ELUCIDATION OF ANALGESIC ACTIVITY OF HYDROETHANOLIC EXTRACT OF *EUPHORBIA NERIIFOLIA* LEAVES IN SWISS ALBINO MICE

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Abstract : The study was carried out to elucidate the analgesic activity and the possible mechanism(s) of action of hydro-ethanolic extract (HEE) of *Euphorbia neriifolia* (EN) leaves using Swiss albino male mice (15-20g). The peripheral analgesic activity of HEE of EN (150, 300 and 400mg/kg body weight, oral) was studied using acetic acid induced abdominal constriction method. The central analgesic activity of HEE of EN was studied using tail immersion and hot plate method in mice. The principle findings of EN at the dose of 150, 300 and 400mg/kg p.o, showed significant ($p<0.01$) decrease in acetic acid-induced writhing, whereas significant ($p<0.05$ and $p<0.01$) increase in latency to tail flick in tail immersion method and elevated mean basal reaction time in hot plate method was also observed. Overall, results demonstrated that HEE of EN possesses significant analgesic activity which confirms the traditional claims of EN mentioned in Ayurveda.

Keywords : Analgesic activity, Aspirin, Albino mice, Acetic acid, *Euphorbia neriifolia*

INTRODUCTION

Approximately 80% of the population of developing countries uses traditional medicines (Perry, 1966; Kumara, 2001). Traditional healing practices are as old as the advent of man and are highly varied, being ethnic, community and eco-system specific. Drugs presently used for the management of pain condition are either narcotics e.g. opioids or non-narcotics e.g. salicylates and corticosteroids e.g. hydrocortisone. All of these drugs present well known side and toxic effects are expensive & limited in number in the market and for the successful introduction of a new product approximately 3000-4000 compounds are to be synthesized, screened and tested where the cost of development ranges from 0.5 to 5 million dollars.

Thus, the greatest drawback in the available potent synthetic analgesic drugs lies in their adverse effect, toxicity and reappearance of symptoms after discontinuation. Due to having adverse side effects, like gastric lesions, caused by NSAIDs (non-steroidal anti-inflammatory drugs) and tolerance and dependence induced by opiates, the use of these drugs as analgesic agents have not been successful in all the cases. Therefore, analgesic drugs lacking ill effects are being searched all over the world as alternatives to NSAIDs and opiates (Kumara, 2001; Zulfiker *et al*., 2010). On the contrary many medicines of plant origin had been used since long time without any adverse effects. Efforts should be made to introduce leads in new medicinal plants to develop cheaper drugs and fewer side effects (Ramesh, 2010). Active constituents from plant sources directly used as therapeutic agent and phytoconstituents are also served as lead molecule for the synthesis of various drugs (Kamboj, 2000; Verma S & Singh, 2006). Plants represent still a large untapped source of structurally novel compounds that might serve as lead for the development of novel drugs (Marston & Hostettmann, 2009). Albeit, systemic study of more plant to analgesic potential of different parts of plants like root, leaf, fruit, whole plant of *Adhatoda vesica, Bauhima racemosus, Ficus glomerata, Plumbago zeylenica, Scopania dulcis, Sida acuta, Stylosanthes fruticosa, Xeromorphis spinosa* etc. which is well documented (Malairajan *et al*., 2006; Chakraborty *et al*., 2010; Mittal *et al*., 2010 & Wahid *et al*., 2010) isolate the active phytoconstituents, investigate their therapeutic, toxic dose and work towards tapping their therapeutic utility.

Euphorbia neriifolia Linn. (Euphorbiaceae) grows luxuriously throughout the Deccan peninsula of India and commonly occurs in the dry hilly rocky grounds of north, south and central India. It is popularly known as "Common milk hedge" in English, "Sehund" or "Siju" in Hindi and "thuhar" in Rajputhana (Nadkarni, 1954; Sharma *et al*., 2011). In addition, EN has been reported to contain flavonoids, alkaloids, saponins and other active phyto-components (Pracheta *et al*., 2011a; b). Ayurveda describes the plant as bitter, pungent, laxative, improves appetite useful in abdominal troubles, tumors, loss of consciousness, delirium, leucoderma, piles, inflammation, enlargement of spleen, anaemia, ulcers, fever and in chronic respiratory troubles (Anonymous, 1952; Nadkarni, 1954; Bigonia & Rana, 2009; Pracheta *et al*., 2011c; Janmeda *et al*., 2011; Sharma *et al*., 2011). The global changing scenario is showing a tendency towards use of toxic plant products having good traditional medicinal background. This plant can be used safely for longer duration as a cheap source of active therapeutics for alleviation of commonly occurring ailments by the poor and underprivileged people of India.

The explicit aim of the present study was to screen out the analgesic activity and the possible mechanism(s) of action at three different dose levels (100, 200 & 400 mg/kg body weight, p.o) of the hydro-ethanolic extract of *Euphorbia neriifolia* leaves in different experimental models of analgesia.

MATERIAL AND METHOD

Drugs and Chemicals- All chemicals used in the study were of analytical reagent grade and of highest quality available, and are purchased from reliable firms and institutes (SRL, MERCK, HIMEDIA and SUYOG).

Experimental plant- *Euphorbia neriifolia* leaves were collected from Botanical garden of Banasthali University, Banasthali, India, in the month of September 2009. The plant was identified with the help of available literature and authenticated by Botanist of Krishi Vigyan Kendra, Banasthali Vidyapith, Banasthali, Tonk district.

Preparation of hydroethanolic extract- Freshly harvested *Euphorbia neriifolia* leaves were air dried in shade and coarse powder (500 g) was defatted in 1.5 L of ethanol (70% v/v) using soxhlet apparatus. The extracted mixture was evaporated at 40°C, using a hot air oven (Mvtex, India) and kept in desiccator for two days. The yield of the extract was 20% w/w of the powdered plant material. Dried extract was collected and stored at 4°C in air tight container. The residue was designated as hydro-ethanolic extract and used to assess analgesic activity.

Experimental animals*:* Male Swiss Albino mice (*Mus musculus)* weighing 15-30 g were obtained from Haryana Agricultural University, Hissar (India) for experimental purpose. The animals were acclimatized for a month prior to experiment. All experiments were conducted on adult Swiss albino male mice when they weighed 20-25g (3-4 months old). Colony bred adult male albino mice were maintained under standard laboratory conditions at a temperature of 22 ± 3ºC, relative humidity of 50±5 % and photoperiod of 12h (12h-dark and 12h-light cycle). The mice were housed in polypropylene cages. The Institutional Animal Ethical Committee approved the animal studies.

Assessment of Analgesic activity- The antinociceptive activity of HEE of EN leaves was assessed using three different methods as follows:

Acetic Acid- Induced Writhing test- In this method 30 Swiss albino mice (20-25g) were segregated into 5 groups of six animals each were treated by oral gavage using intragastric tube. Writhing (s) were induced by the method of Koster et al. (1959) and Devi et al. (2010). The groups were as follows: Group 1 served as control (normal untreated mice), received 0.9% saline solution by oral gavage. Group 2: received aspirin (25 mg/kg body weight: p.o), served as standard treated control group. Group 3, 4 and 5 were administered with hydro-ethanolic extract of leaves of EN (150, 300 and 400 mg/kg body weight: p.o), served as EN treated control group. The dose for plant and standard were decided and selected on the basis of LD_{50} calculated in the laboratory and on the basis of previous published reports (Sutar *et al*., 2008; Bigoniya & Rana, 2010; Pracheta et al., 2011c; Janmeda *et al*., 2011;).

One hour after administration of the test drugs, animals were injected intra-peritoneally with 1% acetic acid (1 ml/100 g body weight). The number of writhing responses such as contortions and stretching were recorded for 30 minutes. The results were evaluated by calculating the mean number of contortions per treated group and results compared to results obtained from control animals (0.9% saline). Percentage of protection or pain inhibition against acetic acid induced writhing was taken as an index of analgesia and it was calculated as follows:

$$\text{Pain inhibition (\%)} = \frac{Wc - Wt}{Wc} \times 100$$

Wc: mean number of contortions of the control group
Wt: mean number of contortions per treated group

Tail immersion method/Heat conduction method- This test was performed as described by Tumer, (1971). Group 1 to 5 received the saline, standard drug and test extract as in writhing test. The lower 3-5 cm portion of the tail was marked and immersed in a cap of water having temperature 55±1ºC. Reaction time was recorded before and after administration of saline, test extract and standard drug. The response time was noted at 0, 30, 60, 90, 120, 150, and 180 minutes after administration of standard and test solution, as the sudden withdrawal of the tail from the hot water. The cut off time was considered 10-12sec to avoid damage the tail for all groups.

$$\text{Percentage protection/inhibition} = \frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Latency (Control)}} \times 100$$

Eddy's hot plate method- Hot plate method was performed as described by Eddy's and Leimback,(1953) Group 1 to 5 received the saline, standard drug and test extract as in writhing test. The animals were placed on a hot plate (Analgesiometer, Techno) maintained at a temperature of 55±1ºC. The basal reaction time, when the animals licked their paw or jumping occurred was recorded by a stop watch before 0 and 30, 60, 90, 120, 150, and 180 minutes after administration of standard and test solution. The initial reaction time was measured in each animal before the administration of doses of EN extract (at both doses). The reaction time was recorded 30 min after drug administration and 15 minutes intervals consequently for a period of 180 minutes. A cut off time of 15 sec was used. The increase in reaction time against control was calculated.

$$\text{Percentage protection/inhibition} = \frac{\text{Latency (test)} - \text{Latency (control)}}{\text{Latency (Control)}} \times 100$$

Statistical analysis- All the data are presented as mean ± SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dunnett's test. Comparison between control and drug treated groups were considered to be statistically significant ($p<0.05$) and $P< 0.01$ as highly significant.

RESULT

The results of present study indicate the HEE of EN possesses analgesic effect, which is in accordance with its ethno-medicinal use. Analgesic effect of the extracts was demonstrated in the experimental models using writhing test, heat conduction method and Eddy's hot plate method using thermal stimuli, an increase in reaction time is generally considered an important parameter of analgesic activity.

Acetic acid induced writhing test- The effect of HEE of EN on acetic acid induced writhing is demonstrated in Table 1. The HEE of EN (150, 300 and 400 mg/kg, p.o) reduced writhing counts significantly ($p<0.01$) in mice. The results obtained were in dose dependent manner compared to control group. Maximum inhibition of writhing response by HEE of EN (400 mg/kg) was 88.07 %, which was comparable to aspirin (25 mg/kg) (graph 1). Standard aspirin (25 mg/kg) showed maximum inhibition of writhes (75.21%).

Tail immersion test- The analgesic activity of HEE of EN was evaluated using tail immersion method are presented in table 2. The extract exhibited marked central analgesic effect as evidenced by significant increase ($p<0.01$) in basal reaction time when compared with the control. HE of plant at all doses showed dose dependent increase in tail flick latency period and maximum inhibition was observed for 400 mg/kg. The results were also compared to the standard drug- aspirin in all three methods. The HEE of EN (150 mg/kg) did not show significant increase in latency to flick compared to control group ($p>0.05$). The HEE of EN at doses (250 and 400 mg/kg, p.o) showed significant ($p<0.05$ and $p<0.01$) increase in latency to flick tail compared to control group. The highest nociception inhibition of stimulus by HEE of EN (400 mg/kg) was observed at 30 minutes.

Hot plate test- The analgesic activity of HEE of EN using hot plate test is presented in Table 3. Oral administration of HEE at 150, 300, 400 mg/kg) resulted significant ($p<0.05$ and $p<0.01$) prolongation of latency time in hot plate test. The highest nociception inhibition of stimulus exhibited by HEE of EN (400 mg/kg) was observed at 30 minutes.

In general our results lend support to the recent finding of Gaur et al. (2009) and Venkataswami, (2012) on *E. neriifolia.*

DISCUSSION

Pain is a condition which is regularly dealt with in daily clinical practice. Hence, any attempt to contribute an easily available analgesic drug from the available flora is always accepted without any reluctance. Two different analgesic testing models were employed with the objective of identifying peripheral (acetic acid- induced writhing method) and central analgesic effect (hot plate and tail immersion method) of the test substances.

In the present study, hydro-ethanolic extract of EN leaves showed significant dose dependent anti-nociceptive activity. A large number of herbal drugs used in the indigenous system of medicine possess a variety of actions on the central nervous system. Acetic acid induced writhing test is widely used method for the evaluation of peripheral antinociceptive activity (Javan *et al*., 1997). Acetic acid is an irritating agent which stimulates the local peritoneal receptors to induce pain with characteristic abdominal constrictions when injected into the peritoneal cavity (Vogel & Vogel, 1997). In present study, EN extract markedly reduced the number of mice abdominal constrictions at all the three doses used. Hence it can be concluded that the EN extract showed a dose dependent inhibition of acetic acid induced writhing in mice.

The development of aspirin was a significant landmark in the history of medicine. Aspirin (acetylsalicylic acid -ASA), is a salicylate drug, often used as an analgesic to relieve minor aches and pains. Salicylic acid, the main metabolite of aspirin, is an integral part of human and animal metabolism. While much of it is attributable to diet, a substantial part is synthesized endogenously (John *et al*., 2008). The main undesirable side effects of aspirin taken by mouth are gastrointestinal ulcers, stomach bleeding, and tinnitus, especially in higher doses. In children and adolescents, aspirin is no longer indicated to control flu-like symptoms or the symptoms of chickenpox or other viral illnesses, because of the risk of Reye's syndrome (Macdonald, 2002). Aspirin reduces the leukocytes associated with acute rheumatic fever. When given on a long term basis, it reduces the haemoglobin level. Aspirin use can cause reversible hypo prothrombininemia by interfering with the function of Vitamin K in the prothombin synthesis. Aspirin both directly and indirectly stimulates respiration (Macdonald, 2002). In analgesic doses, aspirin increases oxygen consumption and carbon dioxide production. Therefore a new alternative in the form of phytochemicals are taken under investigations.

The acetic acid-induced writhing in mice is a visceral pain model that has been associated with release of free arachidonic acid from tissue phospholipids via cyclooxygenase (COX), bradykinins and substances prostaglandin (PGE_2 and $PGF_{2\alpha}$) biosynthesis plays a role in nociceptive mechanisms (Duarte *et al.,* 1988;

Ahmed *et al*., 2006; Baird-Lambert & Jamieson, 2007; Sah *et al.*, 2010). In other words, the acetic acid induced writhing has been associated with increased level of PGE_2 and PGF2α in peritoneal fluids as well as lipoxygenase products (Derardt *et al*., 1980). The acetic acid induced writhing method was found effective to evaluate peripherally active analgesics. The agent reducing the number of writhing, render analgesic effect preferably by inhibition of prostaglandin synthesis, a peripheral mechanism of pain inhibition (Duarte *et al*., 1988; Sah *et al.*, 2010; Ferdous et al., 2008). The significant pain reduction in acetic acid-induced writhes by plant extract might be due to the presence of analgesic principles acting with the prostaglandin pathways and suggests that the analgesic effect may be peripherally mediated via the inhibition of synthesis and release of PGs and other endogenous substances.

The hot-plate and tail-immersion methods are useful in elucidating centrally mediated antinociceptive responses, which focuses mainly on changes above the spinal cord level (Vongtau *et al*., 2004). The significant increase in pain threshold produced by HEE of EN in these models suggests involvement of central pain pathways. Pain is centrally modulated via a number of complex processes including opiate, dopaminergic, descending noradrenergic and serotonergic systems (Headley & Shaughnessy, 1985; Wigdor & Wilcox, 1987; Pasero *et al.*, 1999). The analgesic effect produced by the extract may be via central mechanisms involving these receptor systems or via peripheral mechanisms involved in the inhibition of prostaglandins, leucotrienes, and other endogenous substances that are key players in inflammation and pain.

Euphorbia neriifolia contains active constituents such as phenolics like triterpenes (nerrifolione), diterpenes, flavonoids, alkaloids and steroidal saponins (Pracheta *et al*., 2011a; b) and these constituents has been reported that they possess analgesic and anti-inflammatory actions (Hossinzadeh *et al.*, 2002). The increase in reaction time may be due to the presence of flavonoids and traces of salicylic acid in the plant extract. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase (Ramaswamy *et al*., 1985) which is involved in the late phase of acute inflammation and pain perception (Adeyemi et al., 2010). Flavonoids are capable of modulating the activity of enzymes and affect the behaviour of many cell systems, suggesting that the compounds may possess significant antihepatotoxic, antiallergic, anti-inflammatory (Rao *et al*., 2003), antiosteoporotic and antianalgesic activities (Delorme, 1995; Mills & Bone, 2000). Since, prostaglandins are involved in the pain perception; inhibition of their synthesis so produce analgesic effects. Salicylates and allied compounds have analgesic property (John *et al*., 2008). The mechanism by which they act to reduce mild to moderate pain is based on the relationship between these compounds and prostaglandin synthesis. The component which makes up simple phenol includes salicyclic acid. Phenols have been hypothesized to possess traces of salicylic acid, which makes it a strong candidate as an analgesic agent. Some reports supported the role of tannins and saponins in anti-nociptive and anti-inflammatory activities. Saponins have also been reported to inhibit histamine release in vitro (Adeyemi et al., 2010; Rao et al., 2000). Thus, the presence of these active phyto-constituents in hydroethanolic extract of *Euphorbia neriifolia* could have accounted for its pain inhibition activity.

In conclusion, results of the present study reveal that the *Euphorbia neriifolia* leaf extract had potent analgesic activity. The extract exhibited marked central analgesic effect as evidenced by significant increase in reaction time when compared with the control, as this plant has immense potential and have broad spectrum of activity on several ailments.

Table 1: Effect of *E. neriifolia* on acetic acid -induced writhing response in mice
Each value is presented as Mean ± S.E.M.; n= number of animals in each group (6)

Groups	**Treatment mg/kg**	**No. of writhes/30 min**	**Inhibition (%)**
Group I	Vehicle	35.31± 0.26	-
Group II	25	8.75± 0.16*	75.21
Group III	150	10.75± 0.30*	69.55
Group IV	300	8.51± 0.37*	75.92
Group V	400	4.21± 0.31*	88.07

*p<0.01 compared with control values.

Table 2: Effect of HEE of *E. neriifolia* on latency to tail immersion method in mice
Each value is presented as Mean ± S.E.M.; n= number of animals in each group (6)

Groups	Treatment mg/kg	Tolerance	Reaction time in seconds						
			0 min	30 min	60 min	90 min	120 min	150 min	180 min
Group I	Vehicle	Latency (sec)	7.50±0.18	7.75±0.18	7.50±0.28	7.50±0.18	7.75±0.22	7.50±0.22	7.50± 0.31
Group II	25	Latency (sec)	5.10±0.24	4.10±0.14	3.25±0.22a	2.75±0.22*	2.50±0.25*	2.09±0.22*	2.02±0.28*
		% Protection	32.00	45.33	58.06	63.32	67.74	72.13	73.23
Group III	150	Latency (sec)	6.20±0.11	5.70±0.21	4.90±0.21a	4.10±0.11*	3.65±0.25a	3.50±0.18*	2.75±0.22*
		% Protection	17.33	24.00	39.77	45.00	52.9	53.33	63.31
Group IV	300	Latency (sec)	4.80±0.13	4.50±0.18	3.50±0.21*	3.15±0.19*	2.50±0.25a	2.12±0.11*	2.01±0.18*
		% Protection	36.00	40.00	54.83	58.26	67.74	70.53	73.21
Group V	400	Latency (sec)	3.50±0.25	2.50±0.18*	2.25±0.13*	2.01±0.05*	1.75±0.22*	1.51±0.11*	1.25±0.22*
		% Protection	53.33	66.65	70.96	73.2	77.41	79.86	83.33

[a]p<0.05, *p<0.01 compared with control values.

Table 3: Effect of HEE of *E. neriifolia* on latency to hot plate method in mice
Each value is presented as Mean ± S.E.M.; n= number of animals in each group (6)

Groups	Treatment mg/kg	Tolerance	Reaction time in seconds						
			0 min	30 min	60 min	90 min	120 min	150 min	180 min
Group I	Vehicle	Latency (sec)	8.75±0.54	8.5±0.25	8.5±0.25	8.5±0.25	8.5±0.25	8.5±0.25	8.5±0.25
Group II	25	Latency (sec)	5.75±0.11	5.52±0.25	4.05±0.25a	3.02±0.27*	2.25±0.13*	1.90±0.27*	1.75±0.12*
		% Protection	34.28	35.05	52.35	62.94	73.52	77.64	79.41
Group III	150	Latency (sec)	6.25±0.22	5.11±0.11	4.75±0.22 a	4.05±0.18*	3.65±0.21*	3.15±0.11*	2.75±0.13*
		% Protection	28.57	39.88	44.15	52.33	57.05	62.94	67.64
Group IV	300	Latency (sec)	5.50±0.25	4.75±0.22 a	4.05±0.18 a	3.02±0.18*	2.75±0.13*	2.25±0.13*	1.90±0.14*
		% Protection	37.14	44.12	52.35	64.47	67.65	73.52	77.64
Group V	400	Latency (sec)	5.12±0.21*	3.89±0.11*	3.15±0.11*	2.75±0.13*	2.01±0.18*	1.51±0.18*	1.12±0.22*
		% Protection	41.11	54.33	62.94	67.64	76.35	82.23	86.82

[a]p<0.05, *p<0.01 compared with control values.

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