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

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

E. hirta is being used as a traditional source of medicine for many years in Bangladesh. The Tripura tribe in Chittagong hill tracts of Bangladesh uses this plant for increasing lactation after childbirth and to treat body sores, asthma and chronic bronchitis [3]. The Plant is used for the treatment of cough, asthma, chronic bronchitis, bowel complaints, worm infestation, kidney stones, bronchial affections, conjunctivitis. The crude extract is used as Analgesic, Anti–pyretic, Anxiolytic, Sedative, and Anti–inflammatory and also as Anti–coagulant [4]. These well-established traditional use of this plant acted as the driving force to conduct the present study. Although, many studies have been done on *E. hirta,* none of them compared aqueous, ethanol and ethyl acetate extracts of leaf for central and peripheral analgesic and sedative-anxiolytic activities.

2. Materials and Methods

2.1 Collection and Identification of Plant Material

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

2.2 Preparation of Extracts

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

2.3 Animals

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

2.4 Drugs and Chemicals

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

2.5 Acute toxicity test

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

extended up to 14 days. Estimating the LD50 of these extracts in lab was important for choosing a dose for optimum activity.

2.6 Sedative Activity

2.6.1 Hole Cross Test

The test was performed for screening sedative activity in mice. A steel partition was fixed in the middle of a cage having a size of 30×20×14 cm with A hole of 3 cm diameter at height 7.5 cm in the partition. The number of crossing from one chamber to other was counted for a period of 3 min on 0, 30, 60, 90 and 120 min after the oral gavage with test drugs. Diazepam was used in the positive control group as reference standard at the dose of 1 mg/kg [5].

2.6.2 Open Field Test

The experiment was carried out according to the methods described by Nyeem et al. [6]. The floor of the open field of a square meter was divided into several squares. The apparatus was enclosed with 40 cm high wall. The number of squares visited by the mice was counted for 3 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 [7]. 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. [8]. 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. [9]. 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.

2.9 Statistical analysis

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

3. Results

3.1 Acute toxicity test

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

3.2 Hole cross test

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

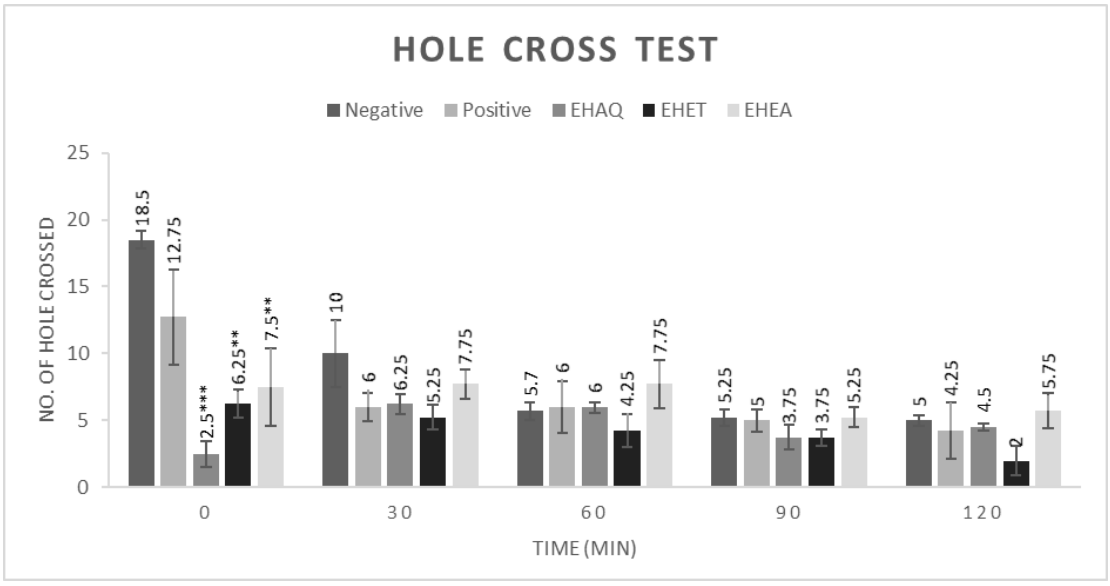

Figure 1: Effect of the aqueous, ethanol and ethyl acetate extracts of leaves of *E. hirta* on hole cross test in mice. Values are mean ± S.E.M., (n = 4); ** $P < 0.01$, *** $P < 0.001$ Dunnet test as compared to control (Vehicle = 0.5 mL/mouse).

3.3 Open field test

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

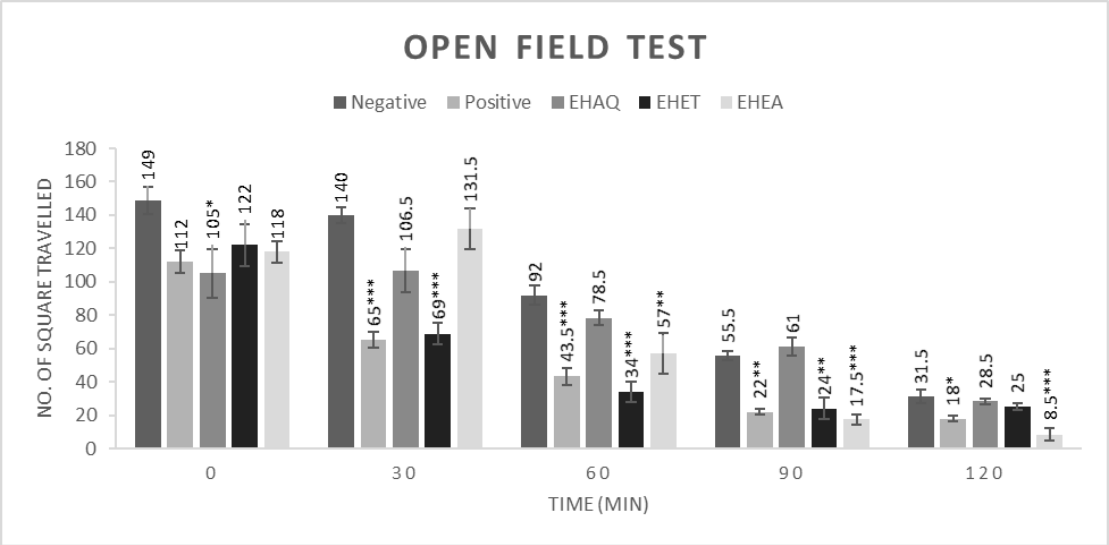

Figure 2: Effect of the aqueous, ethanol and ethyl acetate extracts of leaves of *E. hirta* on open field test in mice. Values are mean ± S.E.M., (n = 4); * $P < 0.05$, ** $P < 0.01$, *** $P <$ 0.001 Dunnet test as compared to control (Vehicle = 0.5 mL/mouse).

3.4 Elevated plus Maze

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

Table 1: EPM test of *E. hirta*

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*

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

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

3.5 Writhing test

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

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

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

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

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

3.6 Formalin test

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

Table 3: Formalin test of *E. hirta*

Dunnett t (2-sided)[a]

Group (N=5)	Early Phase Licking Time (Average ± S.E.M)	% inhibition	Late Phase Licking 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

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

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

4 Discussion

In vivo screening of locomotor activities is considered an effective method to investigate the sedative potential. The ethanol extract significantly decreased the locomotor activity as shown by the results of the open field and hole cross tests. The locomotor activity lowering effect was evident at the 2nd observation (30 min) and continued up to 5th observation period (120 min) (Figure 1& 2). As the major inhibitory neurotransmitter in CNS is the Gamma-amino-butyric acid (GABA) and different anxiolytic, muscle relaxant, sedative-hypnotic drugs showed their action through GABAA, it can be hypothesized that ethanol extract of *E. hirta* also act by membrane hyperpolarization which potentiates GABA-ergic inhibition in the CNS that leads to either decrease in the firing rate of critical neurons in the brain or direct

158 activation of GABA receptor [10]. Thus decreased spontaneous motor activity could be
159 attributed to the CNS depressant activity of the extracts. Moreover, elevated plus-maze test
160 validates psychomotor performance and emotional aspects of rodents. The results showed
161 that extracts of *E. hirta* leaf increased the time spent in open arms to little extent. This effect
162 can be attributed to the action on GABA benzodiazepine receptor complex, stimulation of
163 glucocorticoid production and release in the adrenal cortex [11], after administration of 5-
164 HT1B receptor antagonists and 5- HT1A agonists [12]. Therefore with the present data, it is
165 difficult to predict the precise mechanism for the anxiolytic activity of the *E. hirta* leaf.

166 These three extracts were also evaluated in the formalin and acetic acid-induced writhing test
167 for their analgesic activity. The acetic acid induced writhing response is an established
168 procedure to evaluate peripheral analgesics. The response is thought to be mediated by the
169 prostaglandin pathways, peritoneal mast cells and acid sensing ion channels [13-15].
170 Therefore, the significant pain reduction of the plant extracts may be due to acting with the
171 prostaglandin pathways or interfering with other mediators responsible for peripheral pain.

172 The formalin test is another reliable model of analgesic which is better correlated with
173 clinical pain [16, 17]. This method elucidates central and peripheral activities. The response
174 of early phase is believed to represent a direct chemical stimulation of pain, due to the irritant
175 effect of formalin on sensory C fibers [17]. The late phase response is most likely secondary
176 to the development of an inflammatory response and the release of allergic mediators [18].
177 Inhibition of licking response of the extracts in the early phase and late phase signifies the
178 analgesic effect of the extracts.

179 The medicinal potential of *Euphorbia hirta* (Linn.) is believed to be due to the presence of
180 alkaloids, flavonoids, tannins, saponins, cardiac and cyanogenic glycosides in its crude
181 extract [19]. However, phytochemical screening of each of the crude extract is necessary to
182 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 clinical efficacy as an analgesic and CNS depressant agent. This study provides a scientific acknowledgement of its use and concludes that oral preparation of this plant extract for human use is safe and beneficial.

References

1. Patil, S. B., Naikwade, N. S., & Magdum, C. S.: Review on Phytochemistry and Pharmacological aspects of Euphorbia hirta (Linn.) linn. Journal of Pharmaceutical Research and Health Care. 2009; 1(1):113-133.

2. Rao, K. V., Karthik, L., Elumalai, E. K., Srinivasan, K., & Kumar, G.: Antibacterial and antifungal activity of Euphorbia hirta (Linn.) l. Leaves: A comparative study. Journal of Pharmacy Research. 2010; 3(3):548-549.

3. Hossan, M. S., Hanif, A., Khan, M., Bari, S., Jahan, R., & Rahmatullah, M: Ethnobotanical survey of the Tripura tribe of Bangladesh. American-Eurasian Journal of Sustainable Agriculture. 2009; 3(2):253-261.

4. Nagori, B. P., & Solanki, R: Role of Medicinal Plants in Wound Healing. Research Journal of Medicinal Plants. 2011; 5(4):392-405.

5. Subhan N, Alam MA, Ahmed F, Shahid IJ, Nahar L, Sarker SD. Bioactivity of Excoecaria agallocha. Braz J Pharmacogn. 2008, 18, 521–526. DOI: 10.1590/S0102695X2008000400004

6. Nyeem MAB, Alam MA, Awal MA, Mostofa M, Uddin SJ, Islam N et al. CNS Depressant Effect of the Crude Ethanolic Extract of the Flowering Tops of Rosa Damascena. Iranian J. Pharmacol Ther. 2006, 5, 171-174.

7. Braida D, CapurroV, Zani A, Rubino T, Viganò D, Parolaro D et al. Potential anxiolytic- and antidepressant-like effects of salvinorin A, the main active ingredient of Salvia divinorum, in rodents. British J Pharmacol., 2009, 157, 844-853. DOI: 10.1111/j.1476-5381.2009.00230.x

8. Meera S, Kumar NS and Guptatyam VSSS. Screening of Anti-arthritic, Antiinflmmatory and Analgesic Activity of a Polyherbal Formulation. Int J Pharmacol., 2008, 4(5), 398-402. DOI: 10.3923/ijp.2008.398.402

9. Sharma A, Bhatial S, Kharyaz MD, Gajbhiye V, Ganesh N, Namdeo AG and Mahadik KR: Anti-inflammatory and analgesic activity of different fractions of Boswellia serrata. International Journal of Phytomedicine. 2010; 2:94-99.

10. Khatun M H, Islam M R, Mamun A, Nahar L, Luth F, and Islam M A U: In Vivo Evalu atio n of CNS Dep ressant and Antinociceptive activities of Methanol Extract of *Hibiscus sabdariffa* Fruits . J of Appli Scie Rese. 2011; 7(6):798-804

11. Nishikava H, Hata T, Funakami Y: A role for corticotropin-releasing factor in repeated cold stress-in duced anxiety-like behavior during forced swimming and elevated plu s-maze test in mice. Biol Pharm Bull. 2004; 27(3):352-356.

12. Millan MJ, Hjo rth S, Samanin R, Schreiber R, Jaffard R, De Ladonchamps: B. S1553 5, A novel benzodioxopiperazine ligand of serotonin (%-5HT)1A receptor: II. Modulation of hippocampal serotonin release in relation to p otential an xiolytic properties. J Pharmacol Exp Ther. 1997; 282:148 161.

13. Hossain MM, Ali MS, Saha A, Alimuzzaman M: Antinociceptive activity of whole plant extracts of Paederia foetida. Dhaka University Journal of Pharmaceutical Science. 2006; 5:67-69.

14. Ronaldo AR, Mariana LV, Sara MT, Adriana BPP, Steve P, Ferreira SH and Fernando QC: Involvement of resident macrophages and mast cells in the writhing nociceptive response induced by zymosan and acetic acid in mice. European Journal Pharmacology. 2000; 387:111-118.

15. Voilley N: Acid-Sensing Ion Channels (ASICs): New targets for the analgesic effects of Non-Steroid Anti- inflammatory Drugs (NSAIDs). Current Drug Targets-Inflammation an Allergy. 2004; 3:71-79.

16. Ghannadi A, Hajhashemi V and Jafarabadi H: An investigation of the analgesic and antiinflammatory effects of Nigella sativa seed polyphenols. Journal of Medicinal Food. 2005; 8:488-493.

17. Sandra D, Susana A, Domingo M-H. Peripheral Analgesic and Anti-Inflammatory Effects of Smilax canariensis in an Animal Model. Pharmacology & Pharmacy, 2015, 6, 391-400. DOI: doi.org/10.4236/pp.2015.68040

18. Hunskaar S, Hole K: The formalin test in mice: dissociation between inflammatory and noninflammatory pain. Pain. 1987; 30:103-114.

19. Ogbulie, J. N., Ogueke, C. C., Okoli, I. C., & Anyanwu, B. N.: Antibacterial activities and toxicological potentials of crude ethanolic extracts of Euphorbia hirta (Linn.). African Journal of Biotechnology. 2007; 6(13):1544-1548.