Evaluation of Anti-diarrheal Activity of Gomphrena globosa (L) in Animal Model

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Abstract

Gomphrena globosa (L), known as Bottam phul in Bangladesh is planted as ornamental plant in the garden. It has some folk medicinal uses in our country. The plant sample was collected, washed, sundried and extracted with methanol at room temperature. The concentrated methanolic plant extract at a dose of 200 and 400 mg/kg body weight was subjected to assay for anti-diarrheal activity by following castor oil induced diarrhea in mice. The experimental data was evaluated based on the activity of standard anti-diarrheal drug, Loperamide. The data revealed that the extract of G. globosa at a dose of 400 mg/kg body weight showed statistically significant anti-diarrheal activity in mice model.

Key words: *Gomphrena globosa*, Amaranthaceae, Anti-diarrheal activity, Loperamide, Mice model.

Introduction

Gomphrena globosa grows well in different parts of Bangladesh and used traditionally for many medicinal purposes. *G. globosa* belongs to Amaranthaceae family, commonly known as glove amaranth is an annual branched herb which is cultivated as ornamental flowering herb in garden (Muller and Borsch, 2005). It is native to North-America, South-America, Myanmar and India. It also grows well throughout Bangladesh. *G. globosa* is a folk remedy for oliguria, heat and empacho, hypertension (Yusuf *et al.*, 2009), cough and diabetes (Arcanjo *et al.,* 2011) and expectorant for animals (Asolkar *et al.,* 1992). Since this plant has important medicinal properties, the present study has been undertaken to evaluate the anti-diarrheal activity in mice model in laboratory.

Materials and Methods

Collection of the Plant Sample

Whole plant of *G. globosa* was collected from Dhaka, Bangladesh in November; 2011. This plant was identified by botanists at the Botany Department of the University of Dhaka. The reference sample for this plant has been maintained in the herbarium of Department of Botany, University of Dhaka (DUSH, Accession Number 3557 and calls no 01).

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Preparation of the Test Sample

The whole plant of *G. globosa* was washed properly, cut into small pieces and then sun dried for several days. The pieces were then oven dried for 24 hours at considerably low temperature to facilitate grinding. The pieces were then ground into coarse powder in the laboratory using high capacity grinding machine. About 800 gm of the powdered material was taken in a clean, round bottomed flask (5 liters) and soaked in 3.5 liter of methanol. The container with its content was sealed by foil and kept for a period of 15 days accompanying occasional shaking and stirring. The whole mixture was then filtered through a fresh cotton plug and finally with a Whatman No.1 filter paper. The volume of the filtrate was then reduced using a Buchi Rotavapor at low temperature and pressure. The weight of the crude extract was 29 gm.

Table 1: Test sample and materials used for the experiment

Group	Test samples	Identification	Dose (mg/kg)
Group-I	1% Tween-80 & DMSO in normal saline	Negative control	-
Group-II	1% Tween-80, Loperamide & DMSO in normal saline	Positive control	50
Group-III	Crude methanolic extract	Test sample	400
Group-IV	Crude methanolic extract	Test sample	200

Principle of Anti-diarrheal Activity Test

The anti-diarrheal activity of the crude methanolic extract of *G. globosa* was evaluated by castor oil induced diarrhea in mice (Chattergee TK, 1993). According to this model each mice was fed 1 ml of highly pure analytical grade castor oil to induce diarrhea. Twenty mice were taken and divided into four groups (Group I, Group II, Group III and Group IV). Group I was used as negative control, while Group II served as the positive control or standard group treated with Loperamide. Group III and Group IV were the test groups. Each mouse was fed with the test samples. Then thirty minutes later they were given 1 ml of castor oil to induce diarrhea. The mice were kept under observation for the next four hours. For each mouse the number of times it defecated was recorded. The observation of the experimental groups was compared with the positive control to evaluate the anti-diarrheal activity of the samples.

Experimental Animal and Study Design

Swiss albino mice (25-30 gm) were obtained from the Department of Pharmacy, Jahangirnagar University. They were housed in the animal house of Institute of Nutrition and Food Science of University of Dhaka in standard polypropylene cages and kept under controlled room temperature (24 ± 2ºC; relative humidity 60-70%) in a 12 hour light-dark cycle. The mice were given standard laboratory diet and water ad libitum. Food was withdrawn 12 hour before and during the experimental hours. The ethics of use of experimental animals were followed carefully. Each mouse was weighed properly and the doses of the test samples and control materials were adjusted accordingly.

Experimental Procedure

In order to administer the extract at doses of 400 mg/kg and 200 mg/kg body weight of mice, 100 and 50 mg of crude extract were measured, respectively and triturated in unidirectional way by adding small amount of tween-80 (a suspending agent). After proper mixing of extract and suspending agent, normal saline was slowly added. The final volume of the suspension was made up to 3.0 ml. To stabilize the suspension, it was stirred well by vortex mixture. Test samples and control was given orally by means of a feeding needle to the mice at zero hour. Mice of group I received only 1% Tween 80 and DMSO in normal saline. Mice of group II received loperamide at a dose of 50 mg/kg body weight. Mice of group III and group IV received the crude extract at a dose of 400 and 200 mg/kg body weight. After 30 minutes interval was ensured proper absorption of the administered substances. 1.0 ml of castor oil was given to each mouse for inducing diarrhea. Each mouse was marked in the tail using a permanent marker and kept in a separate container which was pre-cleaned. They were observed for four hours and time of defecation was recorded. The average of total number of defecation by the test groups and average of total number of defecation by the control group was compared. Statistical significance of the data was calculated to evaluate anti-diarrheal activity of the test samples.

Data Collection and Calculation

Total number of defecation by each mouse was taken up to four hours and then the data was evaluated statistically to find its significance.

Table 2: Data representing total number of defecation by each mouse

Group	No. of mouse	No. of defecation of each mouse of each hour				Total no. of defecation by each mouse	Average ± SME
		1st hr	2nd hr	3rd hr	4th hr		
Negative control	M1	5	3	5	4	17	17 ± 0.55
	M2	4	3	4	5	16	
	M3	5	5	6	3	19	
	M4	4	6	4	3	17	
	M5	3	4	3	6	16	
Positive control (Loperamide, 50 mg/kg body weight)	M1	0	1	1	2	4	4.8 ± 0.84
	M2	1	1	2	1	5	
	M3	0	2	2	2	6	
	M4	0	1	1	3	5	
	M5	1	1	1	1	4	
Crude methanolic extract (400 mg/kg body weight)	M1	3	2	3	2	10	9.2 ± 0.21
	M2	2	3	3	1	9	
	M3	3	2	2	2	9	
	M4	3	3	2	2	10	
	M5	2.5	2	2	2	8.5	

Group	No. of mouse	No. of defecation of each mouse of each hour				Total no. of defecation by	Average ± SME
Crude methanolic extract (200 mg/kg body weight)	M1	5	6	4	3	18	16 ± 0.84
	M2	5	4	3	3	15	
	M3	6	5	5	2	18	
	M4	5	3	4	4	16	
	M5	5	3	4	3	15	

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): *P<0.05, All values are means of individual data obtained from five rats (n = 5)

Table 3: Hour-wise average number of defecation by each mouse

Group	Average of stool count ± SEM				
	1st hour	2nd hour	3rd hour	4th hour	Total
Negative control	4.20±0.37	4.20±0.58	4.40±0.51	4.20±0.58	17 ± 0.55
Positive control (Loperamide, 50 mg/kg body weight)	0.40±0.24	1.20±0.20	1.40±0.24	1.80±0.37	4.8 ± 0.84
Crude methanolic extract (400 mg/kg body weight)	2.7 ±0.51	2.4 ±0.37	2.1 ±0.37	2 ±0±0.37	9.2 ± 1.21
Crude methanolic extract (200 mg/kg body weight)	5.2±0.37	4.20±0.58	4.0±0.32	3.00±0.32	16 ± 0.84

Probability values (calculated as compared to control using one way-ANOVA followed by Dunnet's Test): *P<0.05, All values are means of individual data obtained from five rats (n = 5)
Primary data showed that crude methanolic extract at a dose of 400 mg/kg body weight showed significant anti-diarrheal activity by decreasing the total stool count.

Statistical Evaluation of the Data

T-test was carried out using Graph Pad software and t values and P values were calculated. Level of statistical significance was determined for each sample.

Results and Discussion

The statistical evaluation of data confirmed that the crude methanolic extract of *G. globosa* at a dose of 400 mg/kg body weight revealed significant antidiarrheal activity by reducing the count of defecation during 1st, 2nd, 3rd and 4th hour of the study. However at a dose of 200 mg/kg body weight there was slight reduction in the average defecation count. Based on the data obtained it may be concluded that *G. globosa* has important secondary metabolites which are medicinally important. Further research with this plant is necessary to purify and identify the chemical compounds responsible for the antidiarrheal activity.

References

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