Original Research Article

Assessment of analgesic and neuropharmacological activity of different extracts of *Euphorbia hirta* (Linn.) leaf.

Abstract

Aims: The present study was carried out to investigate the possible analgesic and neuropharmacological activities of the aqueous, ethanol and ethyl acetate extracts of *Euphorbia hirta* (Linn.) leaves.

Methods: The analgesic and neuropharmacological potential was studied at the dose of 400mg/kg of body weight in mice. Analgesic potential of the extracts was evaluated using mice writhing method and formalin induced pain tests. In addition, neuropharmacological property of extracts was carried out by hole cross, open field and elevated plus maze tests.

Results: In writhing test, the aqueous extract significantly (89.51%) inhibited the peripheral nociception while in formalin test the ethyl acetate extract significantly ($p>0.001$) inhibited the licking time in both phases. The ethanolic extract exhibited convincing reduction of exploratory behavior in hole cross and open field tests. Furthermore, an increase in the frequency and duration in the open arm of EPM displayed by all three extracts indicates the evidence of their anxiolytic activity.

Conclusion: These results may rationalize the scientific basis for use of this plant in traditional medicine for treatment of analgesia and anxiety related disorders.

Key words: *E. hirta,* writhing, locomotor, formalin, anxiety.

1. Introduction

22 *Euphorbia hirta* (Linn.), belonging to the spurge family of Euphorbiaceae, is an herbaceous
23 plant and very common in tropical countries like Bangladesh. It is a small, erect or ascending
24 annual herb (50 cm high) with hairy stems. The leaves are opposite, elliptical, oblong or
25 oblong-lanceolate with a faintly toothed margin and darker on the upper surface [1]. The
26 leaves produces white or milky juice when cut [2].

27 *E. hirta* is being used as a traditional source of medicine for many years in Bangladesh. The
28 Tripura tribe in Chittagong hill tracts of Bangladesh uses this plant for increasing lactation
29 after childbirth and to treat body sores, asthma and chronic bronchitis [3]. The Plant is used
30 for the treatment of cough, asthma, chronic bronchitis, bowel complaints, worm infestation,
31 kidney stones, bronchial affections, conjunctivitis. The crude extract is used as Analgesic,
32 Anti–pyretic, Anxiolytic, Sedative, and Anti–inflammatory and also as Anti–coagulant [4].
33 Though the aqueous extract has been reported to have central analgesic activity in low dose
34 [5], there was hardly any experimental data found to support the peripheral analgesic
35 properties; alongside no investigation was done for this property with the other two extracts
36 at 400 mg/kg dose. These well-established traditional use of this plant acted as the driving
37 force to conduct the present study. Again, many studies have been done on *E. hirta,* none of
38 them compared aqueous, ethanol and ethyl acetate extracts of leaf for sedative-anxiolytic
39 activities. The reason that these three extracts had been chosen was due to the extraction
40 capacity of the solvents; aqueous (polar compounds), ethanol (slightly polar to non-polar
41 compounds) and ethyl acetate (highly non-polar compounds) and thereby it can be
42 hypothesized that these three extracts retained maximum number of compounds that can be
43 responsible for the activities observed.

44 **2. Materials and Methods**

45 **2.1 Collection and Identification of Plant Material**

46 The plant was collected from Chittagong Hill Tracts (between 21°25'N to 23°45'N latitude
47 and 91°25'E to 92°50'E longitude) of Bangladesh in October 2011 when leaves were in their
48 maximum densities (Accession number DACB 39517).

49 **2.2 Preparation of Extracts**

50 The shade dried leaf was coarsely powdered and 500g extracted with 0.5 L each of water
51 (EHAQ), ethanol (EHET) and ethyl acetate (EHEA) by maceration method at room
52 temperature for a period of 7 days with occasional shaking and stirring. The extracts were
53 filtered and concentrated on rotary evaporator and further dried and weighed about 10% of
54 viscous mass [6].

55 **2.3 Animals**

56 Swiss Albino mice (20-25 g) of either sex were procured from International Centre for
57 Diarrheal Disease Research, Bangladesh (ICDDR, B). The animals were housed under
58 standard conditions of temperature (22 ± 1ºC), relative humidity (55 ± 10%), 12 hr light/dark
59 cycles and supplied with food and water *ad libitum* at the Laboratory Animal House,
60 Department of Pharmacy, East West University, Bangladesh. The animals were divided into
61 five groups (N=4) designated as Control (water), EHAQ (400 mg/kg), EHET (400 mg/kg),
62 EHEA (400 mg/kg) and standard for all experiments.

63 **2.4 Drugs and Chemicals**

64 Diclofenac Sodium and Diazepam were obtained from Square Pharmaceuticals Ltd.,
65 Bangladesh. Acetic Acid were obtained from Mark, Germany. Formalin was purchased from
66 CDH, India. All chemicals used were of analytical reagent grade.

67 **2.5 Acute toxicity test**

68 Randomly grouped (n = 5) mice separately received the aqueous, ethanol and ethyl acetate
69 extracts orally at doses of 500, 1000, 1500 mg/kg. The control group received the vehicle.
70 The animals were observed for possible allergic reactions, and mortality for the next 72 h and
71 extended up to 14 days.

72 **2.6 Sedative Activity**

73 **2.6.1 Hole Cross Test**

74 The test was performed for screening sedative activity in mice. A steel partition was fixed in
75 the middle of a cage having a size of 30×20×14 cm with A hole of 3 cm diameter at height
76 7.5 cm in the partition. The number of crossing from one chamber to other was counted for a
77 period of 3 min on 0, 30, 60, 90 and 120 min after the oral gavage with test drugs. Diazepam
78 was used in the positive control group as reference standard at the dose of 1 mg/kg [7].

79 **2.6.2 Open Field Test**

80 The experiment was carried out according to the methods described by Nyeem et al. [8]. The
81 floor of the open field of a square meter was divided into several squares. The apparatus was
82 enclosed with 40 cm high wall. The number of squares visited by the mice was counted for 3
83 min, on 0, 30, 60, 90 and 120 min immediately after the oral test drug treatment.

2.7 Anxiolytic activity

2.7.1 Elevated plus-maze (EPM) test

The method initially suggested by Handley and Mithani was employed with minor modifications [9]. The procedure was conducted in a sound attenuated room. Sixty minutes after administration of the test drugs, each animal was placed at the center of the maze. During the 5-min test period, the number of open arms entries and duration were recorded. An Entry was defined when the animal places all four paws onto the arm.

2.8 Analgesic Activity

2.8.1 Mouse writhing test

This was based on the method described by Meera et al. [10]. Diclofenac sodium (10 mg/kg, i.p.) was administered as positive control. 30 minutes later all groups received intraperitoneal injection of 0.7%, 0.1 ml/10 gm acetic acid solution. Mouse were observed and the number of writhing or stretches were counted for 20 min immediately after administering acetic acid. Reduction in the number of writhes compared to the control groups was considered as evidence of analgesic effect.

2.8.2 Formalin test

The method was done according to the method described Sharma et al. [11]. 30 minutes after the groups received their respective treatments, 20 μl of 5% formalin was injected subcutaneously into the right hind paw of mice. The time (in sec) spent in licking and biting the injected paw for next 30 min (0-5 and 16-30 min) were taken as an indicator of pain response.

105 **2.9 Statistical analysis**

106 Statistical analysis for animal experiments was carried out using one-way analysis of variance
107 (ANOVA) followed by Dunnett's multiple comparison tests using SPSS 20 for windows. The
108 results obtained were compared with the vehicle control group. P values < 0.05, 0.01 and
109 0.001 were considered to be statistically significant.

110 **3. Results**

111 **3.1 Acute toxicity test**

112 Observations of all three extracts dosing from 500–1500 mg/kg, did not produce any
113 mortality in mice within 72 h and further observation period, suggesting that these extracts of
114 leaves of *E. hirta* have low toxicity profile with LD50 greater than 1500 mg/kg.

115 **3.2 Hole cross test**

116 The number of hole crossed by the mice was moderately reduced by the ethanolic extract.
117 The inhibition was observed from the 2nd to the 5th observation period (Figure 1).

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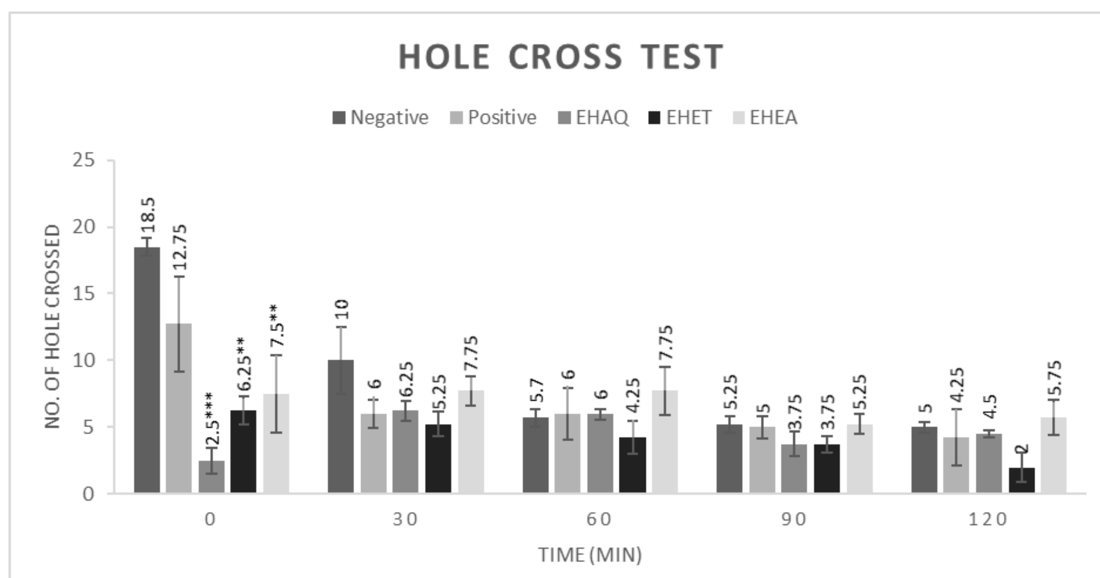

119 **Figure 1: Effect of the aqueous, ethanol and ethyl acetate extracts of leaves of *E. hirta* on**
120 **hole cross test in mice.** Values are mean ± S.E.M., (n = 4); ** $P < 0.01$, *** $P < 0.001$
121 Dunnet test as compared to control (Vehicle = 0.5 mL/mouse).

122 **3.3 Open field test**

123 The ethanolic extract significantly suppressed the number of square travelled by the mice
124 (Figure 2). Maximum suppression was observed from the 3rd observation period and was
125 comparable with the reference drug. The data of ethyl acetate extract was also convincing.
126 The data were statistically significant.

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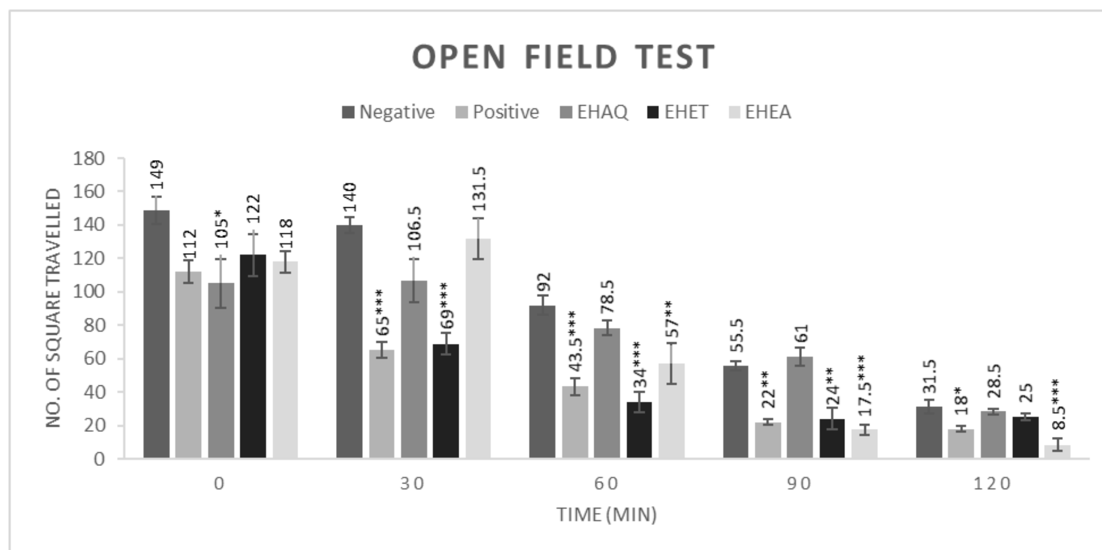

128 **Figure 2: Effect of the aqueous, ethanol and ethyl acetate extracts of leaves of *E. hirta* on**
129 **open field test in mice**. Values are mean ± S.E.M., (n = 4); * $P < 0.05$, ** $P < 0.01$, *** $P <$
130 0.001 Dunnet test as compared to control (Vehicle = 0.5 mL/mouse).

131 **3.4 Elevated plus Maze**

132 Table 1 shows that all extracts effectively increased the percent number of entry into the open
133 arm which indicates its anxiolytic potential.

134 **Table 1: EPM test of *E. hirta***

135 Dunnett t (2-sided)[a]

Group (N=5)	% no. of entry into the open arm	% time spent in the open arms
Positive	77.47 ± 3.037***	79.21 ± 2.789**
Negative	55.71 ± 2.221	51.93 ± 2.080
EHAQ	60.62 ± 0.599	30.77 ± 0.582*
EHET	56.73 ± 1.609	28.95 ± 5.601**
EHEA	59.30 ± 2.061	33.74 ± 5.837*

136 Each value is presented as the mean ± SEM ($n = 5$). ***$P < 0.001$, **$P < 0.01$, *$P < 0.05$

137 a. Dunnett t-test treats one group as control and compares all other groups against it.

138 3.5 Writhing test

139 All three extracts significantly (*P>0.001*) inhibited the nociception induced by acetic acid on
140 mice (Table 2). But the aqueous extract showed maximum inhibition (89.51%) which was
141 comparable with the reference drug (92.30%).

142 **Table 2: Acetic Acid Induced Writhing Test of *E. hirta***

143 Dunnett t (2-sided)[a]

Group (N=5)	No. of writhing (Average ± S.E.M)	% inhibition
Positive	2.75 ± 0.478***	92.30
Negative	35.75 ± 3.099	0
EHAQ	3.75 ± 0.478***	89.51
EHET	9.25±0.661***	74.12
EHEA	10.25±1.089***	71.32

144 ***the mean difference is significant at the 0.001 level.

145 a. Dunnett t-test treats one group as control and compares all other groups against it.

146 3.6 Formalin test

147 Table 3 shows the effect of extracts on formalin induced persistent pain on two phases.
148 EHEA significantly (*P>0.001*) inhibited the licking time in either of the phases.

149 **Table 3: Formalin test of *E. hirta***

150 Dunnett t (2-sided)[a]

Group	Early Phase Licking	%	Late Phase Licking	%

(N=5)	Time (Average ± S.E.M)	inhibition	Time (Average ± S.E.M)	inhibition
Positive	41.5 ± 2.397***	62.94	26.25 ± 1.931***	62.76
Negative	112 ± 6.770	0	70.50 ± 1.707	0
EHAQ	109 ± 4.778	2.67	62.75 ± 2.529	10.99
EHET	94.25±5.344	15.84	61.50±3.476	12.76
EHEA	49.25±1.931***	56.02	34.5±3.926***	51.06

151 ***the mean difference is significant at the 0.001 level.

152 a. Dunnett t-test treats one group as control and compares all other groups against it.

153

154 4 Discussion

155 In vivo screening of locomotor activities is considered an effective method to investigate the
156 sedative potential. The ethanol extract significantly decreased the locomotor activity as
157 shown by the results of the open field and hole cross tests. The locomotor activity lowering
158 effect was evident at the 2nd observation (30 min) and continued up to 5th observation period
159 (120 min) (Figure 1& 2). As the major inhibitory neurotransmitter in CNS is the Gamma-
160 amino-butyric acid (GABA) and different anxiolytic, muscle relaxant, sedative-hypnotic
161 drugs showed their action through GABAA, it can be hypothesized that ethanol extract of *E.*
162 *hirta* also act by membrane hyperpolarization which potentiates GABA-ergic inhibition in the
163 CNS that leads to either decrease in the firing rate of critical neurons in the brain or direct
164 activation of GABA receptor [12]. Thus decreased spontaneous motor activity could be
165 attributed to the CNS depressant activity of the extracts. Moreover, elevated plus-maze test
166 validates psychomotor performance and emotional aspects of rodents. The results showed
167 that extracts of *E. hirta* leaf increased the time spent in open arms to little extent. This effect
168 can be attributed to the action on GABA benzodiazepine receptor complex, stimulation of
169 glucocorticoid production and release in the adrenal cortex [13], after administration of 5-

HT1B receptor antagonists and 5- HT1A agonists [14]. Therefore with the present data, it is difficult to predict the precise mechanism for the anxiolytic activity of the *E. hirta* leaf.

These three extracts were also evaluated in the formalin and acetic acid-induced writhing test for their analgesic activity. The acetic acid induced writhing response is an established procedure to evaluate peripheral analgesics. The response is thought to be mediated by the prostaglandin pathways, peritoneal mast cells and acid sensing ion channels [15-17]. Therefore, the significant pain reduction of the plant extracts may be due to acting with the prostaglandin pathways or interfering with other mediators responsible for peripheral pain.

The formalin test is another reliable model of analgesic which is better correlated with clinical pain [18, 19]. This method elucidates central and peripheral activities. The response of early phase is believed to represent a direct chemical stimulation of pain, due to the irritant effect of formalin on sensory C fibers [19]. The late phase response is most likely secondary to the development of an inflammatory response and the release of allergic mediators [20]. Inhibition of licking response of the extracts in the early phase and late phase signifies the analgesic effect of the extracts.

The medicinal potential of *Euphorbia hirta* (Linn.) is believed to be due to the presence of alkaloids, flavonoids, tannins, saponins, cardiac and cyanogenic glycosides in its crude extract [21]. However, phytochemical screening of each of the crude extract is necessary to attribute to the compound responsible for specific activity.

Conclusion

In vivo study showed that all three extracts possess analgesic or neuropharmacological activity which supports the traditional use of this plant leaf for medical ailments. Though, studies are required on higher animal model and subsequently on human subjects to prove its

193 clinical efficacy as an analgesic and CNS depressant agent. This study provides a scientific
194 acknowledgement of its use and concludes that oral preparation of this plant extract for
195 human use is safe and beneficial.

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