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Evaluation of Anti-diarrhoeal Activity of *Enhydra fluctuans*

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The methanolic and aqueous extracts of *E. fluctuans* was evaluated in experimental diarrhoea, induced by castor oil in mice. Both methanolic and aqueous extracts, given orally at the dose, of 250 mg kg^{-1} body weight, showed significant anti-diarrhoeal activity as compared to that of the control. Both the extracts at the above dose levels also significantly reduced the intestinal transit of charcoal meal in mice. The *in vitro* antimicrobial activity of the extracts showed variable result. The methanolic extract moderately inhibited the growth of *Shigella dysenteriae, Shigella boydii* and *Shigella flexneri*, while the aqueous extract inhibited the growth of *Staphylococcus aureus, S. dysenteriae* and *S. boydii*. But both the methanolic and aqueous extracts did not show any significant effect on *Escherichia coli* and *Pseudomonas aerugenosa*. All the results indicated that the extract of *E. fluctuans* possesses anti-diarrhoeal activity.

Key words: *Enhydra fluctuans*, anti-diarrhoeal activity, intestinal transit, antimicrobial activity

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INTRODUCTION

Diarrhoea is a common disease in all parts of Bangladesh. Every year a large number of people are dying in this disease. There are large number of epidemiological and experimental evidence pertaining to worldwide acute diarrhoeal disease, which is one of the principle causes of death in the infants, particularly in malnourished in developing countries[1]. It thus becomes important to identify and evaluate commonly available natural drugs as an alternative to currently used anti-diarrhoeal drugs, which are not completely free from adverse effect[2].

E. fluctuans Lr. (Family: Compositae), a edible semi-aquatic herbaceous vegetable plant with serrate leaves, grows commonly all over the country. Locally it is known as Helencha and in English as Marsh herb. The leaves are slightly bitter, cure inflammation, leucoderma and good in small pox[3]. The leaves are also antibilious and used in nervous diseases[4]. It possesses nutritional value and its methanolic extract has been reported to have analgesic activity[5]. Chemical constituents like β-carotene[4,6], sesquiterpene lactones[7,8], terpenes[9] have been reported from this plant.

Traditional medicines are still very commonly used in Bangladesh for diarrhoeal disease. As our way to investigate local medicinal plants for their potential therapeutic uses, present study was aimed to investigate the anti-diarrhoeal activity of *Enhydra fluctuans*.

MATERIALS AND METHODS

Plant material and extraction: Whole plant of *E. fluctuans* was collected from the Campus of Khulna University, Khulna in May 2004 and was authenticated by the herbarium authority of Forestry Discipline, Khulna University, Khulna. The dried whole plant was coarsely powdered using a hammer mill and subjected to maceration process with methanol and water separately. After exhaustive extraction, the methanolic and aqueous extracts were dried at low temperature (55-60°C) using rotary evaporator and kept in desiccators. Dark blue-colored residue was obtained. The yields of both the extracts were 9 and 5% (w/w), respectively.

Animals: Swiss albino mice of either sex (20-25 g), obtained from the Animal House, Pharmacy Discipline, Khulna University, Khulna were used. The animals were housed under standard laboratory condition, fed with standard diet and had free access to tap water. All the experiments were performed after an overnight fast.

Anti-diarrhoeal activity studied by castor oil-induced diarrhoea: The animals were divided into four groups of five animals each. Group I received normal saline, which served as the control and the Group II received loperamide (3 mg kg^{-1}; p.o.), which served as the positive control. Group III and IV received methanolic extract (250 mg kg^{-1}; p.o.) and aqueous extract (250 mg kg^{-1}; p.o.), respectively. Half an hour after the treatment, each animal of all the groups received castor oil (Paras Chemicals) at the dose of 0.5 mL orally. Following the castor oil administration, the animals were placed separately in acrylic cages with filter paper, which was changed every hour. The severity of diarrhoea was assessed each hour for 4 h. The total number of faces and wet faces excreted in throughout the total experimental period were scored and compared with control group. The results were expressed as percentage of inhibition[10].

Study on small intestinal transit: Mice were divided in to four groups of five animals each. Group I served as the control and was orally administered with the vehicle (0.5% CMC). The Group II received orally the standard drug, atropine sulphate at the dose of 5 mg kg^{-1}. The Group III and IV received methanolic and aqueous extracts of *E. fluctuans* at the dose of 250 mg kg^{-1} orally. Half an hour after treatment, individual animals were administered orally with 1 mL of charcoal meal (3% deactivated charcoal in 10% CMC). Half an hour after charcoal treatment, each mouse was sacrificed and the total intestinal distance from pylorus to caecum as well as the distance traveled by the charcoal meal was measured and expressed as percentage of distance moved[11].

***In vitro* antimicrobial activity screening:** The methanolic and aqueous extract of *E. fluctuans* leaves were investigated for antimicrobial activity against a number of pathogenic microorganisms *in vitro* by disk diffusion method[12,13]. Sterile Matricel (BBL, Cocksville, USA) filter paper discs were impregnated with known amount of test substances using micropipette and dried. These discs, along with standard disks (Kanamycin, Oxoid Ltd., UK) and control disks were placed in petri dishes containing a suitable agar medium seeded with the test organisms using sterile transfer loop and kept at 4°C to facilitate maximum diffusion. The plates then kept in an incubator (37°C) to allow the growth of the microorganisms. The antibacterial activity of the test agent was determined by measuring the diameter of the zone of inhibition in terms of millimeter. Antimicrobial activity was tested against

Escherichia coli, *Vibrio cholerae*, *Shigella dysenteriae*, *S. boydii*, *S. flexneri*, *S. sonnei*, *Salmonella typhi*, *Enterobacter aerogenes*, *Staphylococcus aureus*, *S. epidermis*, *S. pyogenes* and *Pseudomonas aeruginosa*.

Statistical analysis: Statistical significance was calculated by student's t-test.

RESULTS AND DISCUSSION

The methanolic and aqueous extracts, at the dose of 250 mg kg^{-1} body weight, inhibited castor oil induced diarrhoea and the inhibition was 41.18% ($p<0.001$) and 67.07% ($p<0.001$), respectively. The standard drug loperamide (3 mg kg^{-1}) produced an inhibition of 84.70% ($p<0.001$) (Table 1). The result shows that the effect was less potent with the methanolic extract. Moreover the reduction in the total number of wet faces by the methanolic extract failed to show statistical significance.

Table 1: Effect of *Enhydra fluctuans* methanolic and aqueous extract on castor oil- induced diarrhoea in mice[a]

Groups	Dose mg kg^{-1}; p.o.)	Total No. of faeces in 4 h	Total No. of wet faeces in 4 h	Inhibition (%)
I	-	17.00±0.70	10.00±1.70	-
II	3	2.60±0.74***	1.50±0.37**	84.70
III	250	10.00±1.81**	5.80±0.86	41.18
IV	250	5.60±0.92**	3.40±0.51**	67.07

Table 2: Effect of *E. fluctuans* methanolic and aqueous extract on the intestinal transit of charcoal meal in mice[a]

Groups	Dose (mg kg^{-1}; p.o.)	% Traversed by charcoal meal
I	-	74.70±2.68
II	5	41.22±1.73***
III	250	66.88±3.98
IV	250	60.58±1.21

[a]Values are mean±SE (n=5), ***$p<0.001$, **$p<0.01$ vs. control, Student's t-test

Table 3: Antibacterial activity of *Enhydra fluctuans* methanolic and aqueous extract

Microorganisms	Diameter of zone of inhibition in mm		
	Methanolic extract (500 µg $disc^{-1}$)	Aqueous extract (500 µg $disc^{-1}$)	Kanamycin (30 µg $disc^{-1}$)
Escherichia coli	-	-	30
Vibrio cholerae	-	-	29
Shigella dysenteriae	13	15	29
Shigella boydii	15	16	27
Shigella flexneri	10	5	25
Shigella sonnei	-	-	30
Staphylococcus aureus	6	12	27
Staphylococcus epidermis	-	-	28
Staphylococcus pyogenes	-	-	25
Salmonella typhi	-	-	28
Pseudomonas aeruginosa	-	-	27
Enterobacter aerogenes	-	-	30

In the intestinal transit test, both the extracts delayed gastrointestinal transit of charcoal meal significantly in test animals as compared to the control. The aqueous extract delayed the intestinal transit as compared to the control and the result was statistically significant. On the contrary, though the metahanolic extract delayed the intestinal transit to some extent but the result was not statistically significant (Table 2).

The aqueous extract (500 µg $disc^{-1}$) was found to be sensitive against *Staphylococcus aureus, Shigella dysentery, S. boydii* whereas the methanolic extract (500 µg $disc^{-1}$) was found to be sensitive against *Shigella dysentery*, *S. boydii* and *S. flexineri*. Both the extracts failed to inhibit the growth of *Escherichia coli* and *Pseudomonas aerugenosa* (Table 3).

Castor oil causes diarrhoea through its active metabolite recinoleic acid[14,15], which stimulates the peristaltic activity of small intestine leading to changes in electrolyte permeability of intestinal mucosa. Its action is also associated with stimulation of release of endogenous prostaglandins[16]. *E. fluctuans* has been reported to have analgesic activity[5]; hence the secondary inhibition of prostaglandins may also be a factor in its anti-diarrhoeal activity.

In the present study, the aqueous extract found to reduce the severity of diarrhoeal episode to a grater extent as compared to the methanolic extract. In the gastrointestinal motility test aqueous extract significantly ($p<0.001$) reduced intestinal transit of charcoal meal whereas the result for methanolic extract failed to show statistical significance. Delay in the gastrointestinal transit may also pertain to the greater activity of the aqueous extract in the castor oil induced diarrhoea model.

The antimicrobial activity of any extract would be useful in its anti-diarrhoeal activity when the cause of diarrhoea is respective microorganism. The antimicrobial activity revealed that both the extracts were sensitive against *Shigella dysentery*, *S. Sonnei, S. flexneri, Staphylococcus aureus,* some of the common microorganisms that causes diarrhoea[17] and may contribute to some extent in its anti-diarrhoeal effect.

CONCLUSIONS

This study establishes the use of *E. fluctuans* as an anti-diarrhoeal agent as claimed by the folk practitioner. Aqueous extract seems to be more active than methanolic extract, with significant anti-motility effect on the intestine. Further studies on chemistry of *E. fluctuans* and on other pharmacological factors influencing anti-diarrhoeal activity may provide more insights on its activity.

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