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Screening of antidiarrhoeal activity of three reputed medicinal plants of Bangladesh

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ABSTRACT

Anonna squamosa Linn. (Fam: Anonnaceae), *Calotropis procera* R. Br. (Fam: Asclepiadaceae), and *Piper betel* Linn. (Fam: Piperaceae) leaves have been used in conventional therapies for various disease conditions, including diarrhea. So, the present study was aimed to evaluate the antidiarrhoeal activity of ethanol extracts of the leaves of the above mentioned plants. Antidiarrhoeal activity was evaluated against an experimental model of castor oil-induced diarrhea in mice. In antidiarrheal activity test, oral administration of the mentioned ethanol extracts (250 and 500 mg/kg) showed significant ($P<0.01$) and dose-dependent inhibition. Onset of diarrhea after administration of 250 mg/kg and 500 mg/kg of *A. squamosa* were 88.8 mins and 102.6 mins, of *C. procera* were 83.4 mins and 118.2 mins, and of *P. betel* were 81.0 mins and 115.8 mins, respectively as compared to 70.2 mins and 131.4 mins demonstrated by control (1% Tween-80 in saline) and positive control (Loperamide), respectively. The result indicates the presence of some active principles in the plant extracts which might have a correlation with the revealed anti-diarrhoeal activities of these plants. The results also justify their traditional uses in the treatment of diarrhea.

KEYWORDS: *Anonna squamosa*; *Calotropis procera*; *Piper betel*; Castor oil-induced diarrhea; Anti-diarrhoeal activity;Traditional medicine etc.

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INTRODUCTION

Anonna squamosa Linn. (Fam: Anonnaceae) is a small tree, about 3 to 6 meters high, with oblong to lanceolate leaves, greenish flowers and warty skinned segmented sweet fruits, planted as a fruit plant in different areas of Bangladesh. Leaves and tender stems of it contain the alkaloids, anonaine, roemerine, norcorydine, corydine, isocorydine, nor-isocorydine, norlaurelinen glaucine, xylopine and lanuginosine[1].Its seed oil is rich in the unsaturated fatty acids- oleic and linoleic acids. Leaves and fruits of *Anonna squamosa* Linn. are used for treating tumors and their extracts possess spasmogenic, spasmolytic and oxytocic properties[2,3]. They also show significant anti-cancer and anti-diabetic activities[4] .Leaves, bark and unripe fruits of the plant are used to treat diarrhea and dysentery [5]. Ripe fruit can be used as tonic, laxative and anthelmintic. Root is considered as a drastic purgative. Seeds are abortifacient. Leaves, fruits and seeds are also used as insecticides. The acetogenin and squamotacin possesses antitumor property. The aporphines and oxoaporpines isolated from the plant possess strong antiplatelet and vasorelaxing actions[6].*Calotropis procera* R. Br. (Fam: Asclepiadaceae) is a plant with broad obovate fleshy leaves but it is a white flowered and small in size. It is perennial shrub abounding in milky latex and it grows commonly in wastelands and graveyards in all over Bangladesh. Root bark of the plant is useful in treating chronic cases of dyspepsia, flatulence, constipation, loss of appetite, indigestion and mucous in stool. Extracts of its leaves and roots stimulate respiration and blood pressure in dog, and are used in cardiac arrhythmia, rheumatism and cancer. Flowers of the plant are useful in asthma. Calotropin has digitalis-like actions [7]*Piper betel* Linn. (Fam: Piperaceae) is a stout twining climber with broadly ovate oblong or ovate cordate leaves, tiny yellow-green flowers and small spherical fruits. It is extensively cultivated as a cash crop throughout Bangladesh. Leaves of the plant are popularly used as carminative, astringent, stimulant and antiseptic drug. They are also used in headache and cough of children. Leaf stalk of the said plant is used as a suppository for congestion. Roots of the plant induce permanent sterility in women and extract of its leaves exerts anti-tumor activity in carcinogenesis, and suppresses mutagenic and carcinogenic actions of tobacco specific nitrosamines[8].Plants have been one of the important sources of medicines since the beginning of human civilization. There is a growing demand in the present world for plant based medicines, health products, pharmaceuticals, food supplements, cosmetics etc[9].We,

therefore, report here in the results of preliminary antidiarrhoeal screening of the ethanolic extracts of the above mention plants.

MATERIALS AND METHODS

Collection and preparation of the plant materials

A squamosa and *C procera* leaves were collected from Jahangirnagar University campus, Dhaka, Bangladesh and *P betel* was from the local market of Dhaka, Bangladesh in February 2011, of which voucher specimens (nos. 39316, 37537 and 37536, respectively) have been deposited in the Bangladesh National Herbarium. Leaves of the above mentioned plants were dried under shed for several days after washing[10,11]. The plant parts were then ground to coarse powder using Noka Super Blender, Japan. Each of the powdered materials (300 gm) was then soaked in ethanol (1.5 liter) separately and kept for 14 days at room temperature with occasional shaking. The crude extracts were then filtered through cotton plug followed by Whatman number-1 filter paper (Bibby RE200, UK) individually and the extracts were finally concentrated, one by one, with a vacuum rotary evaporator (Buchi, Switzerland). It rendered solid residues of specific colors which were designated as the ethanol extracts and stored in refrigerator until further investigation[12].

Experimental animals

Young Swiss-albino mice (Webstar strain) of either sex, weighing 20-25 gm, were purchased from the Animal Research Branch of the International Centre for Diarrhoeal Disease and Research, Bangladesh (ICDDR, B). The animals were kept at animal house (Department of Pharmacy, Northern University Bangladesh) for adaptation after their purchase under standard laboratory conditions (Relative humidity 55-65%, Room temperature 25.0±2.0°C and 12 h light-dark cycle) and fed with standard diets (ICDDR, B formulated) and had free access to tap water and libitum. All animal experiments were conducted in an isolated and noiseless condition in accordance with the guidelines of the Animal Ethics Committee of Northern University Bangladesh (112/B/NUB/2012).

Drugs

Loperamide, 1% Tween-80, castor oil (finest cold drawn commercial castor oil)

Antidiarrhoeal test.

Published methods[13,14] were followed for this study with slight modification. The mice were screened initially by giving 0.5 mL of castor oil and only those showing diarrhoea were selected for the experiment. The test animals fastened overnight were randomly divided into four groups consisting of five mice in each group. Group I was denoted as 'control group' which received 0.1% (v/v) Tween 80 in distilled water ; Group II was denoted as 'positive control' and received standard antimotility drug loperamide **(**50 mg/kg body weight**)** as oral suspension. Group III and Group IV were test groups and were treated with the plant extracts at dose of 250 mg/kg and 500 mg/kg body weight, respectively. After one hour treatment with control, positive control or plant extracts, each animal was given 0.5 mL of castor oil by oro-gastric polyethylene catheter and placed in separate cages having adsorbent paper beneath. The characteristic diarrhoeal droppings were noted every hour in five hours study for each mouse. At the beginning of each hour old papers were replaced with the new ones.

Statistical Analysis. All the data obtained were expressed as the mean ± standard error of mean (SEM). Statistical differences between the treatments and the controls were estimated by Statistical Package for Social Science (SPSS, version 11.5) software for Windows followed by the student's t-test. P values less than 0.05 was considered to be statistically significant.

RESULT AND DISCUSSION

Castor oil (0.5 mL, p.o.) induced diarrhoea promptly within approximately one hour in all the animals and produced a considerable amount of stool. The time for diarrhoeal induction was significantly prolonged by administration of ethanol extracts of leaves of *A squamosa* (250 and 500 mg/kg), *C procera* (250 and 500 mg/kg) and *P betel* (250 and 500 mg/kg), in mice in dose-dependent manner (Table 2). Onset of diarrhea after administration of 250 mg/kg and 500 mg/kg of *A squamosa* were 88.8 mins and 102.6 mins, of *C procera* were 83.4 mins and 118.2 mins, and of *P betel* were 81.0 mins and 115.8 mins, respectively as compared to 70.2 mins and 131.4 mins demonstrated by control (1% Tween-80 in saline) and positive control (loperamide), respectively. None of animals treated

with 500 mg/kg of the plant extracts showed diarrhoea up to at least one and half hour after administration of *A squamosa, C procera P betel.* Incase of dose of 250 mg/kg body weight, none of the animals showed diarrhea up to at least one and half hour after administration of *A squamosa* and *C procera*. On the other hand the plant extracts significantly reduced the number of diarrhoeal episodes in comparison to control animals (Figure 1). From the result of diarrhoeal episode it also can be observed that the order of antidiarrhoeal activity are *P betel* > *A squamosa* > *C procera* in both of the experimental doses. The standard antimotility and antisecretory[15]drug, loperamide (50 mg/kg) also profoundly prolonged, and reduced the onset of castor oil-induced diarrhoea and the number of diarrhoeal episodes, respectively. So on the basis of the results , it can be said that the ethanolic extracts of leaves of the studied plants possess antidiarrhoeal activity which is at a higher dose (500 mg/kg) comparable to that of loperamide at a dose of 50 mg/kg. phytoconstituent like flavonoids have been known to inhibit diarrhea induced by castor oil[16].Flavonoids have also been reported to have antimicrobial activities[17].The presence of flavonoids in all the plants extract could be responsible for their anti-diarrheal activity. This justifies the ethnomedicinal uses of the plants.

Table 1. Effect of of *Anonna squamosa* ,*Calotropis procera* and *Piper betel* leaves on onset of diarrhea induced by castor oil and inhibition of defecation.

Groups		**Treatment and dose**	**Route of Administration**	**Onset diarrhea (min)**	**Total no. of stools in**	**%inhibition of defecation**
Controls	Group I: Control	1% Tween-80 in water	Oral	70.2±20.7	14	-
	Group II: Positive control	Loperamide, 50 mg/ kg body wt.	Oral	131.4±17.3 *	07	50%
Experimental	Test group– IIIAS 250	Extract of *A. squamosa* , 250 mg/kg body wt.	Oral	88.8±6.3*	12	14.2%
	Test group - IV AS 500	Extract of *A. squamosa*, 500 mg/kg body wt.	Oral	102.6±17.1	9	21.4%
	Test group– IIICP250	Extract of *C. procera*, 250 mg/kg body wt.	Oral	83.4±5.9*	13	7.1%
	Test group - IVCP 500	Extract of *C. procera*, 500 mg/kg body wt.	Oral	118.2±19.8 *	10	28.5%
	Test group – IIIPB250	Extract of *P. betel*, 250 mg/kg, body wt.	Oral	81.0±5.7*	11	21.4%
	Test roup- IVPB 500	Extract of *P. betel*, 500 mg/kg body wt.	Oral	115.8±19.4 *	08	42.8%

Values are expressed as mean ±SEM (n=5), P < 0.01 vs control, Student's t-test.

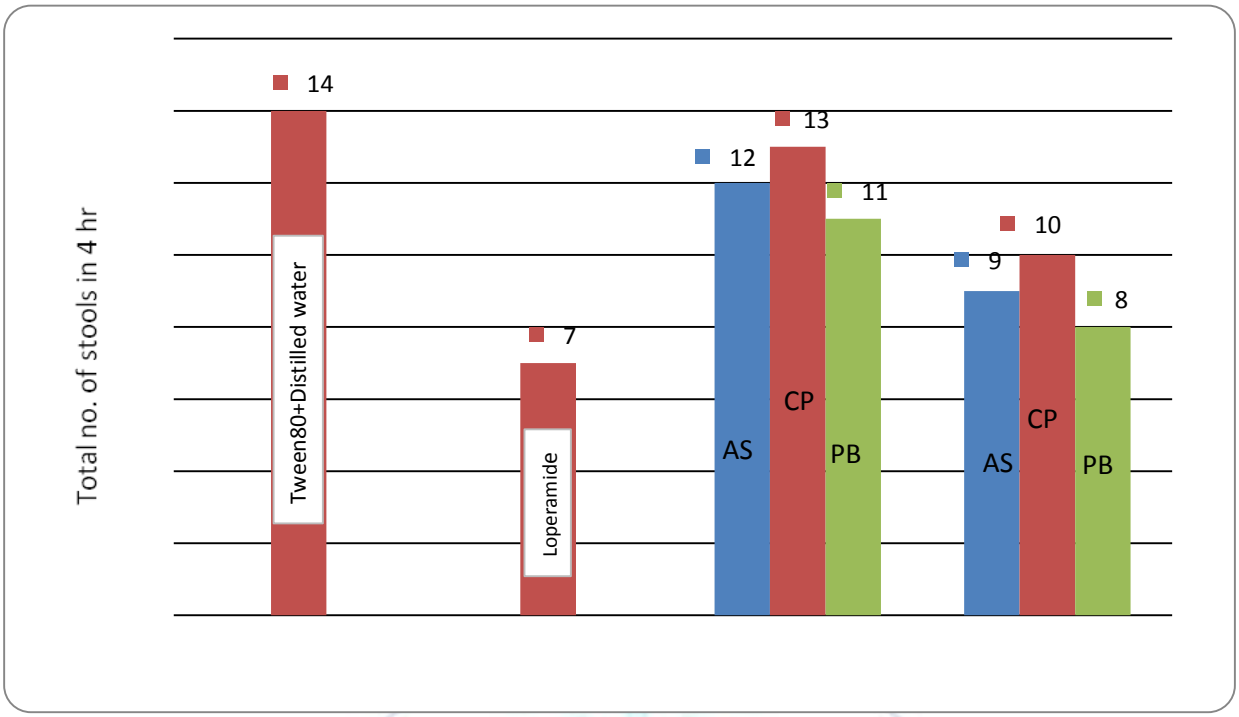

Figure 1: Effects of *Anonna squamosa* ,*Calotropis procera* and *Piper betel* leaves on castor oil-induced diarrhoea in mice

CONCLUSION

In conclusion, the present tudy demonstrates that the ethanol extracts of *A squamosa, C procera* and *P betel contain* pharmacologically active principle(s) possessing significant antidiarrhoeal activity. The present data provided a support for the traditional use of the plant as an antidiarrhoeal remedy. However, further studies will be necessary to elucidate and characterize the active components which are responsible for the antidiarrhoeal effect and to understand exact its mechanisms of action.

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