

Research Article

Anti-diarrheal Potential of Ethanol and Water extracts of *Euphorbia hirta* whole plant on Experimental animals: A Comparative Study

I N Khan¹, S Jahan², M A M Bhuiya¹, K Mazumder^{*1}, B K Saha^{*2},¹ University of Science and Technology Chittagong, Foy's Lake, Pahartali, Chittagong - 4202, Bangladesh² Institute of Food Science and Technology, BCSIR, Dr.Kudrat-I-Khuda Road, Dhanmondi, Dhaka-1205, Bangladesh

Corresponding authors

Dr. Barun Kanti Saha

E-mail: bksbcsir@yahoo.com

Kishor Mazumder, PhD

E-mail: mazumderkis2002@yahoo.com

Abstract: The present study was carried out to evaluate both the ethanol and water extracts of entire plant *Euphorbia hirta* (Family: Euphorbiaceae) for its anti-diarrheal potential against some of the experimental model of diarrhea in rats. The plant showed significant inhibitory activity against castor-oil-induced diarrhea and PGE₂ induced enteropooling in rats. Both the extracts of the plant also showed a significant reduction in gastrointestinal motility in both BaSO₄ and charcoal meal tests. The results obtained established the anti-diarrheal potential of the entire plant though the ethanol extract proofed more potential than the water counter part.

Keywords: *Euphorbia hirta*, ethanol extract, water extract, anti-diarrheal.

1. INTRODUCTION

The major cause of diarrhea among children in developing countries like Bangladesh is malnutrition. To minimize the problem of diarrhea, which is the leading cause of mortality in developing and in least developed countries, the WHO has continued a Diarrheal Disease Control program (CDD), which includes studies of traditional medicinal practices, together with the evaluation of health education and prevention approaches [1].

Euphorbia hirta (Euphorbiaceae) is a common annual herb of subtropical countries like Bangladesh, India, Niger, and Suriname etc. It is also known as cat's hair, asthma weed, basri dudhia, chara, and malnommee. This hairy plant grows up to 2' in height; it has numerous small flowers clustered together with opposite oblong leaves[2]. Previous investigation have revealed that an ethanolic extract for whole Ariel parts of *E. hirta* showed antihistaminic, anti-inflammatory and immunosuppressive, antimalaria [3] as well as broad spectrum of antimicrobial activity [4] recently *E. hirta* ethanolic extract was also found to posses a prominent anti-anaphylactic activity [5]. On the other hand, water leaf extract showed antidiarrheal activity [6] and this is supported by a triple pronounced antibacterial, anti-amoebics and antispasmodic action [7]. However, the present study was done to compare the water and ethanolic extract of whole plant for antidiarrhoeic activity.

2. MATERIALS METHODS

2.1. Plant materials

The whole plants of *Euphorbia hirta* were collected from the experimental plantation area of BCSIR-Laboratory campus, Chittagong, Bangladesh with the help of industrial research division. The whole plants of *Euphorbia hirta* were crushed in a blender and blended parts were dried at room temperature in thick layer for 20 hours. The dried parts were crushed in a blender.

2.2. Preparation of extract

The powdered plant material 1300 gm and 2000 gm was subject to maceration process with 95% ethanol and water respectively at room temperature. After exhaustive extraction, the ethanolic extract (EHEE) and water extract (EHWE) was concentrated under reduced pressure at below 40°C through rotary vacuum evaporator. The solid mass was then freeze dried. A greenish black colored residue was obtained (358gm; yield 27.53 %w/w) from ethanolic extract and greenish colored residue was obtained (260gm; yield 13%w/w) from water extract and stored in a desiccators. For pharmacological studies, a weighted amount of the dried extracts were prepared according to the designed experiments, table-1.

2.3. Animal Used and diet

Albino rats (180-200 g) of either sex were obtained from the animal house of BCSIR- Laboratory, Chittagong, The animals were acclimatized to standard laboratory conditions (temperature 24±1°C, relative humidity 55±5% and a 12h photoperiod) in suspended wire-meshed galvanized cages (4-6 rats/ cages) for one week before the commencement of the experiment. During the entire period of study, the rats were supplied

with semi purified basal diet water ad libitum. All animals were maintained according to the NIH published

guidelines of Care and use of Laboratory Animals as published by [8].

2.4 Dose of Extracts

Table- 1: The doses and routes of the extract used in various experiments

Name of the experiment	Animals used	Route	Dose (mg/kg)	
			Ethanol extract	Water extract
Acute toxicity study	Rats	Orally	Up to 3.2	Up to 3.2
Gastrointestinal motility test with Barium sulphate milk (15% in	Rats	Orally	2.0	2.0
Gastrointestinal motility with activated carbon (10% suspension of activated carbon in 1.0% agar)	Rats	Orally	2.0	2.0
Castor oil induced diarrhea (1ml/Rat)	Rats	Orally	2.0	2.0
PGE ₂ – induced enteropooling	Rats	Orally	2.0	2.0

3. Experimental

3.1 Acute toxicity Study

An acute toxicity study was undertaken relating to the determination of LD₅₀ values using different doses of the extracts according the method described by Devi, B.P.[9]. From the toxicity study it

was found that both ethanol and water extracts are nontoxic and caused no death up to a dose of 3.2 gm/kg body weight given orally, therefore, both ethanol and water extracts are safe and were used in different doses for further studies Table- 2.

Table 2. Acute Toxicity Study

Treatment	Dose mg/kg body weight	No. of animals	No. of survivals	No. of death	LD ₅₀ Values
Control - 1	2 % tween-80 solution	20	20	0	-
Control - 2	Normal saline	20	20	0	-
EHEE	100	20	20	0	-
	200	20	20	0	-
	400	20	20	0	-
	800	20	20	0	-
	1600	20	20	0	-
	3200	20	20	0	>3.2gm/kg
EHWE	100	20	20	0	-
	200	20	20	0	-
	400	20	20	0	-
	800	20	20	0	-
	1600	20	20	0	-
	3200	20	20	0	>3.2gm/kg

EHEE – Euphorbia hirta ethanol extract

EHWE – Euphorbia hirta water extract

3.2 Gastrointestinal Motility Test with Barium Sulphate Milk

This experiment was carried out with the method described by Chatterjee [10]. 12 rats were divided into two experimental (Treated and control)

groups. Treated group received BaSO₄ milk (15% in 0.5% Agar solution) 30 minutes after the administration of the test extract. Control group also received BaSO₄ (15% in 0.5% Agar solution) milk

along with the vehicle (agar solution) of the test extract. The distance traversed by BaSO₄ meals (after 30 minutes) were measured and expressed as a percentage of the total length of small intestine (from pylorus to the ileoceca ljunction).

3.3 Gastrointestinal Motility with Activated Carbon

The modified method of Yegnanarayan [11] was used for this experiment. According to this method, charcoal suspension (10ml/kg of 10% suspension of activated carbon) was administered orally to rats (body weight 180-200 gm), after 30 minutes of the oral dose of extract or vehicle (1.0% agar). In this study, a total of 12 animals were taken for each pre-treatment group. The animals were divided into two experimental groups (control and treated). Animals were sacrificed 30 minutes after receiving the charcoal suspension and the length of the intestine (pyloric sphincter to caecum) and the distance traveled by the charcoal as a fraction of that length was calculated for each animal.

3.4 Castor Oil Induced Diarrhoea in rat

The method of Niemeegers [12] as adopted by Yegnanarayan [11] was followed. Rats, weighing 180-200 gm, were employed for this study. They were all screened initially by giving 1.0 ml of castor oil orally and only those showing diarrhoea were selected for further study. Extract pre-treatment was given orally 1 hour before the rats were administered with the standard dose of 1.0 ml of castor oil. The animals were caged individually and examined for the presence of diarrhea 1/2 hourly for 6 hours after the castor oil challenge. Diarrhoea was defined as the presence in the stool of fluid material that sustained the absorbent paper placed beneath the cage. The number of respondents, the number of stools passed during the 6 hours period was noted for each rat. Purging index (PI) was calculated as follows:

$$\text{Purging index (PI)} = \frac{\% \text{ Respondents} \times \text{Average number of stools}}{\text{Average latent period}}$$

3.5 PGE₂ – induced enteropooling

The modified method of Gunakkunru A [13] was used for this experiment. For this evaluation, rats of the same stock as above were deprive of food and water for 18 h and placed in four cages, with six animals per cage. The first two groups were treated with 2 gm/kg dose of ethanol and water extracts of *Euphorbia hirta*. The third group was treated with 1ml of a 5% v/v ethanol in normal saline (i.p) and then it was treated with 0.5% Tween 80 suspension, which served as negative control. Immediately after the extract administration PEG₂ (Astra Zeneca, India) was administered orally to each rat (100microgram/Kg) in the first three groups. The fourth group was treated with PGE₂ (100 µg/Kg) as well as 0.5% Tween 80 suspension and served as the PEG₂ control group. After 30 minutes following administration of PGE₂, each rat was sacrificed and the

whole length of the intestine from the pylorus to the caecum was dissected out, its content collected in a test tube and the volume measured.

3.5 Statistical Analysis

Data were expressed as Mean ± S. E. (Standard error). Unpaired t tests were done for significant tests. SPSS for WINDOWS @™(Statistical Package for Social Science) for windows was applied for analysis of data with one way analysis of variance followed by Dennett's t-test. Probability (p) value of 0.05 or less (p < 0.05) was considered as significant.

4. RESULTS AND DISCUSSION

Acute toxicity study

From the toxicity study it was observed that the plant extract is non-toxic and caused no death up to a dose of 3.2g/kg orally. It is safe and was used in different doses for further studies.

Effects on gastrointestinal motility

The Ethanol extract of the *Euphorbia hirta*, significantly (p<0.001) reduces the gastrointestinal motility of rats from 68.30cm to 40.58cm with barium sulfate milk at 30 minutes study in comparison to water extract from 66.23cm to 44.16cm (p<0.01). In the case of activated carbon i.e. charcoal meal study, the gastrointestinal motility of rats also significantly (p <0.001) reduced from 68.22cm to 31.44cm and 67.33cm to 40.21cm both in ethanol extract and water extract respectively, so we can say although both extracts exerted inhibitory activity on GI motility but ethanol extract have higher activity than water extract (table 4.1&4.2).

Inhibition of Castor-oil-induced diarrhea

In this study the use of castor oil induced diarrhoea models is logical due to the involvement of autacoids and it was also reported that prostaglandin (PG) have been in the causation of diarrhoea in man and animals [14]. E series of PG has termed as "hormone" [15], which is mainly responsible for that action. The liberation of ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins (PG), which stimulate motility and secretion [16] the castor oil model therefore, incorporates both secretory and motility diarrhoea [11].

In the castor oil induced diarrhoea model on rats, the control group of animals responds 91.66% to diarrhoea but in case of ethanol treated extract group's respond only 55%. In this study, the mean latent period in ethanol extract treated groups is 0.48hr that is significantly (p<0.001) decreasing for treatment compare to their respective controls. The numbers at 1st, 2nd, 5th and 6th hours for ethanol extract treatment significantly (p<0.001) decrease compare to control group (table 4.3). In the ethanol extract, the number of stools at 5th and 6th hours is zero, which indicates that

ethanol extract is more active to prevent the castor oil induced diarrhoea. On the other hand, in case of water extract the castor oil induced diarrhoea model on rats, the control group of animals responds 100% to diarrhoea but the water treated extract group's respond only 60%. In this study, the mean latent period in water extract treated groups is 0.43hr that is significantly ($p < 0.001$) decreasing for treatment compare to their respective controls. The numbers at 1st, 2nd, 5th and 6th hours for water extract treatment significantly ($p < 0.001$) decrease compare to control group (table 4.4). In the water extract, the number of stools at 5th and 6th hours is zero, which indicates that water extract is more active to prevent the castor oil induced diarrhoea.

PGE2 induced intestinal fluid accumulation (enteropooling)

Both extracts were significantly inhibited PGE2 induced intestinal fluid accumulation (enteropooling) in rats at an oral dose of 2mg/kg. PGE2 induced a significant increase in the fluid volume of the rats intestine when compared with control animals received ethanol in normal saline but the comparatively effect was higher in case of ethanol extract than water extracts (table 4.5). It has been shown that E type of prostaglandins cause diarrhoea in experimental animals as well as human beings [17]. Their mechanism has been associated with dual effects on gastrointestinal motility as well as on water and electrolytes transport [18]. PGE2 also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes [15]. These observations tend to suggest that both extracts can inhibit diarrhea at given dose by inhibiting PEG2 induced intestinal accumulation of fluid.

Table 4.1: Effect of Ethanol and Water Extracts of *Euphorbia hirta* on Gastrointestinal Motility with Barium Sulfate Milk on Rat

Groups	Ethanol Extracts				Water Extracts			
	Length of GIT(cm)	Length passed by BaSO4(cm)	Percent Traveled	Mean± SE	Length of GIT(cm)	Length passed by BaSO4(cm)	Percent Traveled	Mean± SE
Control	106	82	77.35	68.30± 2.83	105	65	61.90	66.23± 2.41
	109	80	73.39		97	67	69.07	
	103	63	61.16		103	67	65.04	
	105	68	64.76		99	66	66.66	
	98	68	69.38		107	80	74.76	
	105	67	63.80		105	65	61.90	
Treated	103	47	45.63	*40.58± 3.080	102	35	34.31	^a 44.16± 6.71
	117	49	41.88		108	37	34.25	
	108	44	40.74		106	57	53.77	
	101	46	45.54		101	22	21.78	
	104	44	42.30		124	70	56.45	
	113	31	27.43		104	67	64.42	

*P < 0.001 when compare with control group (n=6)

^aP < 0.01 when compare with control group (n=6)

Table 4.2: Effect of Ethanol and Water Extracts of *Euphorbia hirta* on Gastrointestinal Motility with Activated Carbon on Rat

Groups	Ethanol Extracts				Water Extracts			
	Length of GIT (cm)	Length Passed by BaSO4 (cm)	Percent Traveled	Mean SE	Length of GIT (cm)	Length Passed by BaSO4 (cm)	Percent Traveled	Mean SE
Control	107	81	75.70	68.22±2.67	107	72	67.2	
	108	79	73.14		112	69	61.6	
	105	62	59.04		121	65	53.7	
	95	65	68.42		119	91	76.4	
	105	69	65.71		114	99	86.8	

	101	68	67.32		107	58	54.2	
Treated	111	33	29.72	*31.44±4.36	114	39	34.2	^a 40.21±2.08
	114	36	31.57		112	49	43.7	
	103	22	21.35		102	37	36.2	
	95	20	21.05		99	38	38.3	
	108	46	42.59		106	47	44.3	
	118	50	42.37		103	46	44.6	

*P< 0.001 when compare with control group (n=6)

^aP< 0.001 when compare with control group (n=6)

Table 4.3: Effect of Ethanol Extract of *Euphorbia hirta* on Castor oil Induced Diarrhea on Rat

Groups	Hours (HR)	% Respondents	Mean Latent Period in HR ± SE	No of Stools	Purging Index
Control	1	91.66	0.97±0.01	5.3±0.510	500.82
	2			3.9±0.52	368.52
	3			0.5±0.10	47.24
	4			0.4±0.15	37.79
	5			1.6±0.21	151.19
	6			1.9±0.54	179.54
<i>Euphorbia hirta</i>	1	55	0.48±0.02	^a 1.4±0.51	160.41
	2			^a 1.0±0.15	114.58
	3			0.3±0.02	34.375
	4			0.2±0.01	22.91
	5			^b 0.0±0.0	0.00
	6			^c 0.0±0.0	0.00

^aP< 0.001 when compare with control group(n=6), ^bP< 0.1 when compare with control group (n=6), ^cP< 0.01 when compare with control group (n=6)

Table 4.4: Effect of Water Extract of *Euphorbia hirta* on Castor oil Induced Diarrhea on Rat

Groups	Hours (HR)	% Respondents	Mean Latent Period in HR ± SE	No of Stools	Purging Index
Control	1	100	0.93±0.02	3.4 ± 0.41	365.59
	2			1.0± 0.25	107.52
	3			0.2± 0.32	21.50
	4			0.5± 0.21	53.76
	5			0.2± 0.11	21.50
<i>Euphorbia hirta</i>	1	60	^a 0.43±0.01	0.3± 0.11	32.25
	2			0.4± 0.31	37.20
	3			0.5± 0.20	46.51
	4			0.2± 0.12	18.60
	5			0.1± 0.31	9.30
	6	0.0± 0.0	0.00		
	7	0.0± 0.0	0.00		

^aP< 0.001 when compare with control group (n=6)

Table 4.5: Anti-enteropooling effect of ethanol and water extracts *Euphorbia hirta* in rats

Test drug* Followed by PGE ₂ , p.o	Volume of intestinal fluid (ml)	P-values
Ethanol in saline	0.873±0.016	-
PGE ₂ in ethanol (100 µgm)	3.167±0.022	0.001 ^a
Ethanol extract (2mg/kg)	1.837±0.037	0.001 ^b
Water extract (2mg/kg)	2.135±0.019	0.001 ^b

* The test drug and vehicle were given p.o. Results are expressed as mean ± S.E.M, n=6, Statistical significance test with control was done by ANOVA test.. ^a With respect to ethanol in saline treatment, ^b with respect to PGE₂ treatment.

CONCLUSION:

Regardless of the mechanism of action, based on the results reported here, *Euphorbia hirta* ethanol extracts can be considered to be very effective in preventing castor oil induced diarrhea in comparison to water extract. Further investigation warrant to isolation, identification and characterization of different active compounds from the extracts and their mode of action, which is responsible for this properties, on different biological systems.

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