**Scholars Journal of Applied Medical Sciences (SJAMS)** **ISSN 2320-6691**
**Sch. J. App. Med. Sci., 2013; 1(3):199-204**

(An International Publisher for Academic and Scientific Resources)
www.saspublisher.com

## Research Article

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

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**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 inhibitatory activity against castor-oil-induced diarrhea and $PGE_2$ induced enteropooling in rats. Both the extracts of the plant also showed a significant reduction in gastrointestinal motility in both $BaSO_4$ 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* (Euphorbiaceace) 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**

| | | | Dose (mg/kg) | |
|---|---|---|---|---|
| **Name of the experiment** | **Animals used** | **Route** | **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 $BaS0_4$ milk (15% in 0.5% Agar solution) 30 minutes after the administration of the test extract. Control group also received $BaSO_4$ (15% in 0.5% Agar solution) milk

along with the vehicle (agar solution) of the test extract. The distance traversed by $BaSO_4$ 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:

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

***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*. Te 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 $PGE_2$ (100 µg/Kg) as well as 0.5% Tween 80 suspension and served as the $PEG_2$ control group. After 30 minutes following administration of $PGE_2$, 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 of 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.

**$PGE_2$ 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 diahhroea 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)

[a]P< 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)

[a]P< 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 |
| *Euphorbia hitra* | 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 |

[a]P< 0.001 when compare with control group(n=6), [b]P< 0.1 when compare with control group (n=6), [c]P< 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 |
| | | | | 0.2± 0.11 | 21.50 |
| | | | | 0.3± 0.11 | 32.25 |
| *Euphorbia hirta* | 1 | 60 | [a] 0.43±0.01 | 0.4± 0.31 | 37.20 |
| | 2 | | | 0.5± 0.20 | 46.51 |
| | 3 | | | 0.2± 0.12 | 18.60 |
| | 4 | | | 0.1± 0.31 | 9.30 |
| | | | | 0.0± 0.0 | 0.00 |
| | | | | 0.0± 0.0 | 0.00 |

[a]P< 0.001 when compare with control group (n=6)

**Table4.5: 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 $PGE_2$ 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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