Research Article

Antinociceptive and Anti-Inflammatory Activities of Euphorbia Hirta L. Leaves in Animal Models.

***Dipak Kumar Mishra[1], Annada Prasad Mahapatra[1], Prabhudutta Panda[2].**

1. Institute of Medical Technology, Puri - 752001, Orissa, India.
2. College of Pharmaceutical Sciences, Mohuda, Berhampur-760002, Orissa, India.

ABSTRACT

The present study was done to arrive the potential of chloroform extract of *Euphorbia (E) Hirta L.* (Family-Euphorbiaceae) leaves on antinociceptive, behavioral study and anti-inflammatory effects using various animal models. The dried, powdered leaves of, *Euphorbia Hirta L.* were extracted successively with petroleum ether (60-80°C) and chloroform in soxhlet apparatus. The chloroform extract (yield 5.47% w/w with respected to dry powdered plant material) was selected for all experimental procedure. Two models were taken to derive the effects of nociception, by the tail immersion and hot plate method on Swiss albino mice and anti inflammatory effect were investigated by employing the carrageenan induced rat paw edema test in adult Wister albino rats. Behavioral study was investigated by elevated plus maze method in Swiss albino mice. Results were revealed that the EHCE has significant antinociceptive effect ($P < 0.001$) at the dose levels of 100, 200 and 400 mg/kg, orally in mice and also produced anti-inflammatory effect ($P < 0.001$) at the same dose levels used in the rats. Behavioral study of the EHCE shows that there is no significant anxiolysis effect when used orally. It concludes that, EHCE possessed remarkable antinociceptive effect and anti-inflammatory effect but no anxiolytic effect on animal models.

Keywords: Antinociceptive, Anti-inflammatory, Behavioral study, Chloroform extract, Euphorbia Hirta L. Leaves.

Received 29 June 2012 Received in revised form 14 July 2012 Accepted 19 July 2012

***Address for correspondence:**
Dipak Kumar Mishra
Institute of Medical Technology, Puri - 752001, Orissa, India.
E-mail: dipu_suman2003@yahoo.co.in

INTRODUCTION

Euphorbia hirta L. belongs to the plant family *Euphorbiaceae* is a annual herb, a slender- stemmed, annual hairy plant with branches, including inflorescence and capsules are found tropical Africa and also in South Africa . Within India, it is common in waste ground throughout the hotter parts. It is sufficiently found in the Coastal South Orissa of India. The plant is well known under vernaculars as 'Snake weed' or 'Asthma herb ' in English, 'Haraharia ' in Oriya, and 'doodhli' in Hindi and 'Dugdhika' in Sanskrit [1]. The powdered leaves are useful in intestinal parasites, diarrhoea, peptic ulcers , heartburn, vomiting, amoebic dysentery, asthma, bronchitis, hay fever, laryngeal spasms, emphysema, coughs, colds, kidney stones, menstrual problems, sterility, venereal diseases, worm infestations in children, gonorrhoea, jaundice, pimples, digestive problems and tumours [2]. This plant contains main chemical constituents viz; euphorbianin, leucocyanidol, camphol, quercitrin and quercitol gallic acid, myricitrin, 3, 4-di-O-galloylquinic acid, 2, 4, 6-tri-O-galloyl-D-glucose, 1, 2, 3, 4, 6-penta-O-galloyl-β-Dglucose,1, 3, 4, 6-tetra-*O*-galloyl-β-D-glucose, Triterpenes and phytosterols (β-Amyrin, 24-methylenecycloartenol, and β-Sitosterol, heptacosane, *n*nonacosane [3-12]. The literature reveals that the *E. hirta* leaves are used orally against pain, inflammation and epilepsy in traditional system [13] and most of the phyto constitutes were isolated from leaves of *E.*

hirta. Hence, the leaves of this plant have been used for all pharmacological activities. It has become necessary to search new analgesics due to the limitations of opioid and NSAID therapy and on account of the usefulness of this plant in the traditional treatment of some painful and inflammatory conditions which has not yet been scientifically proved. Hence, an attempt has been made to ascertain the scientific validity to examine the possible antinociceptive, anti-inflammatory and also behavioral study of the crude chloroform extract of *E .hirta L.* leaves in animal models.

MATERIALS AND METHODS

Plant Material

Euphorbia Hirta L. leaves were collected during the month of August from the rural belt of high land of Chandili village, Rayagada Dist, Orissa India, identified and authenticated by Prof. S. K. Dash, HOD, PG Department of Bioscience, College of Pharmaceutical Sciences, Mohuda, comparing with the voucher specimen (EHL-I) present in the herbarium, has been kept in the laboratory for future references. The collected plants were washed and air-dried under the shade, cut into minute pieces, powdered by a mechanical grinder and passed through 40-mesh sieve and stored in a closed vessel for future use.

Preparation of Euphorbia Hirta L. Chloroform Extract

The dried, powdered leaves of *Euphorbia Hirta L.*(1 kg) were extracted successively with 1,200 ml of petroleum ether (60 - 80°C) and 1200ml of chloroform in soxhlet apparatus by following standard TAPPI test Method. A dark greenish black coloured petroleum ether extract was obtained. The same powdered leaves (marc), after proper drying, were extracted with chloroform (5 hr) to produce a greenish brown semisolid mass. The extractions were carried out until the solvents became colourless. These extracts were again dried and concentrated by evaporating the solvent completely under vacuum at the range of boiling points of solvent (Chloroform at 62°C) using rotatory evaporator (Jain Scientific glass works, DTC 201, Ambala cantt, India). The chloroform extract (yield 5.47 % w/w with respected to dry powdered plant material) was selected for all experimental procedure. The chemical constituents of the extract was recognized by qualitative analysis and confirmed by the thin layer chromatography (i.e. hRf values). EHCE was prepared an emulsion by triturating the accurately weighed quantity of the extract with 0.025 % w/v of carboxyl methyl cellulose (CMC) used for the study. All extractive solvents are of analytical grade reagents (AR).

Preparation of Drugs

Tramadol (Contramal, Nicholas Piramal India Limited, Mumbai.) was dissolved in 0.025% w/v of CMC. Diclofenac sodium (Diclomax, Torrent Pharmaceutical Pvt. Ltd., Ahmedabad, India) and carrageenan (Sigma Chemicals Company. St. Louis, MO, USA) were used for the Anti-inflammatory study. The standard drug diazepam (Calmpose, Ranbaxy Lab, India) was used for behavioral study. EHCE and standard drugs were prepared by suspending them in 0.025% w/v CMC at definite concentrations separately for all pharmacological studies.

Preliminary Phytochemical Analysis

The EHCE was subjected to preliminary phytochemical screening for revealing of major chemical groups. In each case test 10% w/v solution of the extract in chloroform was used and unless otherwise mentioned in individual test [14].

Experimental Animals

Adult wister albino rats weighing between 180 and 220 g and Swiss albino mice of either sex between 18 and 22 g were used for the experiments, obtained from M/s Ghosh & Ghosh enterprises, Kolkata, India and were housed in standard polypropylene cages at room temperature of 30 ± 2°C and 60 - 65% relative humidity and had free access to food and water *ad libitum.*. The animals were used for the experiment after an acclimatization period of one week. All procedures described were reviewed and approved by the Institutional Animals Ethical Committee (CPCSEA approval no. 1018/c/06/CPCSEA) of Royal College of pharmacy and Health Sciences, Berhampur, Orissa.

Acute Toxicity Analysis

Toxicity study of the EHCE was performed to get the information, how safe is this extract for the therapeutic use. The LD50 value of

EHCE was derived by following the method [15].The maximum non-lethal dose was found to be 4,000 mg/kg body weight, orally. The 0.025% CMC was used as a vehicle and showed no mortality. The determination of acute toxicity by adopting fixed dose the guideline of CPCSEA and 1/10th of LD50 cut off values [14, 16] of the extracts were taken as screening dose. i.e. 100, 200, 400 mg/kg for subsequent studies.

Antinociceptive Activity

Antinociceptive activity of the EHCE was tested by using the Experimental models of Tail immersion method and Hot plate method. In the tail immersion method, the water bath was maintained at 55 ± 0.5ºC and the tail of mouse was immersed to a constant level (5 cm) in it. The time to flick the tail from water (reaction time) was recorded. A maximum immersion time of 30 s. was maintained to prevent thermal injury to the animals [17]. The reaction time was measured 30 min before test and reference standard. A considerable increase in reaction time compared with control was considered a positive analgesic response. The Hot plate test was carried out using an UGO Basile hot plate apparatus (Socrel model D-S37, Italy). The hot plate test was used to evaluate latency time by following the method [18]. The temperature of the hot plate was maintained at 55 ± 0.5ºC to assess the thermal-induced antinociceptive activity as described [19]. Animals were placed into Perspex cylinder on the heated surface and the time between placement and licking of the paws or jumping was recorded as response latency. After 16 h. fasted mice were divided into five groups of six mice in each. Group-I, served as a control, received 0.025% w/v CMC, 10 ml/kg, orally, Group-II to IV, animals received EHCE at dose of 100, 200 and 400 mg/kg, orally and Group-V, animals were treated with tramadol (50 mg/kg, orally) as a positive-control. Cut off time for the response was set at 60 s to avoid tissue damage to the mice paws [20]. After the determination of baseline response latencies, hot-plate latencies were re-determined at 30min, 60min, and 90min after oral administration of tested drugs and positive-control in this experiment. The pain inhibition percentage was calculated [21,22], according to the following formula. % of (PIP) = Latency (test) - Latency (control) ×100)/Latency (control).

Anti-Inflammatory Assay

According to the technique [23, 24], anti-inflammatory activity was evaluated by using the carrageenan-induced edema in rat paw. After 16 h fasted rats were divided into five groups of six each categorized as Group I to Group V. Group-I, served as a control, received 0.025% w/v CMC at the dose level of 10 ml/kg, orally, Group-II to IV, animals received EHCE at dose of 100, 200 and 400 mg/kg, orally, Group-V, animals were treated with standard drug diclofenac sodium at the dose level of 10mg/kg, orally. Acute inflammation was induced by carrageenan in sub planter side of the right hind paw in rats. The paw was marked with ink at the level of the lateral malleolus and dipped in Perpex cell up to this mark. The measurement of the paw volume was carried out by means of Ugo Basile Plethysmograph model 7150, before and after 4 h after carrageenan injection [25]. Percentage inhibition of edema was calculated using formula [26]. % Pain Inhibition = (1- Vt/Vc) × 100. Where, Vt = Increase in paw volume in drug treated rats and Vc = Increase in paw volume in control group treated rats.

Behavioral Analysis

Behavioral analysis of the animals was evaluated by Elevated plus maze method (EPM). The EPM apparatus consisted of two open arms (30 × 5 cm) and two closed arms (30 × 5 × 20 cm) emanating from a common central platform (5 × 5 cm). The two pairs of identical arms were opposite to each other. The entire apparatus was elevated to a height of 50 cm above the floor level. The animals received the treatment 15 min. before the start of session as per the schedule. At the beginning of the session, a mouse was positioned at the centre of the maze, its head facing the closed arm and allowed to explore the maze for 5 min. All the entries were recorded regarding the time spent in the open arm and the percent entries in the open and closed arms. An entry was defined as the presence of all four paws in the arm [27]. The EPM was carefully wiped with 10% ethanol after each trail to eliminate the possible bias due to the odor of the previous animals [28]. Group-I, served as

control, received 0.025 % w/v CMC, 10 ml/kg, orally, Group-II to IV, animals received EHCE at dose of 100, 200 and 400 mg/kg and Group-IV, animals were treated with standard drug diazepam at the dose level of 4 mg/kg, orally and the average time spent in both open and closed arm in each group of the mice were recorded.

Statistical Analysis

The results were presented as Mean ± S.E.M. and statistical significance ($P < 0.001$) between treated and a control group was evaluated by paired t-test [29].

RESULTS

Preliminary Phytochemical Analysis

Results of different chemical tests on the chloroform extract of *E. Hirta L.* showed the presence of phytoconstituents viz., steroids, terpenoids, flavonoids, saponins and tannins.

Antinociceptive Activity

The effects of the EHCE were used to investigate the antinociceptive effects in animal models by adopting two methods of tail immersion test and hot plate test are shown in (**Table 1 & 2)** respectively. The extract produced about 67 and 75 % of PIP in test animals in case of tail immersion method at the dose of 200 and 400 mg/kg after every one-hour intervals. The results were found to be statistically significant ($P < 0.001$) antinociceptive effects and were comparable to the standard drug tramadol, which showed 71% of PIP at the dose of 50 mg/kg ($P < 0.001$). The centrally acting analgesics generally elevate the pain threshold of mice towards heat. EHCE significantly ($P < 0.001$) increased the reaction time of animals towards the thermal source in a dose-dependent manner. In hot plate test EHCE showed a pain inhibition percentage (PIP) of 60.87% and 64.94 %, respectively whereas tramadol showed a greater PIP of 67.47 % at 90 min after treatment.

Table 1: Antinociceptive activity of *Euphorbia hirta l.* by tail immersion method.

Groups	Dose (mg/kg)	Pretreatment	Post treatment			
			1h	2h	3h	4h
(Group-I)Control (0.025%CMC	10 ml	1.6 ± 1.05	1.6 ± 1.13	1.6 ± 1.13	1.6 ± 1.13	1.6 ± 1.13
(Group-II) EHCE	100 mg / kg	1.7 ± 1.52	1.71 ± 1.01 (6.43%).	1.85 ± 1.0 (13.51%).	1.87 ± 1.0 (14.44%).	4.22 ± 1.02 (62.00%).
(Group-III) EHCE	200 mg / kg	1.8 ± 0.9	4.9 ± 0.9a (67.35%).	6.5 ± 1.5a (75.38%).	6.6 ± 1.21a (75.76%).	5.5 ±1.12a (70.91%).
(Group-IV) EHCE	400 mg / kg	1.9 ±1.02	6.3 ± 1.05 (74.60%).	6.6 ± 1.12a (75.76%).	6.4 ± 1.1a (75.00%).	7.1 ± 1.21a (77.46%).
(Group-V) Tramadol + control (Positive control)	50 mg / kg	1.8 ±1.2	5.5 ± 1.12 (70.91%).	10.8 ± 1.0 (85.19%).	12.2 ± 1.0 (86.88%).	14.3 ± 1.01 (88.81%).

Results expressed as mean ± S.E.M. aP < 0.001 significantly different from control ; Paired t - test (n = 6).

Figures in the parentheses indicate % of (PIP) in mice.

Anti-inflammatory activity

Indigenous drug systems can be a source of variety of new drugs, which can provide relief in inflammation but their claimed reputation has to be verified on scientific basis. The present investigation revealed that the anti-inflammatory activity of *E. Hirta* on carrageenan induced paw edema in rats is shown in (**Table 3)**. These results indicate that, EHCE showed significant reduction ($P < 0.001$) in edema volume at oral dose of 100, 200 and 400 mg/kg of body weight, which is comparable to the standard drug diclofenac sodium at the dose of 10 mg/kg in acute inflammatory model.

Table 2: Antinociceptive activity of *Euphorbia hirta l.* leaves by hot plate method.

Treatment	Dose (mg/kg)	Paw ticking time in seconds				Paw jumping time in seconds			
		0	30	60	90	0	30	60	90
(Group-I) control (0.025%CMC)	10 ml	3.1 ± 0.1	2.9 ± 0.3	2.7 ± 0.21	2.8 ± 0.1	3.1 ± 1.0	2.9 ± 0.8	2.8 ± 0.6	2.7 ± 0.4
(Group-II) EHCE	100 mg / kg	3.7 ± 0.31	3.7 ± 0.2b (21.62%)	4.6 ± 0.1b (41.30%)	5.1 ± 0.12b (45.09%)	3.7 ± 0.3	6.3 ± 0.1b (53.97%)	6.7 ± 0.2b (58.21%)	6.6 ± 0.4b (59.09%)
(Group-III) EHCE	200 mg/kg	3.6 ± 0.12	4.8 ± 0.15 (39.58%)	4.5 ± 0.1b (40.00%)	5.7 ± 0.12b (50.88%).	3.8 ± 0.3	6.8 ± 0.4b (57.35%)	6.6 ± 0.5b (57.58%)	6.9 ± 0.6a (60.87%)
(Group-IV) EHCE	400 mg/kg	3.8 ± 0.12	4.7 ± 0.2 (38.30%)	4.9 ± 0.8 (44.90%)	5.0 ± 0.5a (44.00%)	4.1 ± 0.6	5.9 ± 0.7 (50.85%)	5.8 ± 0.12 (51.72%)	7.7 ± 0.5a (64.94%)
(Group-I) control + Tramadol	50 mg/kg	3.5 ± 0.2	4.7 ± 0.3 (38.30%)	5.6 ± 0.12a (51.79%)	6.01 ± 0.15a (53.41%)	3.4 ± 0.1	6.6 ± 0.3a (56.06%)	7.7 ± 0.8a (63.64%)	8.3 ± 0.3a (67.47%)

Results expressed as mean ± S.E.M.bP<0.05 , aP < 0.001 significantly different from control ; Paired t - test (n = 6).

Figures in the parentheses indicate % of (PIP) in mice.

Table 3: Anti-inflammatory activity of *Euphorbia hirta l.* leaves on carrageenan induced paw edema in albino rats.

Treatment	Dose (mg/kg)	Percentage of inhibition of paw edema after carrageen injection			
		1h	2h	3h	4h
(Group-I) Control (0.025 % CMC)	10mg / kg	13.22 ± 1.97	35.28 ± 4.05	41.20 ± 4.06	41.00 ± 5.12
(Group-II) EHCE	100mg / kg	30.90 ± 2.77a	70.83 ± 9.63a	100.70 ± 8.67b	110.54 ± 10.38b
(Group-III) EHCE	200 mg / kg	24.74 ± 4.20a	46.28 ± 1.01a	70.62 ± 7.80a	80.09 ± 4.56a
(Group-IV) EHCE	400 mg / kg	19.64 ± 0.705a	40.09 ± 0.71a	62.92 ± 7.604a	69.79 ± 4.26a
(Group-V) Diclofenac sodium	10 mg / kg	44.34 ± 2.46a	99.80 ± 3.64a	130.04 ± 2.53a	134.76 ± 5.34a

Results expressed as mean ± S.E.M.bP<0.05 , aP < 0.001 significantly different from control ; Paired t - test (n = 6).

Behavioral Study by EPM

In EPM, the behaviour, which was absorbed that, confirmed the anxiolytic activity of diazepam as reported. The effect of the EHCE on behavioral study by EPM in mice was depicted in Table 4. In administration of diazepam 4 mg/kg, i.p. dose produce significant anxiolytic effect indicated by increase in the open arm entries, time spent in open arm. However, there was no significant anxiolysis effect or impairment in behavioral of the animals observed with EHCE at the dose levels of 100, 200 and 400 mg/kg of body weight when administered orally.

Table 4: **Behavioral study of *Euphorbia hirta l.* leaves by elevated plus maze (EPM) in mice.**

Treatment	Dose	Number of arm entry		Time spend in arms(seconds)	
		open	closed	open	closed
(Group-I)Control (0.025% CMC)	10ml	2.3 ± 2.2	4.5 ± 2.3	48.42 ± 1.98	251.63 ± 1.92
(Group-II) EHCE	100mg / kg	0.8 ± 1.15	1.4 ± 1.97	24.20 ± 2.13	227.0 ± 1.99
(Group-III) EHCE	200 mg / kg	1.6 ± 2.1	2.0 ± 2.18	24.2 ± 2.28	275.70 ± 2.02
(Group-IV) EHCE	400 mg / kg	1.5 ± 2.18	2.10 ± 2.17	27.2 ± 2.02	274.6 ± 2.13
(Group-V) Control + Diazepam	4 mg / kg i.p.	5.7 ± 2.15	1.2 ± 2.01	267.0 ± 1.89	31.2 ± 1.98

Administration of the diazepam 4mg / kg , i.p. dose produce significant anxiolytic effect compared to that of the control group.

However, there was no significant anxiolysis effect in EHCE when administered orally.

DISCUSSION

In acute toxicity study, oral administration of EHCE did not produce any mortality in mice upto a dose level of 4 g/kg. This may be due to broad non-toxic range of the plant, where the plant extract showed a high LD50 and relatively safety. The antinociceptic effect of EHCE was investigated by two well-established assay procedures. The antinociceptic action of all the tested compounds was clearly evident by a dose dependent reduction on tail immersion test and hot plate test are shown in Table 1 & 2 respectively. These methods for investigating antinociceptive were selected such that centrally mediated effects were investigated. Even though the present day armamentarium is rich in potent analgesic agents, the search for novel and safe analgesic drugs continues and vigorously pursued in many parts of world. The reasons are very obvious; the most potent opiate group of analgesics is associated with many undesirable side effects and also carries a potential for drug addiction. The other prominent groups of analgesics viz. NSAID are notorious for their ulcerogenic [30] and nephrotoxic potential [31]. In this regard, it is interesting to note that many flavonoids isolated from various plants exhibited potent analgesics and anti-inflammatory action [32-35]. It is also believed that those flavonoids ability to influence the said activities occur through modulation of the pro-inflammatory gene expression, such as inducible NO synthase and cyclooxygenase-2 [36]. Due to these valid reasons, the plant *E.Hirta* was explored for its antinociceptive and anti-inflammatory activities. The EHCE at the doses of 100, 200 and 400 mg/kg, p.o. tested was shown to possess antinociceptive activity in tail immersion method. It has been assumed that thermally motivated and tonic tests elicit the selective stimulation of Aδ and C fibers, respectively [37]. It is tempting to propose that EHCE or its metabolites may interfere with the transmission of both fibers or with a common pathway, such as spinal and thalamic pathways. The hot-plate test was selected to investigate central analgesic activity, because it had several advantages, particularly the sensitivity to strong analgesics and limited tissue damage. Hence, the hot plate method was employed to verify if the extract could show any central analgesic effect, as the test is specifies analgesic test [38]. It was demonstrated that the EHCE at dose of 100, 200 and 400 mg/kg, p.o. widely used acute

inflammatory model for studying anti-inflammatory agent. The EHCE were found to be statistically significant ($P < 0.001$) antinociceptive effects and were comparable to the standard drug tramadol at the dose of 50 mg/kg. Edema represents the early phase of inflammation in carrageenan induced paw edema and is the simplest and most widely used acute inflammatory model for studying anti-inflammatory agent. The development of Carrageenan-induced edema is believed to be biphasic of which the first phase is mediated by release of histamine, serotonin and kinine in the first hour after injection of carrageenan and the second phase is related to release of prostaglandin like substances in 2-3 h. [39-41]. The EHCE showed significant anti-inflammatory activity at 4 h against carrageenan injection suggesting that the extract predominantly inhibits the release of prostaglandin like substances from phlogenic stimuli. There are reports that flavonoid possesses anti-inflammatory activity [33-35,42] and some of them also act as phoshpholipase inhibitors [43-45]. Also, there are few reports on the experimental models, the non selective antagonist of opiod receptors apparently acts by antagonizing the action of endogenous opiods involved in pain or stress [46]. In the present study, the maximum anti-inflammatory effect of EHCE may be attributed to presence of flavonoids as evident by preliminary phytochemical investigations. From the results it could be concluded that the extracts exhibit anti-nociceptive activity by central as well as peripheral mechanism(s). The behavioral study of the animals was evaluated by EPM. The EPM test is based on a premise where the exposure to an EPM evoked an approach avoidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm [27, 47]. The decrease in aversion to the open arm is the result of an anxiolytic effect expressed by the increase time spent and entries in the open arm. Most of the sedatives and hypnotics drugs were implied by the method of EPM. Generally sedatives and hypnotics suppress cerebral activity. They also depress the CNS beginning with the cerebral cortex and descending with increasing dose to the medullury centers causing medullary paralysis [48]. It was reported that the administration of diazepam 4 mg/kg, i.p.dose produce significant anxiolytic effect [49], indicated by increase in the open arm entries, time spent in open arm and closed arm. The control group and the dose levels of 100, 200 and 400 mg/kg of body weight of EHCE was not produce significant anxiolysis effect when compare to standard drug. Finally, it concluded that the EHCE possess remarkable antinociceptive, anti-inflammatory activity but no anxiolytic activity. However, more detailed phytochemical studies are necessary to identify the active principles and exact mechanisms of action.

CONCLUSION

There has been a growing trend towards natural medicines and the use of dietary supplements for modern healthcare, as people throughout the world are becoming increasingly dissatisfied with the possible side-effects, lack of noticeable long-term results and high cost associated with allopathic drugs. Therefore, the leaves of the plant *Euphorbia Hirta L.* (Family-Euphorbiaceae) were selected for the present investigation. In traditional system of medicine, the plant *Euphorbia hirta L.* leaves were found to be very useful for the treatment of various human ailments. The plants have been reported by different researchers to have various medicinal values. Finally, it concluded that the EHCE possess remarkable antinociceptive, anti-inflammatory activity but no anxiolytic activity.

ACKNOWLEDGEMENTS

Authors are thankful to the authority of the Royal College of pharmacy and Health Sciences, Berhampur, Orissa for laboratory facilities and also thankful to the Prof. S. K. Dash, HOD, PG Department of Biosciences, College of Pharmaceutical sciences, Mohuda, Berhampur, for the identification of the plant.

REFERENCES

1. Sandeep BP, Nilofar SN, Chandrakant SM. Review on Phytochemistry and Pharmacological Aspects of Euphorbia hirta Linn. J. Pharma. Res. Health Care 2009; 1:113-133.
2. Kirtikar KR, Basu BA. Indian medicinal plants. 2nd ed. New Delhi: Periodical Experts Books Agency; 1991. pp.856-860.
3. Rastogi RP, Mehrotra BN. Compendium of Indian medicinal plants, CDRI. New Delhi: Lucknow and publication and information directorate; 1995. 4:543.
4. Sood SK, Bhardwaj R, Lakhanpal TN. Ethnic Indian Plants in cure of diabetes. India: Scientific Publishers; 2005. pp.64-65.
5. Gupta DR. A chemical examination of Euphorbia hirta Linn. Bull Chem Soc Jpn 1966; 39(11):2532-2534.
6. Blanc P, Bertrand P, De saqui-Sannes G. Flavonoids of Euphorbia hirta. Plants Med. Phytother 1972; 6:106-109.
7. Aqil M. Euphorbianin, a new glycoside from Euphorbia hirta Linn. Global Journal of Pure and applied science 1999; 5(3):371.
8. Chen L. Polyphenols from leaves of Euphorbia hirta L. Zhongguo Zhong Yao Za Zhi 1991;16(1):38-39.
9. Yoshida T, Chen L. Tannins and related polyphenols of euphorbiaceous plants IV.Euphorbins A and B, novel dimeric dehydroellagitannins from euphorbia hirta L. Chemical and Pharmaceutical bulletin 1988;36(8):2940.
10. Yoshida T, Namba O, Okuda T. Hydrolysable tannin oligomers from euphorbiaceae. Tennen Yuki Kagobutsu Toronkai Koen Yoshishu 1989;31:601.
11. Martinz V. Antiinflammatory compounds from the n hexane extract of euphorbia hirta. Reviews sociedad Quimica De mexico 1999;43:103.
12. Gnecco S. Distribution pattern of n-alkanes in Chilean species from the Euphorbiaceae family. Boletin de la Sociedad Chilena de Quimica 1996; 41(3): 229.
13. Lanhers MC, Fleurentin J, Dorfman P, Mortier F, Pelt JM. Analgesic,antipyretic and anti-inflammatory properties of Euphorbia hirta. Planta Med 1991;57:225-231.
14. Patil MB, Jalalpure SS, Ashraf A. Preliminary phytochemical investigation and wound healing activity of the leaves of Argemone mexicana Linn. Indian Drugs 2001;38:288-293.
15. Litchfield JT, Wilcoxon F. A simplified method of evaluating dose-effect experiments. J. Pharmacol Exp Ther 1949;96:99-113.
16. Shivakumar H, Sankara SLVJ, Vaidya VP. Anti-inflammatory activity of the unripe fruits of Ficus glomerata. Indian Drugs 2006;44:48-50.
17. Janssen PAJ, Niemegeers CJE, Dony JGH. The inhibitory effect of fentanyl and other morphine like analgesics on the warm water induced tail withdrawal reflex in rats. Arzneimittel Forschung. Drug research 1963;6:502-507.
18. Hosseinzabeh H, Ramezani M, Salmani G. Antinociceptive, anti-inflammatory and acute toxicity effects of Zataria multiflora Boiss extracts in mice and rats. J. Ethnopharmacol 2000;73:379-385.
19. Turner RA. Analgesics. In: Turner RA (ed.) Screening method pharmacology. London: Acadamic press; 1965. pp.100-113.
20. Junping KOU, Yun NI, Na LI, Jingrong WANG, Liang LIU, Zhi-Hong JIANG. Analgesic and Anti-inflammatory activities of total extract and individual fractions of Chinese medicinal ants Polyrhachis lamellidens. Biol. Pharm. Bull 2005;28:176-180.
21. Wu Tin, Tian-Shan Wang, Fang-Zhou Yin, Bao-Chang Chi. Analgesic and anti- inflammatory properties of brucine and brucine-N-oxide extracted from seeds of Strychnos nuxvomica. J. Ethnopharmacol 2003;88:205-214.
22. Owoyele BV, Olaleye SB, Oke JM, Elegbe RA. Anti-inflammatory and analgesic activities of Nothospondias Staudtii. Niger J. Physiol. Sci 2004;19:102-105.
23. Winter CA, Risley EA, Nuss CW. Carrageenan induced oedema in hind paw of rats an assay for anti-inflammatory drugs. Proc. Soc. Exp. Biol. Med 1962;111:544-547.
24. Satyanarayana D, Joshi AB, Chandrashekar KS, Vijaynarayana K. Anti-inflammatory activities of the flowers Tabernamonatana divaricata L. Indian Drugs 2004;41:405-407.
25. Harris JM, Spencer PSJ. A modified Plethysmographic apparatus for recording volume changes in rat paw. J. Pharm. Pharmacol 1962;14:464-466.
26. Mohammed A, Kumar S. Synthesis of some hydrazone derivatives and their anti-inflammatory activity. Indian Drugs 2005;42:75.
27. Guaraldo L, Chagas DA, Konno AC, Korn GP, Pfiffer T, Nasello AG. Hydroalcoholic extract and fractions of Dailla rugosa Poiret: effects on spontaneous motor activity and elevated plus-maze behaviour. J. Ethnopharmacol 2000;72:61-67.
28. Lister RG. The use of a plus-maze to measure anxiety in the mouse. Psychopharmacology 1987;92:180-185.
29. Woodson RF. Statistical Methods for the

Analysis of Biomedical Data. New York: Wiley Series in Probability and Mathematical statistics, Wiley; 1986. pp.315-316.
30. Robert ll LJ, Morrow JD, ln: Hardman JG, Limbird LE, Gilman AG. Goodman and Gilman's The Pharmacological Basis of Therapeutics. 10th ed. New York: McGraw Hill; 2001. pp.687.
31. Consucio H, Jordi C, Cristina VI, Luis Alberto GR. Non steroidal anti-inflammatory drugs and risk of ARF in the general population. Am. J. Kidney Dis 2005;45:531-539.
32. Brasseur T. Anti-inflammatory properties of flavonoids. J. Pharm.Belg 1989;44:235-241.
33. Ferrandiz ML, Alcaraz MJ. Anti-inflammatory activity and inhibition of archidonic acid metabolism by flavonoids. Agents Actions 1991;32:283-287.
34. Ballesteros JF, Sanz MJ, Ubeda A, Miranda MA, Paya M, Alcarz MJ. Synthesis and pharmacological evaluation of 2-hydrox3r chalcones and flavones as inhibitors of inflammatory mediators. J. Med. Chem 1995;38:2794.
35. Sudheesh S, Kumar P, Kumar V, Vijayalakashmi NR. Hypolipidemic effect of flavonoids from Solanum melongena. Plant Foods Hum Nutr 1997;51:321-330.
36. Dawson TM, Snyder SH. Gases as biological messengers: Nitric oxide and carbon monoxide in the brain. J. Neurosci 1994;14:5147-5159.
37. Yeomans DC, Pirec V, Proudfit HK. Nociceptive responses to high and low rates of noxious cutaneous heating are mediated by different nociceptors in the rat behavioral evidence. Pain 1996;68:133-140.
38. Sulaiman MR, Somchit MN, Israf DA, Ahmad Z, Moin S. Antinociceptive effect of Melastoma malabathricum Ethanolic extract in mice. Fitoterapia 2004;75:667-672.
39. Vinegar R, Schreiber W, Hugo R. Biphasic development of carrageenan oedema in rats. J.Pharmacol. Exp. Ther 1969;166:96-103.
40. Di Rosa M, Giround JP, Willoughby DA. Studies of the acute inflammatory response induced in rats in different sites by carrageenan and turpentine oil. J. Pathol 1971;104: 15-29.
41. Brooks PM, Day RO. Nonsteroidal anti-inflammatory drugs differences and similarities. N. Engl. J. Med 1991;324:1716-1725.
42. Jadhav RB, Kharya MD. Plant Flavonoids: A versatile class of phytoconstituents with potential anti-inflammatory activity. Indian Drugs 2005;42:8.
43. Fowzy AA, Vishwanath BS, Franson RC. Inhibition of human non-panereate phospholipases A2 by retinoid and flavonoids. Agents Actions 1988;25:394-400.
44. Mikayw A, Yamamota H, Kubota E, Hamaguchi O. Inflammatory response to 12-o-tetradecanolyphorbol-13-acetate and carrageenan by ym-26734, a selective inhibitor of Extra cellular group l phospholipase A2. Br. J. Pharmacol 1993;110: 447.
45. Aitchdrfoun M, Mounnier C, Heymons F, Binistic C, Bon C, Godfroid JJ. 4-alkoxybenzamidines as new potent phospholipase A2 inhibitors. Biochem. Pharmacol 1996;51:731.
46. Faden AI. Role of thyrotropin-releasing hormone and opiate receptor antagonists in limiting central nervous system injury. Adv. Neurol 1998;47:531-546.
47. Murugesan T, Ghosh L, Das J, Pal M, Saha BP. CNS activity of Jussiaea suffruticosa Linn. Extract in Rats and mice. Pharm. Pharmacol. Commun 1999;5:663-666.
48. Grollman A, Grollman E. In. Pharmacology and Therapeutics. 7th ed. Great Britain: Henry Kimpton Ltd; 1970. pp.159-180.
49. Rabbani M, Sajjadi SE, Zarei HR. Anxiolytic effects of Stachys lavandulifolia Vahl on the elevated plus-maze model of anxiety in mice. J. Ethnopharmacol 2003;89:271-276.