dennett's t test)

Table 3: Effects of ethanol extracts of *Leucas cephalotes* leaves on acetic acid-induced writhing test

Treatment groups	Dose mg/kg	No of writhing	% of inhibition	p value
Control	10 mL/kg	27.60±2.93	-	
Diclofenac sodium	100 mg/kg	11.60±1.28	57.97	.000
ELCL	200 mg/kg	18.80±2.01	31.88	.013
ELCL	400 mg/kg	12.80±1.93	53.62	.000

Numbers of writhing are presented as (mean ± standard error of mean). P<0.05, vs. control; (Dennett's t test)

Table 4: Effects of ethanol extracts of *Leucas cephalotes* leaves on castor oil-induced diarrhea test

Group	Dose	Number of diarrheal feces	% of inhibition of diarrhea
Control	10 mL/kg	8.00±0.97	-
Loperamide HCL	3 mg/kg	2.20±0.37	72.50
ELCL	200 mg/kg	5.60±0.68	30.00
ELCL	400 mg/kg	3.60±0.55	55.00

Numbers of feces are presented as mean ± SEM (standard error of mean). P<0.05, vs. control (Dennett's t test)

Table 5: Effects of ethanol extracts of *Leucas cephalotes* leaves on MgSO₄ induced diarrhea test

Group	Dose	Number of diarrheal feces	% of inhibition of diarrhea
Control	10 mL/kg	6.40±0.68	-
Loperamide HCL	3 mg/kg	1.40±0.24	78.13
ELCL	200 mg/kg	3.80±0.58	40.62
ELCL	400 mg/kg	2.80±0.58	56.25

Numbers of feces are presented as mean ± SEM (standard error of mean). P<0.05, vs. control (Dennett's t test)

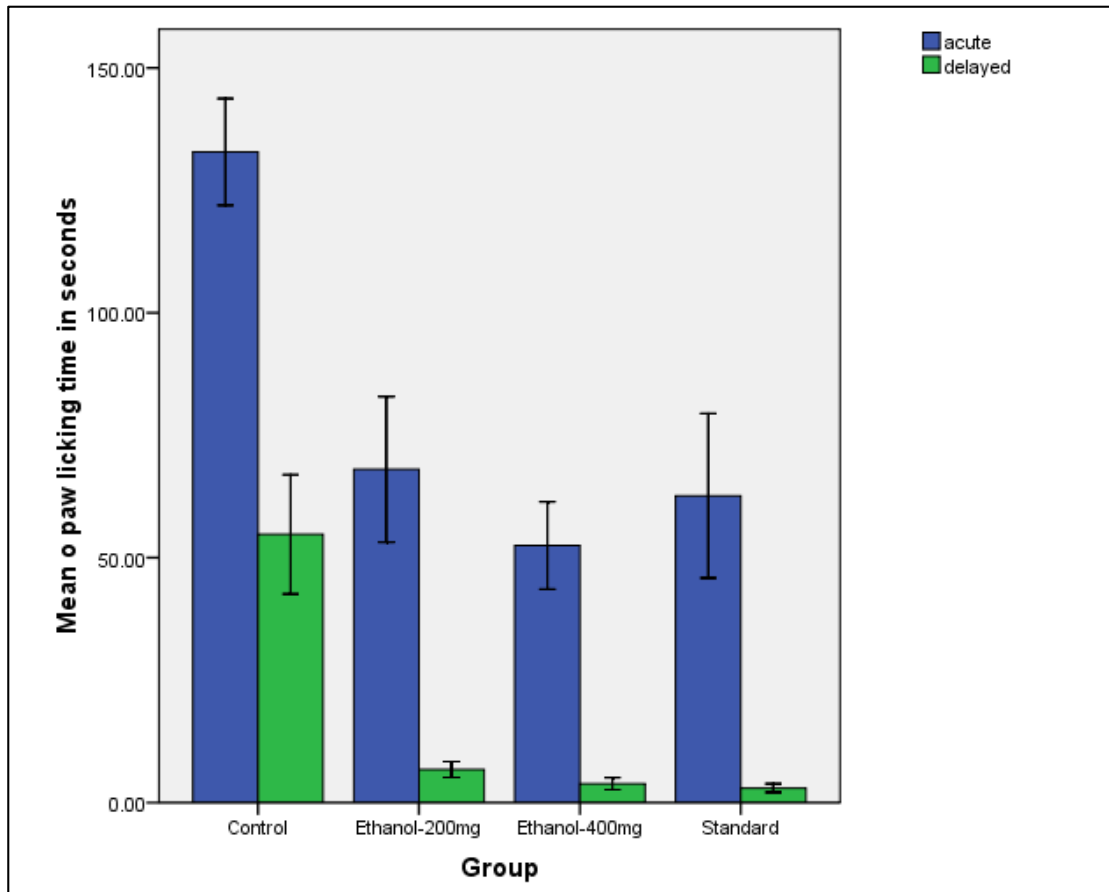


Fig 1: Effect of ethanol extract of *Leucas cephalotes* leaves in formalin induced paw licking test X-axis – group of experimented animal Y-axis – number of paw licking time Level of Significance = $p < 0.05$ compared to control (*Dennett's t test*).

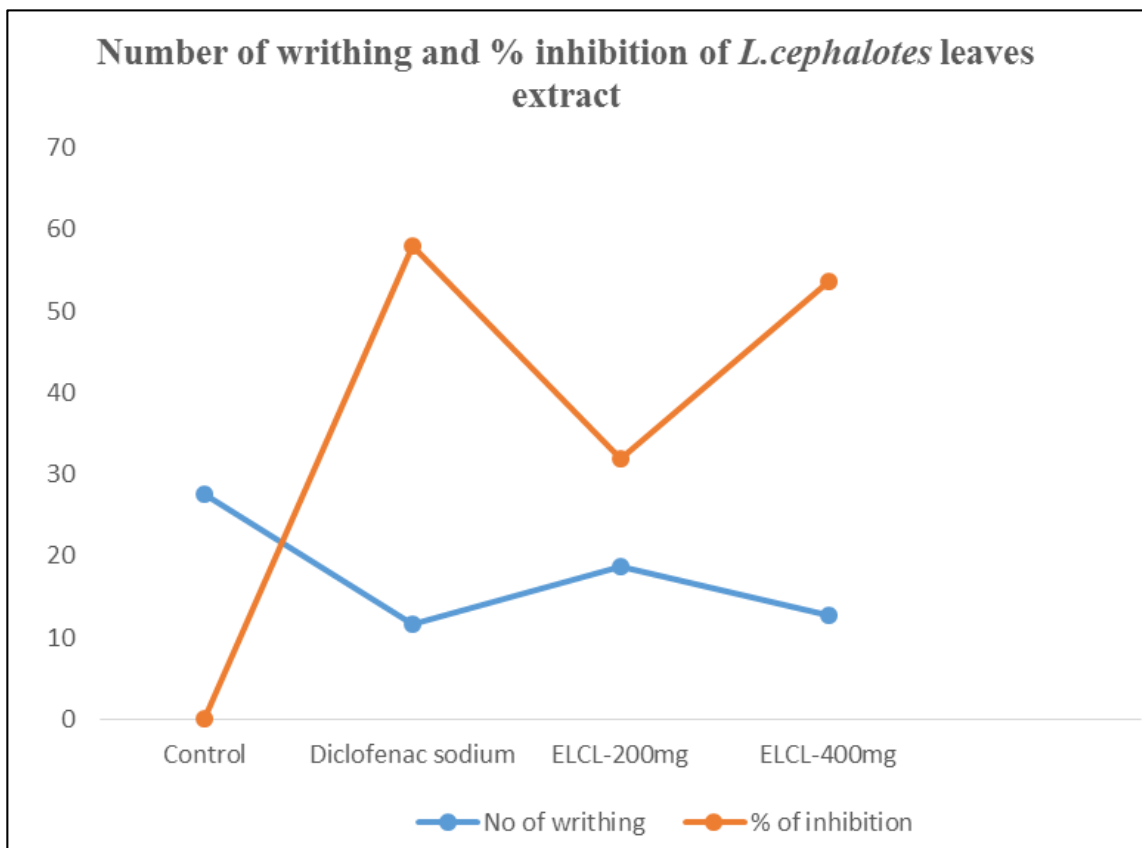


Fig 2: Effect of ethanol extract of *Leucas cephalotes* leaves in acetic acid-induced writhing test X-axis – group of experimented animal Y-axis – number of writhing and % of inhibition Level of Significance = $p < 0.05$ compared to control (*Dennett's t test*).

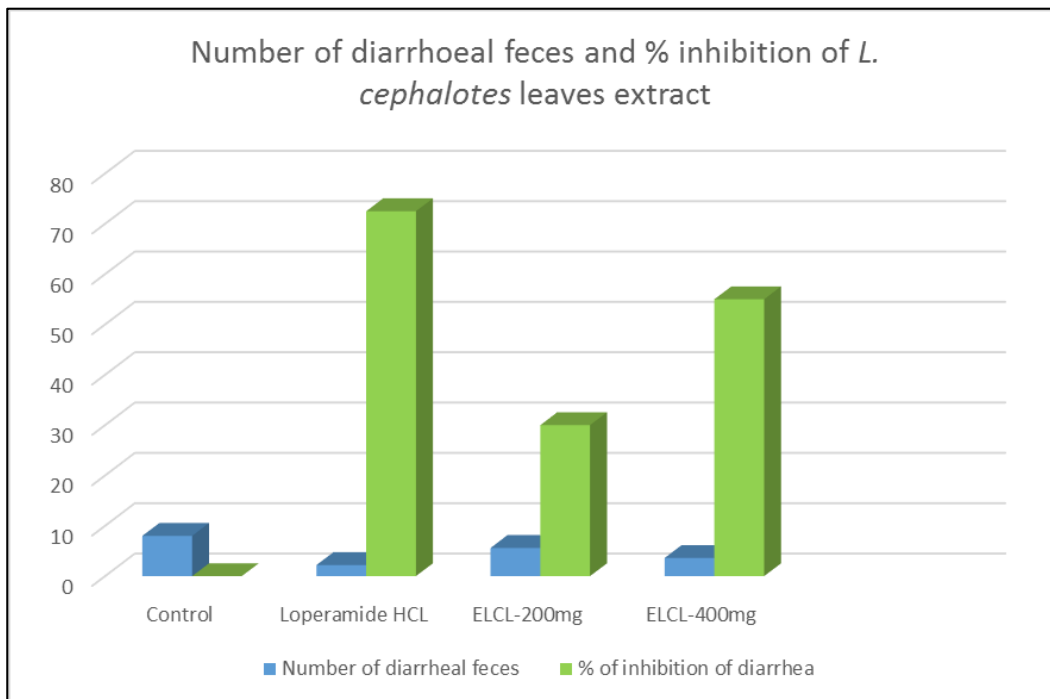


Fig 3: Effect of ethanol extract of *Leucas cephalotes* leaves in castor oil-induced diarrheal test X-axis – group of experimented animal Y-axis – number of diarrheal feces and % of inhibition Level of Significance = $p < 0.05$ compared to control (*Dennett's t test*).

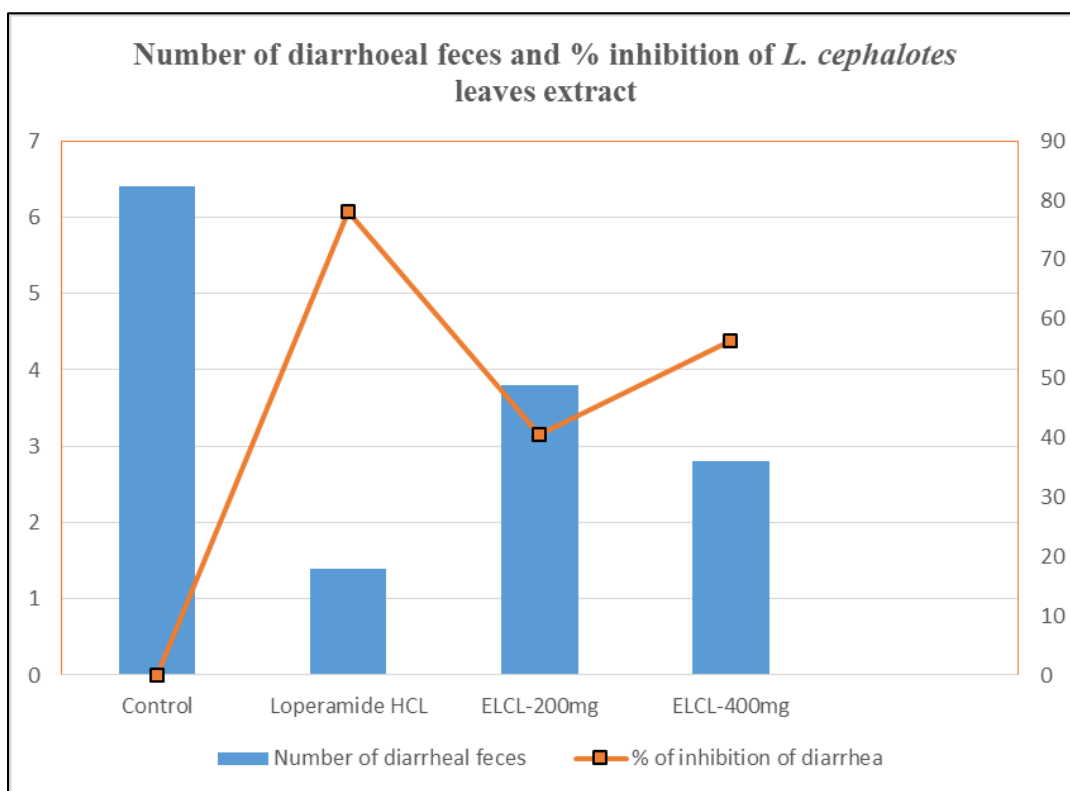


Fig 4: Effect of ethanol extract of *Leucas cephalotes* leaves in $MgSO_4$ induced diarrheal test X-axis – group of experimented animal Y-axis – number of diarrheal feces and % of inhibition Level of Significance = $p < 0.05$ compared to control (*Dennett's t test*).

5. Conclusion

The results proposed that the Ethanol extract of *Leucas cephalotes* leaves might possess analgesic and antidiarrhoeal activities. Data obtained in this study showed that all activities were dose dependent and statistically significant. The presence of flavonoids, sitosterol, tannin and phenolic compounds might be responsible for these activities and which are probably mediated via inhibition of various autocoids formation and release. Now it is under investigation of isolating and determining the exact active constituents and

structures of *Leucas cephalotes* leaves that are responsible for these activities. The genotoxicity study of this extract may be a promising area for the researchers. Moreover, it could be potential source for novel ‘lead’ discovery for analgesic and antidiarrhoeal drug development.

6. Conflict of Interests

The authors declare that there is no conflict of interests regarding the publication of this manuscript.

7. Acknowledgements

For providing facilities throughout the work, the authors would like to thank Department of Pharmacy, Jessore University of Science & Technology, Jessore, Bangladesh.

8. References

- Rauf A, Uddin G, Siddiqui BS, Muhammad N, Khan H. "Antipyretic and antinociceptive activity of *Diospyros lotus* L. in animals," Asian Pacific Journal of Tropical Biomedicine, vol. 4, supplement1, 2014, S382-S386,
- Parrotta JA. Healing plants of peninsular India. New Delhi: CABI publishing, 2001, 436-437.
- Baburao B, Reddy ARN, Kiran G, Reddy YN, Mohan GK. Antioxidant, analgesic and anti-inflammatory activities of *Leucas cephalotes* (roxb. Ex roth) spreng. Brazilian J. Pharma. Sci. 2010; 46:525-529.
- Miyaichi Y, Segawa A, Tomimori T. Studies on Nepalese crude drugs. XXIX. Chemical constituents of Dronapuspi, the whole herb of *Leucas cephalotes* SPRENG. Chem Pharm Bull (Tokyo) 2006; 54(10):1370-9.
- Bahadur KD, Sen AB. Chemical examination of *Leucas cephalotes*. Quart J Crude Drug Res. 1969; 9:1453-1454
- Kirtikar KR, Basu BD. Indian Medicinal Plants. International book Distr, 2005; 3:2017-2018.
- Johns T, Faubert GM, Kokwaro JO, Mahunnah RL, Kimanani EK. Anti-giardial activity of gastrointestinal remedies of the Luo of east Africa. J. Ethnopharmacol. 1995; 46(1):17-23.
- Pushpangadan P, Atal CK. Ethno-medico-botanical investigations in Kerala I. Some primitive tribals of western ghats and their herbal medicine. J. Ethnopharmacol. 1984; 11(1):59-77.
- Saha JC, Kasinathan S. Ecobolic properties of Indian medicinal plants. II. Indian J Med. Res. 1961; 49:1094-1098.
- Caius JF. The medicinal and poisonous plants of India. Jodhpur (India): Scientific Publ; 1986. 397-399. 4. Ghosh NC. *Comparative materia Medica*. Kolkata (India): Hanneman Publ. Co. Private Ltd, 1988.
- Qamaruddin, Parveen N, Khan NU, Singhal KC. *In vitro* antifilarial potential of the flower and stem extracts of *Leucas cephalotes* on cattle filarial parasite *Setaria cervi*. J Nat Rem. 2002; 2(2):155-163.
- Shivendu Mishra. Antidiabetic Activity of *Leucas cephalotes* (Roth) Spreng. in Alloxan Induced Diabetic Rats. International Conference on Promotion and Development of Botanicals. Kolkata, 2005. SNPS/CB/5S-003
- Sofowora A. Screening plants for bioactive agents. In: Medicinal plants and traditional medicine in Africa. 2nd ed. Sunshine House, Ibadan: Spectrum Books Ltd, 1993, 134-156.
- Harborne JB. Phytochemical methods, a guide to modern techniques of plant analysis. 2nd ed. London: Chapman and Hall. 1998, 54-84.
- Evans WC. Pharmacology. Singapore: Harcourt Brace and Company. 1997, 226.
- Raaman N. Phytochemical techniques. Pitam Pura, New Delhi: New India Publishing Agency, 2006, 22.
- Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. African Journal of Biotechnology. 2005; 4(7):685-688.
- Walum E. Acute oral toxicity. Environ Health Perspect. 1998; 106(2):497-503.
- Hunskar S, Hole K. The formalin test in mice: dissociation between inflammatory and non-inflammatory pain, Pain, 1987; 30(1):103-114.
- Koster R, Anderson M, De-Beer EJ. Acetic acid for analgesic screening," Federation Proceedings, 1959; 18:412-417.
- Shoba FG, Thomas M. Study of antidiarrhoeal activity of four medicinal plants in castor-oil induced diarrhoea," Journal of Ethnopharmacology. 2001; 76(1):73-76.
- Doherty NS. Inhibition of arachidonic acid release as the mechanism by which glucocorticoids inhibit endotoxin-induced diarrhoea, British Journal of Pharmacology, 1981; 73(2):549-554.
- Alabri THA, Musalami AHSA, Hossain MA, Al-Riyami AMWQ. Comparative study of phytochemical screening, antioxidant and antimicrobial capacities of fresh and dry leaves crude plant extracts of *Datura metel* L. J King Saud Univ Sci. 2014; 26:237-243.
- Gutiérrez-Rebolledo GA, Garduño-Siciliano L, García-Rodríguez RV, et al., "Anti-inflammatory and toxicological evaluation of *Moussonia deppeana* (Schldl. & Cham) Hanst and Verbascoside as a main active metabolite, Journal of Ethnopharmacology, 2016; 187:269-280.
- Sahni C, Shakil NA, Jha V, Gupta RK. Screening of Nutritional, Phytochemical, Antioxidant and Antibacterial activity of the roots of *Borassus flabellifer* (Asian Palmyra Palm). Journal of Pharmacognosy and Phytochemistry. 2014; 3(4):58-68.
- Jothy SL, Zakaria Z, Chen Y, Lau YL, Latha LY, Sasidharan S. Acute oral toxicity of methanolic seed extract of *Cassia fistula* in mice. Molecules. 2011; 16:5268-5282.
- Okokon JF, Davis K, Nwidi LL. Anti-inflammatory and antinociceptive activities of *Solenostemon monostachyus* aerial part extract in mice," Avicenna Journal of Phytomedicine. 2016; 6(3):284-294.
- Jaman MU, Sultana F, Chowdhury MAR, Hossain MT, Haque MIU. *In vivo* assay of analgesic activity of methanolic and petroleum ether extracts of *Manilkara zapota* leaves. Br J Pharm Res. 2014; 4(2):186-191.
- Shinde UA, Phadke AS, Nair AM, Mungantiwar AA, Dikshit VJ, Saraf MN, Studies on anti-inflammatory and analgesic activity of *Cedrus deodara* (Roxb.) Loud. Wood oil, J Ethnopharmacology. 1999; 65:21-27.
- Ronaldo AR, Mariana LV, Sara MT, Adriana BPP, Steve P. Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. Eur J Pharmacol. 2000; 387:111-118.
- Voilley N. Acid-sensing ion channels (ASICs): new targets for the analgesic effects of non-steroidal anti-inflammatory drugs (NSAIDs). Curr Drug Targets Inflamm Allergy. 2004; 3:71-79.
- Hossain MM, Ali MS, Saha A. Antinociceptive activity of whole plant extracts of *Paederia foetida*. Dhaka Univ J Pharm Sci. 2006; 5:67-69.
- Collier HO, Dinneen LC, Johnson CA, Schneider C. The abdominal constriction response and its suppression by analgesic drugs in the mouse. Br. J. Pharmacol. Chemother. 1968; 32:295-310.
- Abdullahi AL, Agho MO, Amos S, Gamaniel KS, Wambebe C. Antidiarrhoeal activity of the aqueous extract of *Terminalia avicennioides* Roots. Phytotherapy Research. 2001; 15:431-434.

35. Ghani A. Medicinal Plants of Bangladesh / Chemical Constituents and Uses. Asiatic Society of Bangladesh, Dhaka, 1998.
36. Yoshio K, Kazuko S, Bunsyo M, Kazunory H, Atsushi I, Yasuhiro K. Relationship between antidiarrhoeal effects of Hnge-Shashin-To and its active components. *Phytotherapy Research*. 1999; 13:468-473.
37. Capasso F, Mascolo N, Autone G, Romano V. Laxatives and the production of autacoids by rat colon. *J Pharm Pharmacol*. 1986; 38:627-629.
38. Tenorio JAB, do Monte DS, da Silva TMG, da Silva TG, Ramos CS. *Solanum paniculatum* root extract reduces diarrhea in rats, *Revista brasileira de Farmacognosia*, 2016; 26(3):375-378
39. Singh A, Saharan VA, Ram V, Bhandari A. Evaluation of antidiarrhoeal activity of *Elytraria acaulis* extracts on magnesium sulphate- and castor oil-induced diarrhoea in wistar rats. *Malay J Pharm Sci*. 2013; 11(2):31-39.
40. Tripathi KD. Essentials of Medical Pharmacology. Jaypee Brothers Medical Publishers (P), New Delhi, 1994, 775.