

Full Length Research Paper

## Anti-inflammatory activity of methanolic extract of *Eclipta prostrata* L. (Asteraceae)

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The methanolic extract of leaves of *Eclipta prostrata* Linn was investigated for anti-inflammatory activity in albino Wistar rats. The methanolic extract administered by the oral route at a concentration of 100 and 200 mgkg<sup>-1</sup> showed the significant dose dependent anti-inflammatory activity in carrageenin and egg white induced hind paw oedema in rats. Anti-inflammatory activity of the tested extract was comparable with that of the standard drug indomethacin (10 mgkg<sup>-1</sup>) and cyproheptadine (8 mgkg<sup>-1</sup>). The results lend support to the traditional use of *E. prostrata* in the treatment of inflammatory diseases.

**Key words:** *Eclipta prostrata*, methanolic extract, albino Wistar rats, anti-inflammatory activity.

### INTRODUCTION

*Eclipta prostrata* Linn (Family-Asteraceae) is a common plant and abundantly grows throughout India up to 6000 ft height of hills. It is commonly known as Trailing Eclipta in English, Bhamgra in Hindi and Kayyantakara in Tamil. It is an erect or prostrate annual herb and the leaves are opposite, sessile and lanceolate. The leaves are densely arranged on both sides of the stem and rooting at the nodes and the flower-heads are white (Asolkar et al., 1992). *E. prostrata* Linn has great traditional reputation of being used as a medicinal agent in India. Various parts of the plant is used by the rural people of Tamil Nadu for several human illnesses like kidney and liver weakness, inflammatory conditions, ophthalmic and digestive disorders. It is also regarded as the best remedy for hair in Ayurvedic medicines and act as haematinic, diuretic and anthelmintic (Anonymous, 1952; Kirthikar and Basu, 1998).

The extract of the plant has the ability to act as an antidote for snake venom (Melo et al., 1994; Mors et al., 1989). Previous studies on this plant proved its usefulness in modification of immune function, cytological responses, serine proteinase inhibition, lipid lowering and liver function (Ge and Wan, 1990; He et al., 1992; Konarev, 2002;

Kumari et al., 2006; Lans, 2001). Recent reports showed that the triterpenoid saponins isolated from this plant has antimicrobial, immunosuppressant, anti-guardian and anti-venom potentials (Liu et al., 2000; Pithayanukul et al., 2004; Sawangjaroen et al., 2005; Zhang Guo, 2001; Zhao et al., 2001; Wiart et al., 2004). Phytochemically, *E. prostrata* is rich in wadeolactone, eclalbasaponin,  $\beta$ - amyryrin, stigmasterol and luteolin-7- glucoside ((Asolkar et al., 1992).

Wagner and Fessler (1986) reported the effectiveness of the 5-lipoxygenase inhibition of wedelolactone isolated from *Eclipta alba* (L.) and *Wedelia calendulacea* Less in *in-vitro* porcine-leukocytes test system. Hence, the present study was initiated to evaluate, anti-inflammatory activity of the methanolic extract of leaf of *E. prostrata* Linn in albino Wistar rats.

### MATERIALS AND METHODS

#### Plant material

The leaves of *E. prostrata* Linn were collected from the mature plant in and around the city of Tirunelveli, Tamil Nadu, India during July 2005 and dried under shade, pulverized by a mechanical grinder and passed through sieve # 40 to get the fine powder.

The plant was identified by Prof. P. Jayaraman, Taxonomist, Plant Anatomy Research Centre, Chennai and the voucher specimen was deposited at S. A. Raja Pharmacy College, Raja Nagar,

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**Table 1.** Inhibitory effect of methanolic extract of leaves of *E. prostrata* against carrageenin induced paw oedema in albino Wistar rats.

Treatment	% Increase in paw volume, Mean $\pm$ S.E (n = 6)					% Inhibition in paw vol.
	Post insult time of assay (min)					
	0	60	120	180	240	
Propylene glycol (5 ml kg <sup>-1</sup> )	39.04 $\pm$ 1.05	68.39 $\pm$ 3.25	96.72 $\pm$ 5.17	108.49 $\pm$ 7.39	109.12 $\pm$ 8.19	--
MEEP (100 mg kg <sup>-1</sup> )	26.35 $\pm$ 1.08	47.98 $\pm$ 2.25	73.19 $\pm$ 5.15	71.58* $\pm$ 4.33	70.94* $\pm$ 5.07	34.02
MEEP (200 mg kg <sup>-1</sup> )	24.42 $\pm$ 1.64	45.54 $\pm$ 2.26	68.16 $\pm$ 5.18	66.39* $\pm$ 4.54	63.94* $\pm$ 5.07	38.80
Indomethacin (10 mg kg <sup>-1</sup> )	27.8 $\pm$ 0.92	33.8 $\pm$ 1.83	38.8 $\pm$ 2.21	55.9** $\pm$ 2.29	58.82** $\pm$ 3.92	48.47

\*p<0.01 Vs control; \*\* p< 0.001 Vs control by student's 't' test. MEEP: Methanolic extract of leaves of *E. prostrata*

Vadakkangulam.

### Preparation of extracts

The leaf powder was macerated in 95% methanol as solvent for 72 h with occasional shaking at room temperature. The extract was collected in a conical flask, filtered through Whatman No.1 filter paper and the filtrate was evaporated to dryness under reduced pressure. The yield of the prepared extract was around 8.7% w/w.

### Animals

Albino Wistar rats of either sex (160 – 180 g) were used for the study of anti-inflammatory activities. They are housed for at least one week before starting experiment in standard plastic cages at room temperature. The animals had free access to standard food in pellets and tap water.

### Preliminary phytochemical group test

The preliminary phytochemical screening of methanolic extract of leaves of *E. prostrata* was performed by the standard methods (Tyler et al., 1993; Trease and Evans, 1996; Plummer, 1985).

### ANTI-INFLAMMATORY ACTIVITY

#### Carrageenin-induced rat paw oedema

The rats weighing 160 - 180 g were divided into four groups, and each group consisting of six animals. Paw oedema was induced by subplantar injection of 0.1 ml of freshly prepared 1% carrageenin suspension into the right hind paw of each rat. The paw volumes were measured using a plethysmometer before as well as 60, 120, 180 and 240 min after the injection of carrageenin (Winter et al., 1962). The methanolic extract of leaves of *E. prostrata* at 100 and 200 mgkg<sup>-1</sup> were administered orally to first two groups of rats. The third and fourth group of rats received 5 mlkg<sup>-1</sup> propylene glycol as vehicle control or 10 mgkg<sup>-1</sup> indomethacin as drug control respectively, for comparative pharmacological assessment. Test drugs and vehicle were given 1 h before the injection of carrageenin. The relative potency of the drugs under investigations was calculated based upon the percentage inhibition of the inflammation.

#### Egg white induced hind paw oedema

Albino Wistar rats of either sex weighing about 160 - 180 g were divided into four groups of six animals each. The methanol extract of leaves of *E. prostrata* at 100 and 200 mgkg<sup>-1</sup> was administered orally

to first two groups of rats. The third and fourth group of rats received 5 mlkg<sup>-1</sup> propylene glycol as vehicle control or 8 mgkg<sup>-1</sup> cycloproheptadine as drug control respectively, for comparative pharmacological assessment. All the drugs and vehicle were given 1 h prior to the study. Freshly taken egg white (0.1 ml) was injected into the sub plantar tissue of the left hind paw of the rat. The volumes of the injected paws were measured at 0, 60, 120, 180 and 240 min using a plethysmometer. The percent increase in paw oedema of the treated group was compared with that of the control and the inhibitory effects of the drugs were studied (Andres, 1967). Percentage inhibition was calculated for both models by using the following formula:

$$V_C - V_T / V_C \times 100$$

V<sub>C</sub> = Control (% increase in paw volume in 3<sup>rd</sup> hour), V<sub>T</sub> = Test (% increase in paw volume in 3<sup>rd</sup> hour).

### STATISTICAL ANALYSIS

The results were expressed as mean  $\pm$  S.E and the significance were evaluated by student's t-test compared with control (Woodson, 1987).

### RESULTS AND DISCUSSION

#### Preliminary phytochemical group tests

Preliminary phytochemical screening showed the presence of steroids, triterpenoids, flavanoids, reducing sugar, tannins and saponins in methanolic extract of leaves of *E. prostrata* Linn.

#### Anti-inflammatory activity

The anti-inflammatory potential of the methanolic extract of leaves of *E. prostrata* was investigated using Carrageenin-induced rat paw oedema and egg white induced hind paw oedema methods. The results of methanolic extract of leaves of *E. prostrata* in carrageenin induced hind paw oedema were presented in Table 1. The results revealed that the methanolic extract of leaves of *E. prostrata* at 100 and 200 mgkg<sup>-1</sup> exhibited 34.02 and 38.80% inhibition respectively in carrageenin induced hind paw oedema; while indomethacin showed 48.47% (Table 1). The results of egg white induced hind paw oedema test showed that the oedema suppression by methanolic extract of leaves *E. prostrata* at 100 and 200 mgkg<sup>-1</sup> was 35.05 and 38.23%

**Table 2.** Anti-inflammatory activity of methanolic extract of leaves of *E. prostrata* against egg white induced paw oedema in albino Wistar rats.

Treatment	% Increase in paw volume Mean $\pm$ S.E (n = 6)					% Inhibition in paw vol.
	Post insult time of assay (min)					
	0	60	120	180	240	
Propylene glycol (5 ml kg <sup>-1</sup> )	19.53 $\pm$ 1.20	81.83 $\pm$ 5.22	88.93 $\pm$ 3.92	95.20 $\pm$ 7.7	99.03 $\pm$ 7.21	--
MEEP (100 mg kg <sup>-1</sup> )	24.50 $\pm$ 1.81	45.31 $\pm$ 3.12	76.32 $\pm$ 3.52	61.83* $\pm$ 4.22	60.97 $\pm$ 3.92	35.05
MEEP (200 mg kg <sup>-1</sup> )	19.28 $\pm$ 0.83	70.38 $\pm$ 4.73	63.2 $\pm$ 2.50	58.8* $\pm$ 3.83	55.8 $\pm$ 2.81	38.23
Cyproheptadine (8 mg/kg)	14.2 $\pm$ 0.88	33.5 $\pm$ 1.83	38.9 $\pm$ 2.81	41.8* $\pm$ 3.2	58.82 $\pm$ 2.90	56.09

\*p < 0.001 Vs Control by student's 't' test. MEEP: Methanolic extract of leaves of *E. prostrata*

respectively; whereas cyproheptadine (8 mgkg<sup>-1</sup>) produced 56.09% (Table 2). Anti-inflammatory intensity produced by methanolic extract of whole plants of *E. prostrata* is comparable to that of the standard drugs indomethacin and cyproheptadine used in this study.

The earlier studies had indicated the use of egg-albumin as a phlogistic agent causes oedema in rat hind paw. Carrageenin-induced rat paw oedema and egg white induced hind paw oedema methods are suitable for screen agents for anti-inflammatory activity which are frequently used to assess the anti-oedematous effect of natural products (Akah et al., 1993; Amos et al., 2002).

Several inflammatory mediators like complement, histamine, kinins, prostaglandins and pro-inflammatory cytokines have been suggested to play a role in the mechanism of inflammation (Rosa et al., 1971; Hirschelmann and Bekemeier, 1981). It is assumed that at least some of these mediators are subjects of inhibition by the methanolic extract of leaves of *E. prostrata*.

Oedema which develops after carrageenin inflammation is a biphasic event (Vinegar et al., 1969). The initial phase is attributed to the release of histamine and serotonin. The oedema maintained between the first and the second phase is due to kinin like substances (Crunkhon and Meacock, 1971). It has been reported that the egg white acts prominently on the mast cells. Oedema induced by it, appears to be mediated by histamine and serotonin. Inflammatory processes in which mast cells are prominently involved are inhibited by antihistaminic and antiserotonin compounds in the rat. The anti-oedematous effect showed by methanolic extracts of leaves of *E. prostrata* was significant during the first phase of oedema development and significantly maintained in the second phase of the oedema development, suggesting an inhibitory effect on the release of active pain substance such as histamine, serotonin, polypeptides or prostaglandins.

## Conclusion

Oral administration of methanolic extract of leaves of *E. prostrata* at a concentration of 100 mgkg<sup>-1</sup> and 200 mgkg<sup>-1</sup> showed the significant dose dependent anti-inflammatory activity in carrageenin and egg white induced hind paw oedema in rats. The preliminary phytochemical

screening of leaves of *E. prostrata* indicated the presence of steroids, triterpenoids, flavanoids, tannins, reducing sugar and saponins. The steroids, alkaloids and triterpenoids present in the extract may be responsible for this anti-oedematous effect. Thus, further work is essential to fractionate, purify and identify the active principle(s) presenting this extract, as well as to understand the precise mechanism of action in anti-inflammatory activities by the methanolic extract of leaves of *E. prostrata*.

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