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EVALUATION OF ANTIDIARRHEAL ACTIVITY OF *Clerodendrum viscosum* Vent.

M.M. RAHMAN[1*], M.N. HASAN[2] AND F. SABRIN[1]

[1]Department of Biotechnology and Genetic Engineering , Mawlana Bhashani Science and Technology University, Santosh, Tangail-1902, Bangladesh ; [2]Department of Genetic Engineering and Biotechnology, Jessore Science & Technology University, Jessore-7408, Bangladesh.

[*]Corresponding Author & address: Md. Masuder Rahman, E-mail: masudmbstu@gmail.com
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ABSTRACT

Rahman MM, Hasan MN, Sabrin F (2011) Evaluation of antidiarrheal activity of *Clerodendrum viscosum* Vent. *J. Innov. Dev. Strategy* 5(2), 56-61.

Clerodendrum viscosum Vent. (Verbenaceae), a medicinal shrub, commonly known as glorybower, has been reported to possess antidiabetic, antidiarrheal, antidysenteric, antihematuria and antihemorrhagic properties. However, the scientific basis of its antidiarrheal potential is still unknown. The antidiarrheal activity of ethanolic leaf extract of *C. viscosum* Vent. was evaluated on castor oil-induced diarrhea, gastrointestinal transit, intestinal fluid accumulation and gastric emptying in mice. The ethanolic extract of the leaves showed inhibitory activity against castor oil induced diarrhea. A significant reduction ($p< 0.001$) in the gastrointestinal motility was observed in charcoal meal test in mice. The extract also decreased volume of intestinal secretion significantly induced by castor oil in the test animals ($p< 0.001$) as evident by gastric emptying as compared to controls. Inhibition of the gastrointestinal propulsion and fluid secretion by the extract suggested that the extract might have exerted its antidiarrheal activity by antisecretory mechanism.

Key words: *antidiarrheal, castor oil, fluid accumulation, gastrointestinal transit, Clerodendrum viscosum*

INTRODUCTION

Clerodendrum is a genus of about 400 species of flowering plants in the family Verbenaceae (or Lamiaceae). The genus is native to tropical and warm temperate regions of the world, with most of the species in tropical Africa and southern Asia, but a few in the tropical America and northern Australasia, as well as extending north into the temperate zone in eastern Asia. Common names include glorybower, bagflower and bleeding-heart. They are shrubs, lianas, and small trees, growing up to height of 1-12m with opposite or whorled leave. *Clerodendrum* species are used as food plants by the larvae of some Lepidoptera species including *Endoclita malabaricus* and *Endoclita sericeus*.

Diarrhea is too frequent, often too passage of poorly formed stools. In, pathological term, it occurs due to passage of excess of water in faeces (Tripathi 2001). In the ileum and colon, active Na^+K^+-ATPase mediated salt absorption occurs and water follows iso-osmotically. In addition, glucose facilitated Na^+ absorption takes place in the ileum. This mechanism remains intact even in severe diarrhea. Diarrhea associated with carcinoid (secreting 5-HT) and medullary carcinoma of thyroid (secreting calcitonin) is mediated by cAMP. Excess of bile acids also causes diarrhea by activating adenyl cyclase (Tripathi 2001).

Khatry *et al.* (2005) studied antinociceptive, anti-inflammatory and diuretic activities of methanolic extract of the aerial parts of *C. viscosum* Vent. The effect of MeCV (Methanolic extract of *C. viscosum*) on acetic acid-induced writhing at doses of 150 and 300 mg/kg body weight was compared to that of aminopyrine at a dose of 50 mg/kg body weight. MeCV exhibited statistically significant ($p<0.001$) inhibition of acetic acid-induced writhing by 37.95 and 54.91% at doses of 150 and 300 mg/kg body weight respectively, in a dose-dependent manner (correlation co-efficient, r = 0.98). Ahmed *et al.* (2007) studied on anti-inflammatory, antinociceptive, neuropharmacological and the effect of MeCV on acetic acid-induced writhing at doses of 150 and 300 mg/kg body weight and was compared to that of aminopyrine at a dose of 50 mg/kg body weight with the activities of *C. viscosum* Vent. The crude methanolic leaf extract of *C. viscosum* Vent. was evaluated for these purposes. In the present study, ethanolic extract of the leaves were investigated for its potential activities against antidiarrheal properties in mice.

MATERIALS AND METHODS

Extraction

For the purpose of hot extraction, Soxhlet apparatus was used. First, a thimble was made by thick filter paper & 170 gm ground powder was placed inside the thimble, and was then loaded into the main chamber of the Soxhlet extractor. The Soxhlet extractor was placed onto a flask containing the extraction solvent. The Soxhlet was then connected with a condenser. The solvent was heated to reflux. The solvent vapour traveled up in the distillation arm and was then flooded into the chamber housing the thimble of solid. The condenser ensured that cool solvent vapour, dripped back down into the chamber housing the solid material. The chamber containing the solid material was slowly filled with warm solvent. Some of the desired compound was then dissolved into warm solvent. When the Soxhlet chamber was almost full, the chamber was automatically emptied by a siphon side arm, with the solvent running back down to the distillation flask. This cycle was repeated several times for proper extraction. During each cycle, a portion of the non-volatile compound was dissolved into the solvent. After several cycles the desired compound was concentrated into the distillation flask. The advantage of this system was that instead of many portions of warm solvent being passed through the sample, just one batch of solvent was recycled.

After extraction the solvent was removed, firstly by means of a water bath and in the oven, yielding the extracted compound. The non-soluble portion of the extracted solid remained in the thimble, and was discarded. The obtained concentrate was reddish in color. The concentrate was then designated as crude ethanolic leaf extract of *C. viscosum* Vent.

Study of Antidiarrheal Activity of by Castor Oil Induced Diarrhea in Mice

Principle

Diarrhea was defined by the presence of stool or any fluid material staining the absorbent paper placed beneath the cage. Time taken before the first defecation was the 'Latent period'. The total count stool and latent period of test group were compared with positive control groups. Antidiarrheal agent was supposed to increase latent period and decrease total stool count.

Mechanism of Diarrheal Action induced by Castor Oil

Upon oral administration, castor oil mixes with bile and pancreatic enzymes and liberates ricinoleic acid from triglyceride (Fig. 1). A small amount of ricinoleic acid is absorbed in the gastrointestinal tract and metabolized like any other fatty acid but most remains in the intestine where it produces its anti-absorptive or secretory effect. The ricinoleic acid thus liberated readily forms ricinoleate salts with sodium and potassium in the lumen of the intestine. The ricinoleate salt formed as such behaves like a soap or surfactant within the gut and at the mucosal surface. The precise interaction with serotonin (5-HT) has been termed as "diarrheagenic hormones" in action. The mechanism how these ricinoleate salts induce diarrhea is not still known. But most agreed view is that it stimulates the intestinal epithelial cell's adenyl cyclase, releases prostaglandins and specially prostaglandins of the E series along" (Tripathi 2001).

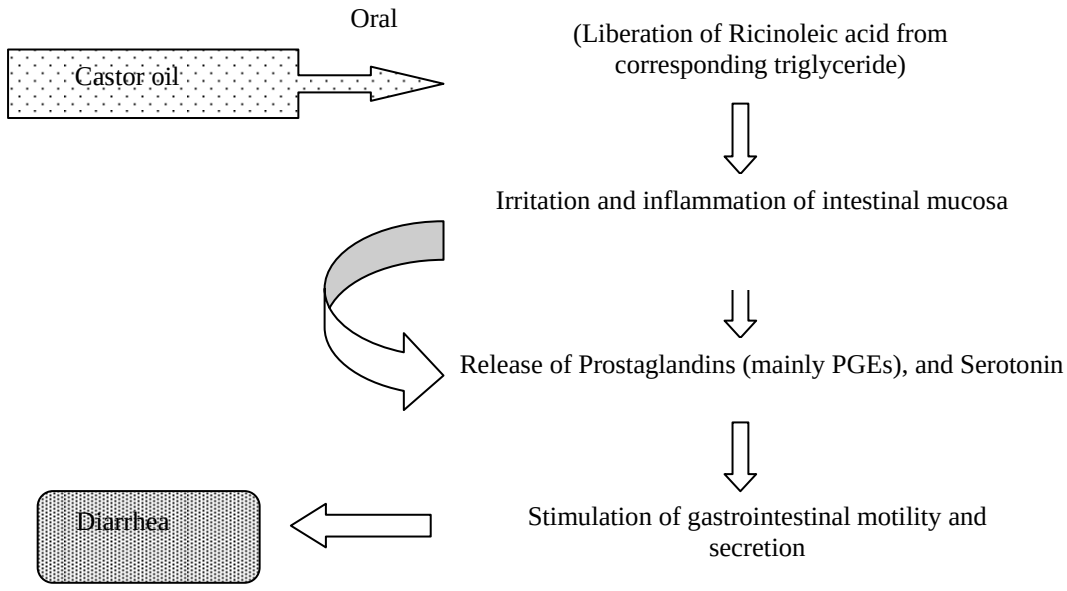

Fig. 1. Mechanism of diarrheal action of castor oil

Study Design

Young Swiss-albino mice aged 4-5 weeks with average weight 18-25gm were used for the experiment. The mice were purchased from the Jahangirnagar University, Dhaka, Bangladesh. They were kept in standard environmental condition for a week for adaptation after purchasing and fed properly. The mice were all screened initially by giving 0.3 ml of castor oil and only those showing diarrhea were selected for the final experiment. The test animals were randomly chosen and divided into four groups having five mice in each; they were accurately weighed & properly marked. Of the experimental groups, group-I or the control received only distilled water containing 1% Tween-80. Group-II or the positive control received standard ant-motility drug, Loperamide at a dose of 3mg/kg-body weight as oral suspension. The test groups (I & II) were treated with suspension of leaves' extract of *C. viscosum* Vent. at the oral dose of 250 & 500 mg/kg body weight.

Preparation of Sample

To prepare suspension of the test samples at the doses of 250 & 500 mg/kg per body weight, 250 & 500mg of crude ethanol extracts were measured respectively. The extract was triturated in unidirectional manner by the addition of small amount of Tween-80. After proper mixing of extract and Tween-80, the distilled water was slowly added. The final volume of the suspensions was made 10 ml allowing final concentration 50 & 25mg/ml

respectively. For the preparation of standard drug, 3mg of Loperamide was taken & was triturated in unidirectional manner by the addition of small amount of Tween-80. After proper mixing, distilled water was slowly added up to the final volume 10 ml and thus concentration became 0.3mg/ml. The amount to be administered for obtaining desired concentration was calculated using the formula (Body weight of mice × 0.01) ml.

METHODOLOGY

The method, described by Taufiq *et al.* (2005), was followed for this study. Test samples, control and Loperamide were given orally by means of a feeding needle (Table 1). The mice were fed with the samples, control and Loperamide 40 min prior to the oral administration of castor oil at a dose of 0.3ml per mouse. Individual animals of each group were placed in separate cages having adsorbent paper beneath and examined for the presence of diarrhea every hour in four hours' of study after administration of castor oil. Number of stools or any fluid material that stained the adsorbent paper were counted at each successive hour during the 4 h period and were recorded for each mouse. The latent period of each mouse was also counted. At the beginning of each hour fresh papers were replaced for the old ones. During an observation period of 4 h, the total number of faecal output including diarrheic faeces excreted by the animals was recorded. A numerical score based on stool consistency was assigned as normal stool equivalent to 1 while watery stool to 2.

Table 1. Experimental design showing leaf extract of *C. viscosum* on castor oil induced diarrhea in mice.

Animal Group	No. of mice	Treatment	Dose (/kg.body wt.)	Route of administration
I (Control)	5	Water containing 1% Tween80	10ml	Oral
II (Positive control)	5	Loperamide	50mg	Oral
III (Test group-1)	5	Leaves extract of *C. viscosum*	250mg	Oral
IV (Test group-2)	5	Leaves extract of *C. viscosum*	500mg	Oral

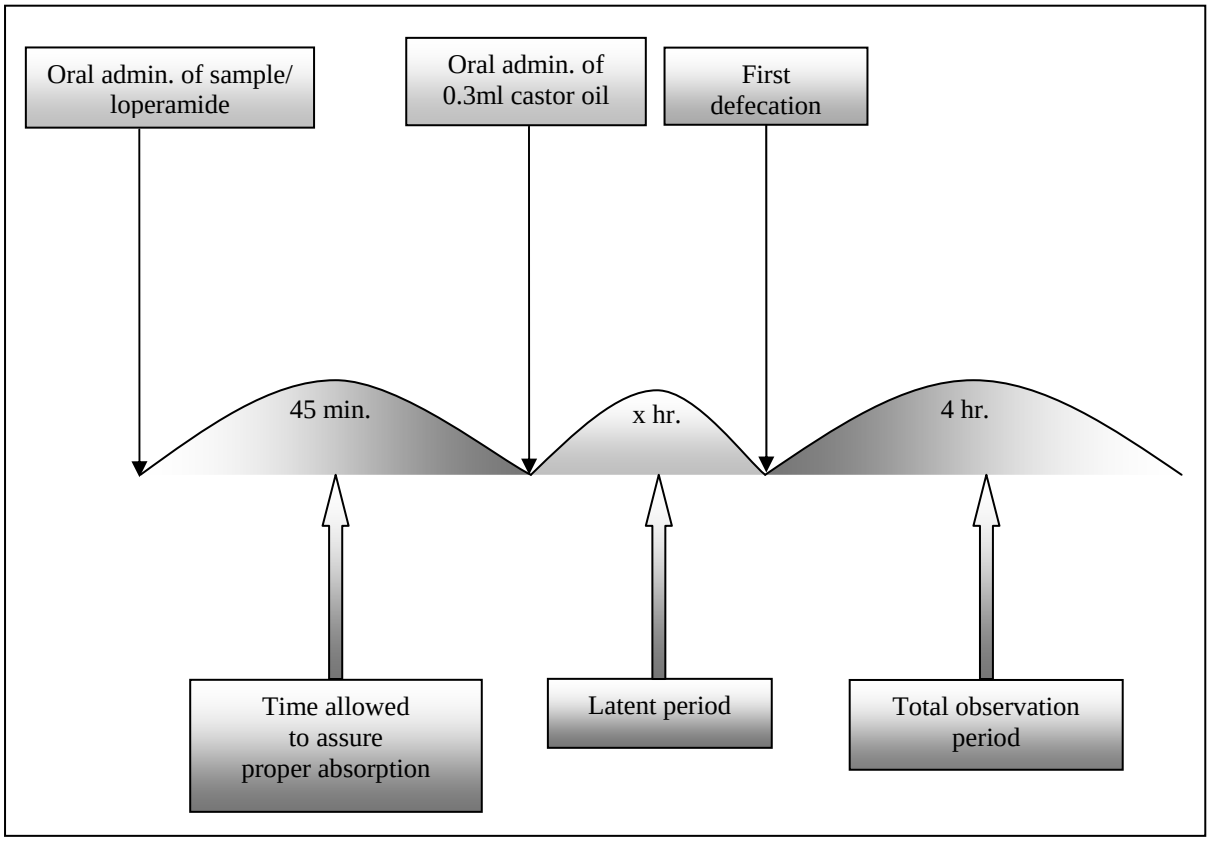

Fig. 2. Schematic representation for study design of anti-diarrheal activity observation in test mice with castor oil induced diarrhea

RESULTS AND DISCUSSION

Evaluation of Antidiarrheal Activity

The amount of extract was calculated which represented 22.26% of plant material. The result of the phytochemical screening revealed the presence of alkaloids, flavonoids, glycosides, saponins and volatile oil.

Table 2. Effect of the leaves of *C. viscosum* Vent. on the latent period of castor oil induced diarrheal episode in mice

Group (dose)	Number-ing of mice	Weight (gm)	Latent period (h)	Mean of latent period(h)	Standard Deviation (SD)	Standard Error (SE)	t-test (P-value)
I (Control)	1	22gm	0.75	0.98	0.29	0.15	_
	2	20 gm	1.47				
	3	19 gm	0.92				
	4	20 gm	0.68				
	5	21 gm	1.17				
II (Standard /positive control) Loperamide, 50mg/Kg	1	21 gm	1.93	1.97	0.26	0.13	5 (p<0.01)
	2	22gm	2.20				
	3	18 gm	1.77				
	4	23gm	1.65				
	5	20 gm	2.33				
III (Test group) 250mg/kg	1	22gm	1.83	2.13	0.20	0.10	6.37 (P<0.001)
	2	24gm	2.41				
	3	20gm	2.16				
	4	22gm	2.00				
	5	23gm	2.25				
IV (Test group) 500mg/kg	1	24 gm	2.08	2.16	0.15	0.07	7.12 (P<0.001)
	2	20gm	2.25				
	3	24 gm	2.33				
	4	22 gm	2.25				
	5	24 gm	1.91				

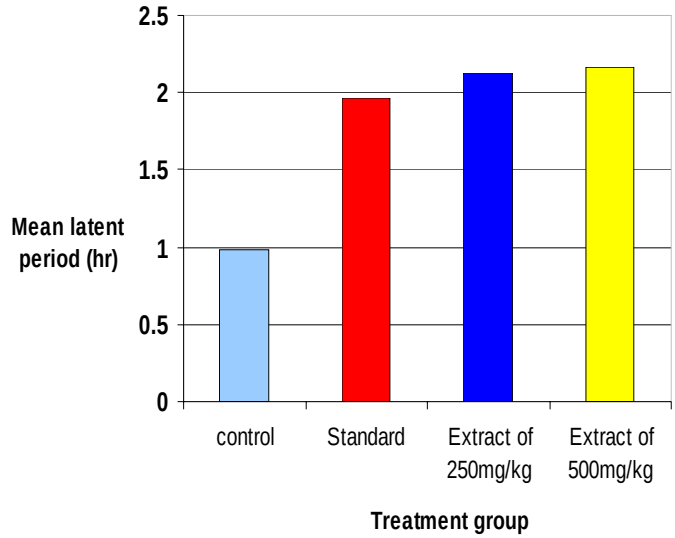

Fig. 3. Effect of crude extract of *C. viscosum* Vent. leaves on the latent period of castor oil induced diarrhea in mice

Table 3. Effect of the leaves of *C. viscosum* Vent. on the basis of mean stool count of castor oil induced diarrheal episode in mice

Group	Treatment	Mice no.	Number of faeces	Mean of faeces	SD	SE	t-test (P-value)
I Control	Water containing 1% Tween 80	C1	3	7.6	2.87	1.43	_
		C2	10				
		C3	8				
		C4	11				
		C5	6				
II Positive control	Loperamide (50mg/kg)	P1	2	2.4	1.01	0.51	3.43 (P<0.001)
		P2	3				
		P3	1				
		P4	4				
		P5	2				
III Test group-1	Et. extract (250mg/kg)	S1	7	6	0.89	0.44	1.06 (P<0.25)
		S2	5				
		S3	7				
		S4	5				
		S5	6				
IV Test group-2	Et. extract (500mg/kg)	T1	6	4.2	1.60	0.80	2.07 (P<0.1)
		T2	4				
		T3	2				
		T4	3				
		T5	6				

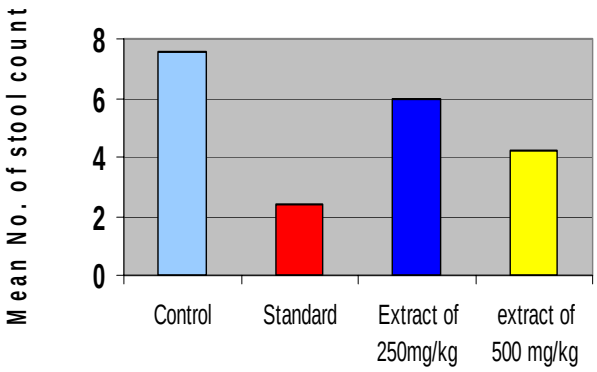

Fig. 4. Effect of leaf extracts of *Clerodendrum viscosum* Vent. on the basis of mean stool count on castor oil induced diarrhea in mice

For testing whether any antidiarrheal activity of this plant existed, a method was employed which involved the induction of diarrhea in mice by castor oil, and was subsequently treated with standard drug as control and test extract respectively. The treatments were compared in regard to latent period as well as stool count. The mice which were treated only with castor oil showed increased stool count, and when treated with standard drug, stool count was reduced. If the extract under investigation was able to reduce the stool count, it was said to possess antidiarrheal activity.

Table 2 and Fig. 3 showed the effect of *C. viscosum* Vent. on the latent period of castor oil induced diarrheal episode in the dose of 250mg/kg and 500mg/kg body weight where as Table 3 and Fig. 4 showed the effect of *C. viscosum* Vent. on the basis of mean stool count of castor oil induced diarrheal episode in mice. The extract significantly increased the latent period in comparison to standard drug. The leaf extract of *C. viscosum* Vent. could reduce the total number of faeces as well as of diarrheic faeces in a dose dependent manner, and the result

was found to be significant. So from the study it could be interpreted that the leaves of *C. viscosum* Vent. possessed antidiarrhoeal activity. A further investigation could put more insight into elucidating the mechanism of action and the clinical importance of the plant.

CONCLUSION

The present study was aimed to evaluate the pharmacological properties of the leaves of *C. viscosum* Vent**.** based on parameters both latent period and number of stools. The ethanolic leaf extract of of *C. viscosum* Vent. showed moderate anti-diarrhoeal activity at both 250 mg/Kg and 500 mg/Kg body weight doses compared to the standard antidiarrhoeal drug Loperamide (50 mg/kg body weight). As all the experiments were conducted with some limitations, further pharmacological and toxicological study is required to establish the therapeutic potential of this plant.

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