

Full Length Research Paper

Study of antidiarrheal and anthelmintic activity methanol extract of *Commelina benghalensis* leaves

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The objective of this study was to examine the restorative effects of methanol extract of *Commelina benghalensis* leaves in antidiarrheal activity in Swiss albino mice model and anthelmintic activity in *Tubifex tubifex*. Antidiarrheal activity study in Swiss albino mice were divided into four groups. Group I was treated as the control group and received 10 ml/kg of 2% Tween-80 orally; Group II served as a positive control and took standard drug (loperamide) in 5 mg/kg orally; Groups III and IV were the test groups which received the methanol extract of *C. benghalensis* orally at 200 and 400 mg/kg, respectively. Dependent upon the model comprehensive weight of dry feces, aggregate weight of wet defecation, length of intestinal travel and intestinal weight were gathered. At long last, information were investigated using restricted analysis of variance (ANOVA) took after by Dunnett test. Anthelmintic action on aquarium worm *T. tubifex* by utilizing three focuses of 10, 5, and 2.5 mg/ml of *C. benghalensis* movement was assessed by *in vitro* system. The property of the extract on defecation, intestinal transit and intestinal fluid accumulation (antienterpooling) were assessed in castor oil induced diarrhea. Leaves extract of *C. benghalensis* at 200 and 400 mg/kg exhibited significant ($p < 0.05$, 0.01 and 0.001) and dose dependent antidiarrheal probable in castor oil induced diarrhea. The diarrhea was inhibited by 27.59 and 55.17 at doses of 200 and 400 mg/kg in that order. The extract were found to have an antienterpooling in castor oil affected test Swiss albino mice's by decreasing both weight and volume of intestinal substance, and obviously reducing the intestinal travel similar to that of the standard medication, loperamide (5 mg/kg). Anthelmintic activity of methanol extract *C. benghalensis* show the time taken for paralysis (12.38, 16.25 and 22.17 min) and time taken for death (21.39, 45.64 and 57.25 min) at doses of 10, 5, and 2.5, respectively. The methanol extract of *C. benghalensis* demonstrated antidiarrheal effect in Swiss albino mice model and anthelmintic action on aquarium worm *T. tubifex*. In addition, the extract was discovered to be safe at measurements of 1000, 2000, 3000, and 4000 mg/kg in mice model. The discoveries propose the legitimacy of the much lauded impact of *C. benghalensis* as antidiarrheal and anthelmintic specialists in expected home grown medication.

Key words: *Commelina benghalensis*, anti-diarrhea, Swiss albino mice, anthelmintic, *Tubifex tubifex*.

INTRODUCTION

Nature has been a recurrent source of pharmacologically dynamic atoms and curative herbs have been utilized by incalculable human eras (Zheng et al., 2015). *Commelina*

benghalensis Linn. (Commelinaceae) is a perennial herb native to tropical Asia and Africa, commonly known as Bengal day flower or Dew flower. It is a large, straggling

annual herb up to 40 cm long with rooting at basal nodes and characterized by attractive small bluish-violet flowers. Leaves are ovate-elliptic or oblong, shortly triangular, bright green in color and 4 to 7 cm long. The spathes are green, funnel-shaped, compressed and about 1.5 cm long. Capsules are broadly ovoid-oblong and 4 to 5 mm long (Zheng et al., 2015).

C. benghalensis is utilized as a part of customary medication outline to treat different diseases. It is used for the treatment of headache, constipation, leprosy, fever, snake bite and jaundice (Hasan et al., 2008; Yusuf et al., 1994; Kirtikar and Basu, 1980). It is also used in the treatment of mouth thrush (Ssenyonga and Brehony, 1993), insanity (Tabuti et al., 2003), epilepsy (Okello and Ssegawa, 2007) and psychosis (Adjanohoun, 1993). In Lesotho, it is applied to treat infertility in women and in India it is used as bitter, laxative, anti-inflammatory, demulcent, emollient and depressant (Jayvir et al., 2007). In China, it is used as diuretic and febrifuge (Hong and Deflipsis, 2000). In Pakistan it is used as vegetable (Qaiser and Jafri, 1975). In Nepal, the paste of the plant is utilized to treat smolders and juice of the roots is utilized to treat acid reflux (Manandhar and Sanjay, 2000).

In a past study, antimicrobial action of watery extract of *C. benghalensis* was assessed (Sharma and Sharma, 2010). Potential calming and anxiolytic exercises of diverse portions of the plant are accounted for in the writing (Hasan et al., 2009). The plant is also reported with remarkable antioxidant, antitumor and anticancer, and thrombolytic activity (Zheng et al., 2015; Mbazima et al., 2008; Rahman et al., 1999; Chowdhury et al., 2015). Protective activity of the roots extract against paracetamol induced hepatic damage in Wistar rats has been reported (Paresh and Chanda, 2008). Phytochemical investigations on *C. benghalensis* have revealed the existence of alkaloid, volatile oil, wax (Raju et al., 2007), vitamin C, vitamin A and β -carotene (Guerrant et al., 2001). Diarrhea can be characterized as a modification in the ordinary solid discharge, portrayed by a circumstance in which a grown-up every day stools surpasses 300 g and contains 60 to 95% water (Zavala et al., 1998). Looseness of the bowels is one of the primary drivers of newborn child passing particularly in underdeveloped nation (Galheigo et al., 2015). It represents more than 5 million passing in overall every year in newborn children and offspring of under 5 years. Therapeutic plants are potential wellsprings of antidiarrheal medications (Van et al., 2015; Chowdhury et al., 2015).

In Bangladesh, one third of the total child death burden is due to diarrhea (Patzl et al., 2015). Use of traditional medicines to combat the episodes of diarrhea has been emphasized by WHO in its Diarrhea Control Program

(Wansi et al., 2007). Medicinal plants have been reported for their efficiency in the treatment of diarrhea, thanks to the contribution of many researchers (Dooley et al., 2015). Gastrointestinal nematodes (GIN) cause genuine monetary misfortunes and is the most imperative element restricting sheep generation around the world (Prada et al., 2014; Valcarcel et al., 2015; Debebe et al., 2015). Anthelmintic are medications that demonstrate or systemically to kill grown-up helminths or formative stages that attack organs and tissues (Lou et al., 2014). *T. tubifex* is a provincially to oust worms from the gastro-intestinal tract cosmopolitan nauidid annelid sensu (Lou et al., 2014) representing one of the major components of the benthic fauna in freshwater communities (Schmelz et al., 2015). Also present in polluted waters, *T. tubifex* is widely used in laboratories for ecotoxicology research (Mendez et al., 2014) and as a model organism for the study of annelid development (Mendez et al., 2013). *T. tubifex* is characterized by considerable variability in its morphological features (Urbisz et al., 2015) and by a mixed reproductive strategy, with parthenogenesis (Marotta et al., 2014), self-fertilization (Gavrilov, 1935), and biparental reproduction through cross-mating (Sarker et al., 2015).

The aim of the present study is to identify the antidiarrheal activity by using different experimental methods and anthelmintic activity of methanol extract of *C. benghalensis* leaves using *T. tubifex* worm. However, no earlier studies have been conducted experimentally to characterize the antidiarrheal and anthelmintic effect of this leaves.

MATERIALS AND METHODS

Plant collection and identification

The leaves of *C. benghalensis* were collected from Chittagong, Bangladesh in the month of October, 2014. The leaves were taxonomically identified by Dr. Shaikh Bokhtear Uddin (Associate Professor, Department of Botany, University of Chittagong, Bangladesh). A voucher specimen (Accession No. Pharm P&D 71/09-15/30) was deposited at the Department of Pharmacy, International Islamic University Chittagong, Bangladesh for further reference.

Extract preparation

The leaves were dried for a period of 2 weeks under shade and ground. The ground leaves (250 g) were soaked in sufficient amount of methanol for one week at room temperature with occasional shaking and stirring, and then filtered through a cotton plug followed by Whitman filter paper number 1. The solvent was evaporated under reduced pressure at room temperature to yield semisolid. The extract was then preserved in a refrigerator till

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further use.

Experimental animals

Swiss albino mice, weighing about 25 to 30 g, were collected from Jahangir Nagar University, Savar, Bangladesh. The animals were provided with standard laboratory food and distilled water and maintained at natural day-night cycle having proper ventilation in the room. All the experiments were conducted in an isolated and noiseless condition. The study protocol was approved by the P&D Committee, Department of Pharmacy, International Islamic University of Chittagong, Bangladesh (Grant No. Pharm P&D 71/09-15/30). The animals were acclimatized to laboratory condition for 7 days prior to experimentation.

Experimental worms

Experimental worms were collected from local aquarium shop. Then, authenticated by local zoologist (Authentication No. Wb2015).

Chemicals and reagents

Loperamide (Square Pharmaceuticals Ltd., Bangladesh), castor oil (WELL's Health Care, Spain), normal saline solution (0.9% NaCl) and charcoal meal (10% activated charcoal in 5% gum acacia), and albendazole were used for anthelmintic activity tests. All other reagents were of analytical grade.

Preparation of test doses

The extracts were suspended in the vehicle. Various strengths were prepared from a stock solution of 40 mg/ml. The solutions were prepared, and the freshly prepared solutions were administered orally.

Acute toxicity study

For acute toxicity study, forty Swiss albino female mice were used. According to the method of Walum, mice were divided into four groups of five animals each (Walum, 1998). Different doses (1000, 2000, 3000 and 4000 mg/kg) of methanol extract of *C. benghalensis* leaves were administered by stomach tube. Then, the animals were observed for general toxicity signs.

In vivo antidiarrheal activity

Castor oil-induced diarrhea

The experiment employed the method described by Awouters et al. (1978). Mice were fasted for 18 h before the test with free access to water and divided into four groups of five animals each. Group I treated as control (saline 2 ml/kg body weight intraperitoneally), Group II received standard drug (loperamide 5 mg/kg body weight p.o.), Groups III and IV received methanol extract of *C. benghalensis* (200 and 400 mg/kg body weight p.o.). Then, 1 h later, castor oil was administered orally to these animals to induce diarrhea. The mice's were then housed singly in cages lined with white blotting paper. The papers were changed every hour. The total number of both dry and wet feces excreted were counted every hour for a period of 4 h and compared with the control group. The total number of diarrheal feces of the control group was considered 100%.

Castor oil induced enteropooling

Intraluminal fluid accumulation was determined by the method of Robert et al. (1976). Mice were fasted for 18 h and divided into five groups of four animals each. Group I served as control (saline 2 ml/kg body weight intraperitoneally), Group II received standard drug (loperamide 5 mg/kg body weight ip), Groups III and IV received methanol extract of *C. benghalensis* (200 and 400 mg/kg body weight po). Then, 1 h later, castor oil was administered orally to these animals to induce diarrhea. Two hours later, the mice were sacrificed by overdose of chloroform anesthesia, and the small intestine was ligated both at the pyloric sphincter and at the ileocecal junctions and dissected out. The small intestine was weighed. The intestinal contents were collected by milking into a graduated tube and the volume was measured. The intestines were reweighed and the differences between full and empty intestines were calculated.

Gastrointestinal motility test

This experiment was carried out by the method described by Mascolo et al. (1994). Mice were fasted for 18 h and divided into four groups of five animals each. Castor oil was administered orally to these animals to induce diarrhea. One hour later, Group I received saline 2 ml/kg body weight intraperitoneally, Group II received standard drug (loperamide 5 mg/kg body weight ip), Groups III and IV received methanol extract of *C. benghalensis* (200 and 400 mg/kg body weight po). One hour after intraperitoneal administration of treatments, animals received 1 ml of charcoal meal (10% charcoal suspension in 5% gum acacia) orally. One hour later, the animals were sacrificed by overdose of chloroform anesthesia and the distance traveled by the charcoal meal from pylorus to caecum was measured and expressed as a percentage of the total distance of the intestine.

In-vitro anthelmintic assay

The anthelmintic activity of methanol extract of *C. benghalensis* was carried out as per the procedure of Ajaiyeoba et al. (2001) with some minor modifications. The aquarium worm *T. tubifex* were used in the present study, because it has anatomical similarity and belongs to the same group of intestinal worm, that is, annelid (Verma et al., 2013; Raju et al., 2013; Rajagopal et al., 2013). The worms were collected from the local market of Chittagong, and average size of the worms, 2 to 2.5 cm in length were used for the study. The standard drug levamisole (1 mg/ml) and three different concentrations of methanol extract of *C. benghalensis* (2.5, 5 and 10 mg/ml) in double distilled water (Satish and Ravindra, 2009; Iqbal et al., 2001) were prepared freshly and used for the study of anthelmintic activity. One group composed of water and it was considered as the controlled group. The anthelmintic activity was determined at two different stages 'time of paralysis' and 'time of death' of the worms. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Death was concluded when the worms lost their motility followed with fading away of their body colors (Grime et al., 2006). Death was also confirmed by dipping the worms in slightly warm water. The mortality of parasite was assumed to have occurred when all signs of movement had ceased (Temjenmongla and Yadav, 2005).

Statistical analysis

The results are expressed as mean \pm standard error of the mean (SEM). Data were analyzed using one way factorial ANOVA tests

Table 1. Effect of methanol extract of leaves of *C. benghalensis* on castor oil (0.5 ml) induced diarrhea in Swiss albino mice.

Group	Treatment	Total number of feces	Inhibition of defecation (%)	Total number of diarrheal feces	Inhibition of diarrhea (%)
I	Saline (2 ml/kg p.o.)	13.4±0.245	-	5.8±0.2	-
II	Loperamide (5 mg/kg p.o.)	5.4±0.4 ^c	59.70±2.99	2.2±0.374 ^b	62.07±6.45
III	MECB (200 mg/ml p.o.)	8.8±0.2 ^c	34.33±1.49	4.2±0.2 ^b	27.59±3.45
IV	MECB (400 mg/ml p.o.)	7±0.547 ^c	47.76±4.09	3.2±0.2 ^c	55.17±3.45

Values are mean ± SEM ($n = 5$); ^a $p < 0.05$, ^b $p < 0.01$ and ^c $p < 0.001$, Dunnett test as compared to negative control (saline). Statistical representation of the total number of feces and total number of diarrheal feces by *C. benghalensis* methanol extract, positive antidiarrheal control (Loperamide, 5 mg/kg p.o.) processed by paired t-test analysis (Dennett's test). Data were processed by paired t-test analysis by using SPSS for windows, version 16.0.

Table 2. Effect of methanolic extract of leaves of *C. benghalensis* on castor oil induced enteropooling in Swiss albino mice.

Group	Treatment	Volume of intestinal content (ml)	Weight of intestinal content (g)	Inhibition of intestinal content (%)
I	Saline (2 ml/kg p.o.)	0.24±0.013	0.628±0.033	-
II	Loperamide (5 mg/kg p.o.)	0.13±0.001	0.316±0.002 ^b	48.2±0.40
III	MECB (200 mg/ml p.o.)	0.17±0.007	0.408±0.02 ^b	33.11±3.64
IV	MECB (400 mg/ml p.o.)	0.15±0.005	0.334±0.01 ^b	45.25±1.84

Values are mean ± SEM ($n = 5$); ^a $p < 0.05$, ^b $p < 0.01$ and ^c $p < 0.001$, Dunnett test as compared to negative control (saline). Statistical representation of the weight of intestinal content (g) by *C. benghalensis* methanol extract, positive antidiarrheal control (Loperamide, 5 mg/kg p.o.) processed by paired t-test analysis (Dennett's test). Data were processed by paired t-test analysis by using SPSS for windows, version 16.0.

using SPSS Data Editor for Windows, Version 16.0 (SPSS Inc., USA) followed by Dennett's tests on each group except the control group for anthelmintic. The results obtained were compared with the negative control group for antidiarrheal activity and $P < 0.05$, $P < 0.01$ and $P < 0.001$ was considered to be statistically significant in Dennett's tests. Statistical program GRAPHPAD PRISM® (version 6.00; GraphPad Software Inc., San Diego, CA, USA) was used for graphical presentation.

RESULTS

Acute toxicity test

None of the animals showed behavioral, neurological or physical changes characterized by symptoms, such as reduced motor activity, restlessness, convulsions, coma, diarrhea and lacrimation at the limit dose of 4000 mg/kg for methanol extract of *C. benghalensis* during the observation period. In addition, no mortality was observed at the test dose. Thus, the median lethal dose (LD₅₀) of the plant extract was found to be greater than 4000 mg/kg.

In vivo antidiarrheal activity

Castor oil-induced diarrhea

In the castor oil-induced diarrhea conduct experiment, the leaves extract of *C. benghalensis* produced a noticeable

antidiarrheal result in the mice's, as shown in Table 1. At doses of 200 and 400 mg/kg, the extract produced significant ($p < 0.01$) defecation. The total number of wet feces produced upon administration of castor oil decreased (4.2 ± 0.2 , at 200 mg/kg and 3.2 ± 0.2 , at 400 mg/kg) compared to the control group (5.8 ± 0.2) while loperamide decreased to 2.2 ± 0.374 at the dose of 5 mg/kg.

Castor oil induced enteropooling

Castor oil caused accumulation of water and electrolytes in intestinal loop. Treatment with the *C. benghalensis* extract (200 and 400 mg/kg) produced a significant and dose-dependent reduction in intestinal weight and volume (Table 2). The intestinal volume was decreased by 33.11 ± 3.64 and $45.25 \pm 1.84\%$ at doses 200 and 400 mg/kg, respectively. The standard drug, loperamide (5 mg/kg), also significantly inhibited ($p < 0.01$) intestinal fluid accumulation ($48.2 \pm 0.40\%$).

Gastrointestinal motility test

The consequence of *C. benghalensis* extract on the intestinal transit is shown in Table 3. All doses of the extracts successful produced significant alteration in the percent of intestinal motility compared to the negative control. The negative control (saline) resulted in $84.85 \pm 2.88\%$

Table 3. Effect of methanolic extract of leaves of *C. benghalensis* on charcoal induced gut transit changes in Swiss albino mice.

Group	Treatment	Intestine cross by marker (%)	Inhibition (%)
I	Saline (2 ml/kg p.o.)	84.85±2.88	
II	Loperamide (5 mg/kg po)	45.35±1.72 ^c	43.6±2.14
III	MEHO (200 mg/ml po)	57.95±0.71 ^b	27.92±0.88
IV	MEHO (400 mg/ml po)	51.99±1.2 ^c	35.34±1.5

Values are mean ± SEM (n = 5); ^ap < 0.05, ^bp < 0.01 and ^cp < 0.001, Dunnett test as compared to negative control (saline). Statistical representation of the % of intestine cross by marker by *C. benghalensis* methanol extract, positive antidiarrheal control (Loperamide, 5 mg/kg p.o.) processed by paired t-test analysis (Dennett's test). Data were processed by paired t-test analysis using SPSS for windows, version 16.0.

Table 4. Anthelmintic activity of methanol extract of *C. benghalensis*.

Treatment	Time taken for paralysis (min)	Time taken for Death (min)
Control (Water)	0	0.00
Levamisole (1 mg/ml)	3.3±0.38	6.5±0.76
<i>C. benghalensis</i> (10 mg/ml)	12.38±0.43 ^b	21.39±0.50 ^b
<i>C. benghalensis</i> (5 mg/ml)	16.25±0.48 ^b	45.64±1.01 ^b
<i>C. benghalensis</i> (2.5 mg/ml)	22.17±0.49 ^b	57.25±1.18 ^b

Values are mean ± SEM, (n = 3); ^ap < 0.05, ^bp < 0.01 and ^cp < 0.001, Dennett's test as compared to positive control (Levamisole, 1 mg/ml). Statistical representation of the effective paralysis and dead time by *C. benghalensis* methanol extract, positive anthelmintic control (Levamisole, 1 mg/ml) processed by paired t-test analysis (Dennett's test). Data were processed by paired t-test analysis using SPSS for windows, version 16.0.

intestinal motility by the marker-charcoal meal. The 200 and 400 mg/kg oral dose of the extracts of *C. benghalensis* exhibited 57.95±0.7 and 51.99±1.2^c intestinal motility (Table 3). And the extracts significantly inhibited 27.92±0.88 and 35.34±1.5 % at all doses in intestinal motility. However, the standard drug, loperamide (5 mg/kg) demonstrated a significant inhibition (43.6±2.14%) in intestinal motility.

Anthelmintic activity

Consequences of the study were recorded as shown in Table 4 and Figure 1 as in the form of time required to get the following attacks of paralysis and at the end time required for complete death of parasite. From the aforementioned study, it was seen that the methanolic extract showed dose dependent anthelmintic activity as compared to a standard drug levamisole. Methanol extract *C. benghalensis* show the time taken for paralysis (12.38, 16.25, and 22.17 min) and time taken for death (21.39, 45.64, and 57.25 min) at dose 10, 5, and 2.5, respectively compared to a standard drug levamisole (1 mg/ml) that showed the time taken for paralysis (3.3±0.38 min) and time taken for death (6.5±0.76 min).

DISCUSSION

It is generally realized that castor oil is metabolized into

ricinoleic corrosive in the gut which is in charge of looseness of the bowels generation (Darracq et al., 2015). The peristaltic action of small digestive tract is expanded if ricinoleate is introduced in the small digestive system, thus Na⁺ and Cl⁻ porousness changed in intestinal mucosa (Tariq et al., 2015). Emission of endogenous prostaglandin is empowered additionally by ricinoleate (Beubler and Juan, 1979; Sadraei et al., 2014). The leaves extract of *C. benghalensis* at 200 and 400 mg/kg measurements showed enormous decrease of the quantity of diarrheal and total feces which may be because of the initiation of prostaglandin biosynthesis with resultant abatement in emission of liquid into the lumen or may be because of advancement and ingestion of water and electrolytes in the gut. The standard drug, loperamide (5 mg/kg) also produced statistically significant (p<0.01) diarrheal inhibition (62.07%). The intestinal volume was decreased by 33.11 and 45.25% at doses of 200 and 400 mg/kg, respectively. The aforementioned hypothesis was further upheld by the inhibitory activity of the extract on intestinal charcoal supper motility. Methanol extract of *C. benghalensis* smothered the propulsive development or travel of charcoal supper through the gastrointestinal tract which fundamentally shows that the extract may have the probability to decrease the recurrence of stooling in diarrheal conditions.

Anthelmintic medications act quickly and specifically on neuromuscular transmission of nematodes. Three major

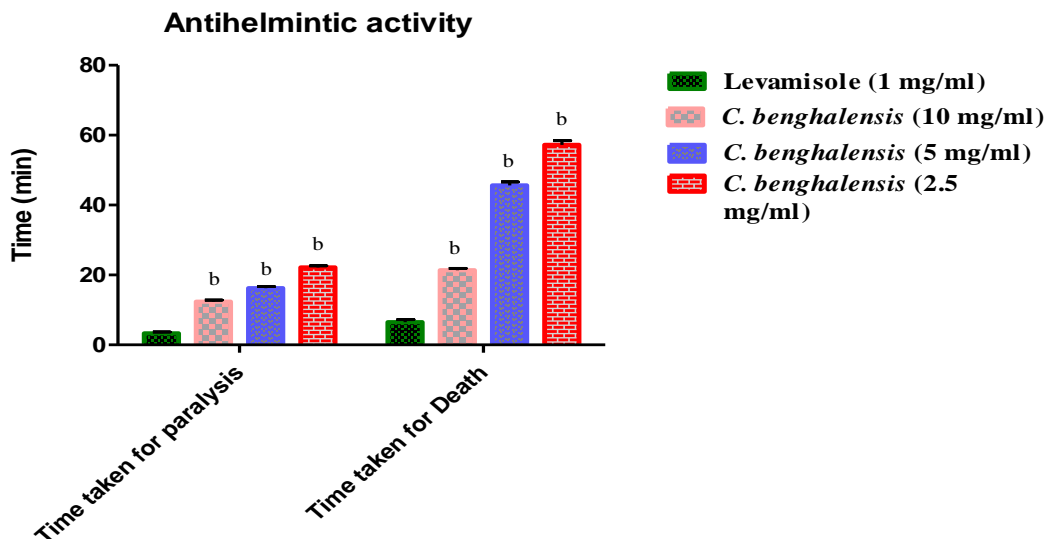


Figure 1. Anthelmintic activity of methanol extract of *C. benghalensis*. Values are mean \pm SEM ($n = 3$); ^a $p < 0.05$, ^b $p < 0.01$ and ^c $p < 0.001$, Dennett's test as compared to positive control (Levamisole, 1 mg/ml). Statistical representation of the effective paralysis and dead time by *C. benghalensis* methanol extract, positive anthelmintic control (Levamisole, 1 mg/ml) processed by paired t-test analysis (Dennett's test). Data were processed by paired t-test analysis using SPSS for windows, version 16.0.

groups of helminths (worms), the nematodes, trematodes, and cestodes- infect human (Mwale and Masika 2015; Pawluk et al., 2015; Romero et al., 2014). Nematodes are elongated roundworms that possess a complete digestive system, including both mouth and an anus. These cause the infections of the intestine as well as the blood and tissues. The trematodes (flukes) are leaf-shaped flatworms that are generally characterized by the tissues they infect. For example, they may be categorized as liver, lung, intestinal, or blood flukes. The cestodes or true tapeworms typically have a flat, segmented body and are attach to the hosts intestine. Levamisole are agonists at nicotinic acetylcholine receptors of nematode muscle and reason spastic loss of motion.

The methanol extract of *C. benghalensis* showed anthelmintic activity at a concentration of 10, 5 and 2.5 mg/ml. The anthelmintic effect of the extract is comparable with that of the effect produced by the standard drug levamisole (1 mg/ml) which also produced statistically significant ($p < 0.01$) anthelmintic activity time taken for paralysis (3.3 min) and time taken for death (6.5 min). The activity was concentration dependent. The plant possesses significant anthelmintic activity at 10, 5, and 2.5 mg/ml concentration measured by time taken for paralysis (12.38, 16.25, and 22.17 min) and death of the earth worm (21.39, 45.64, and 57.25 min) in that order.

Conclusion

This study underpins that the methanol extract of *C. benghalensis* leaves are the planned sources of

antidiarrheal and anthelmintic specialists in the conventional drug framework. In this case, studies are obliged to assist to distinguish the dynamic constituent(s) of the portions to comprehend the pharmacological activity of the antidiarrheal and anthelmintic impacts. The discoveries propose the legitimacy of the much lauded impact of *C. benghalensis* as antidiarrheal and anthelmintic specialists in expected home grown medication.

Conflict of Interests

The authors have not declared any conflict of interests.

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