

ANTI-INFLAMMATORY AND ANALGESIC ACTIVITY OF HYDRO-ALCOHOLIC LEAVES EXTRACT OF *EUPHORBIA NERIIFOLIA* LINN.

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This work have done for the determination of the anti-inflammatory and analgesic activity of 70% v/v hydro-alcoholic extract of dried leaves of *Euphorbia neriifolia* by oral administration at dose of 400 mg/kg/day of body weight to healthy albino rats. The hydro-alcoholic extract was studied for their anti-inflammatory activity by using carrageenan-induced hind paw edema in rats and the mean increase in paw volume and % inhibition in paw volume were measured plethysmometrically at different time intervals after carrageenan (1% w/v) injection. The hydro-alcoholic extract was also evaluated for analgesic activity using Eddy's hot plate method and tail-flick method in albino rats. The extract of *Euphorbia neriifolia* showed significant ($P < 0.05$) reduction in the carrageenan-induced paw edema in rats and analgesic activity evidenced by increase in the reaction time by Eddy's hot plate method and tail-flick method in albino rats. The hydro-alcoholic extract showed a greater anti-inflammatory and analgesic effect when compared with the standard drugs, indomethacin and diclofenac sodium respectively. The present observation indicated significant ($P < 0.001$) activity of the hydro-alcoholic extract of *Euphorbia neriifolia* in the treatment of inflammation and pain.

Keywords: Paw edema, flavonoid, *Euphorbia neriifolia*, inflammation, pain.

INTRODUCTION

Euphorbia neriifolia Linn. (*Euphorbiaceae*) grows luxuriously around the dry, rocky, hilly areas of North, Central and South India. It is a herb full of spine, popularly known as Sehund, Thohar and Milk Hedge. The leaves are thick succulent, 6-12 inch long, ovular in shape. In the traditional system leaves are used as aphrodisiac, diuretic, cough and cold, and also used in the treatment of bronchitis, bleeding piles, ano-rectal fistula. The tribal population of Chattishgarh region uses the milky latex as an ingredient of aphrodisiac mixture. Latex is used to deroot skin warts, earache and in arthritis¹. The aqueous extract of the latex of *Euphorbia neriifolia* facilitated the wound healing process as evidenced by increase in tensile strength, DNA content, epithelization and angiogenesis². Plants is bitter, laxative, carminative, improves appetite, useful in abdominal troubles, bronchitis, tumors, leucoderma, piles, inflammation, enlargement of spleen, anemia, ulcers, fever and in chronic respiratory troubles³⁻⁵.

Phytochemical investigations on *Euphorbia neriifolia* yielded in the isolation of several classes of secondary metabolites, many of which expressed biological activities such as triterpenes (nerrifolione), flavonoids and steroidal saponins⁶⁻⁷. The present study was undertaken to find out the possible actions of *Euphorbia neriifolia* leaves for its anti-inflammatory and analgesic activity.

Materials and Methods

Indomethacin, Micro Labs, Bangalore; Carrageenan,

Sigma Chemicals, USA and Diclofenac sodium, Apex Labs, Chennai; were used in the experiment. All other chemicals were used of analytical grade.

Collection of plant

Euphorbia neriifolia Linn. leaves were collected from Hoshangabad, MP, India, in the month of September 2007. The plant was identified with the help of available literature and authenticated by Dr. A. P. Shrivastava, Principal, Pandit Khushilal Sharma Govt. Ayurveda College and Institute, Bhopal, India. A voucher specimen was deposited in the herbarium of department (No. 1085).

Preparation of extract

Freshly collected leaves were dried in shade and coarse powder was extracted by macerating 500 g in 1.5 L of ethanol (70% v/v) for one week with occasional stirring. The macerated mixture was filtered through muslin cloth and evaporated under reduced pressure and at 40°C up to one third of initial volume, remaining solvent was completely evaporated at 40°C, using a rotary vacuum evaporator (Superfit, India). The dried residue was designated as hydro-alcoholic extract and used for further studies. The dose 400 mg/kg/day of body weight was selected range from 1/6 to 1/15 of LD₅₀ based on the preliminary study conducted at our laboratory and data are not shown in this paper.

Animals

Wister albino rats (120-200 g) and Swiss albino mice (20-30 g) of either sex supplied from Ravi Chand & Sons,

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Ahmedabad, India were used. The animals housed under standard laboratory conditions maintained at 25 ± 1°C and under 12/ 12 h light / dark cycle and fed with standard pellet diet (Gold Mohur brand, Lipton India Ltd.) and water ad libitum. Animal experiments were approved by the Institutional Animal Ethical Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), constituted under the directives of Ministry of Social Justice and Empowerment, Government of India.

paw volume was measured and percent inhibition was calculated (Table 1).

$$\text{Percent inhibition} = 100 (1 - V_t / V_c)$$

Where, V_c= Edema volume in control and V_t= Edema volume in test / standard compound.

ANALGESIC ACTIVITY STUDY

Analgesic activity by tail flick method

The albino mice were divided into three groups consisting of six animals in each group. Group I received 0.2 ml of

TABLE- 1 Effect of *Euphorbia neriifolia* on Carrageenan-induced Paw Edema

Treatment	Dose (mg/kg)	Mean Paw Volume (ml)						Percent Inhibition
		0 min.	15 min.	30 min.	60 min.	120 min.	180 min	
Control	Saline	0.81±0.04	1.07±0.10	1.38±0.16	1.76±0.12	1.84±0.11	1.54±0.04	---
Extract	400	0.74±0.11	1.11±0.18	1.38±0.10	1.36±0.10	0.85±0.14	^a 0.69±0.20	55.12
Indomethacin	10	0.81±0.08	1.11±0.10	1.11±0.10	1.11±0.10	0.74±0.17	^a 0.57±0.04	63.27

n= 6, Values are expressed as mean ± SEM, ^aP < 0.001 When compared with control

Anti-inflammatory activity study

The albino rats of either sex were divided into three groups consisting of six animals in each group. Group I received 0.2 ml of 2% w/v carboxy methyl cellulose suspension orally as a control group, group II received 400 mg/kg body weight of hydro-alcoholic extract of *Euphorbia neriifolia* orally and group III received 10 mg/kg of body weight of indomethacin intraperitoneally as a standard drug. Acute inflammation was induced in all groups by injecting 0.1 ml of 1% w/v carrageenan into the sub-plantar region of the right hind paw of rats. Mean paw volume was measured 1 h prior to carrageenan injection using plethysmometer and at 0, 15, 30, 60, 120 and 180 min after the carrageenan injection . Mean increase in the

2% w/v carboxy methyl cellulose suspension orally as a control group, group II received 400 mg/kg body weight of hydro-alcoholic extract of *Euphorbia neriifolia* orally and group III received 1.0 mg/kg of body weight of diclofenac sodium intraperitoneally as a standard drug. The reaction time was recorded using tail flick analgesiometer at 0, 30, 60, 120 and 180 min time interval after the drug administration . The temperature was maintained at 50-55°C and data are represented in Fig. 1

Analgesic activity by Eddy's hot plate method

Mice were divided into three groups consisting of six animals in each group and treatments were given as per tail-flick method. Animals were placed on the Eddy's hot plate maintained at 55±1°C. The reaction time in control

FIGURE 1. Analgesic activity of *Euphorbia neriifolia* by tail-flick method. n= 6, Values are expressed as mean ± SEM, ^aP < 0.001 When compared with control

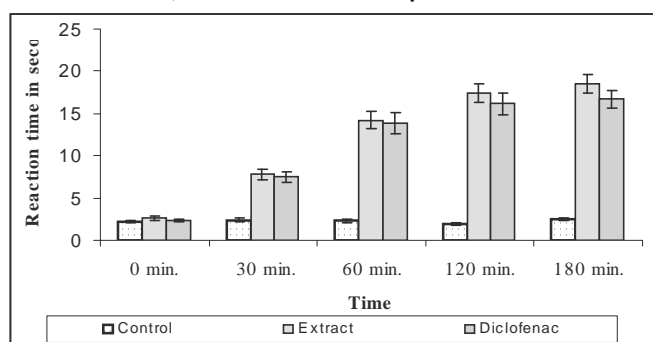
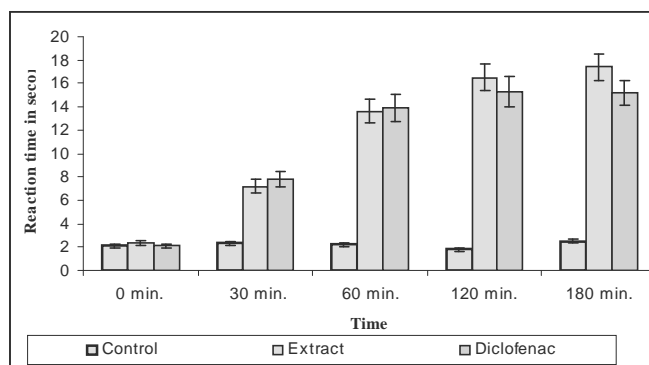


FIGURE 2. Analgesic activity of *Euphorbia neriifolia* by Eddy's hot plate method. n= 6, Values are expressed as mean ± SEM, P < 0.001 When compared with control



and treated animals was recorded at 0, 30, 60, 120 and 180 min after the treatment¹⁰ and data are represented in Fig. 2

Statistical analysis: Results were expressed as Mean \pm SEM, statistical significance was calculated by applying one way ANOVA. $P < 0.05$ was considered as significant.

RESULTS

Carrageenan-induced Paw edema

Anti-inflammatory effect of hydro-alcoholic extract were observed and found to be significant at the level of $P < 0.001$ when compared with the vehicle 0.2% CMC (control group) and indomethacin (Standard). The percent inhibition in paw edema after 3 h were recorded 63.27 % in case of indomethacin and 55.12 % in hydro-alcoholic extract of *Euphorbia neriifolia*.

Analgesic activity determined using tail-flick method and Eddy's hot plate method

The analgesic activity of hydro-alcoholic extract of *Euphorbia neriifolia* determined by using tail flick and Eddy's hot plate methods respectively (Fig. 1 and 2). The extract exhibited marked central analgesic effect as evidenced by significant increase in reaction time when compared to the control. The results were also comparable to the standard drug, diclofenac sodium in both methods.

DISCUSSION

Inflammation has different phases the first phase is caused by an increase in vascular permeability, the second one by infiltrate of leucocytes and the third one by granuloma formation. We determined anti-inflammatory activity by using inhibition of carrageenan-induced inflammation which is one of the most feasible methods to screen anti-inflammatory agents. The development of carrageenan-induced edema is bi-phasic; the first phase is attributed to the release of histamine, serotonin and kinins and the second phase is related to the release of prostaglandins and bradykinins¹¹⁻¹³. We observed that hydro-alcoholic extract of *Euphorbia neriifolia* at the given dose i.e. 400 mg/kg p.o. possess significant inhibition against carrageenan-induced paw edema in rats. This response tendency of the extract in carrageenan-induced paw edema revealed good peripheral anti-inflammatory properties of the extract. This anti-inflammatory effect of hydro-alcoholic extract of *Euphorbia neriifolia* may be due to the presence of flavonoids. It has been reported that a number of flavonoids possess anti-inflammatory¹⁴ and analgesic¹⁵ activities. Flavonoids are known to inhibit the enzyme prostaglandin synthetase, more specifically the endoperoxidase¹⁶ and reported to produce anti-inflammatory effects¹⁷. Since,

prostaglandins are also involved in the pain perception; inhibition of their synthesis.

We used eddy's hot plate and tail-flick methods for the determination of analgesic activity. The hydro-alcoholic extract of *Euphorbia neriifolia* possess good analgesic activity by increase in the reaction time (increase threshold potential of pain) may be due to the above mentioned mechanism

Thus, we concluded that the crude hydro-alcoholic extract of leaves of *Euphorbia neriifolia* produces significant anti-inflammatory and analgesic activities.

ACKNOWLEDGEMENT

The authors are grateful to Micro Labs, Bangalore, India and Apex Labs, Chennai for providing the gift sample of drugs.

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