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Evaluation of Anti –Inflammatory Activity of *Eclipta alba* in Rats

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ABSTRACT:

The anti-inflammatory effect of the plant of Eclipta alba (Family – Asteraceae) was evaluated using carrageenin, mediators such as histamine and serotonin induced paw oedema, and cotton pellet induced granuloma tests for their effect on acute and chronic phase inflammation models in rats. Maximum inhibition (55.85%) was noted at the dose of 500 mg/kg after 3 hr of drug treatment in carrageenin induced paw oedema, whereas the Indomethacin (standard drug) produced 61.30% of inhibition. In the chronic model (cotton pellet induced granuloma) the CEEA and standard drug showed decreased formation of granuloma tissue by 49.7,41.5,22.1% and 53.48 % respectively. The results indicate the potent anti-inflammatory effect and therapeutic efficacy of *Eclipta alba* extract on animal models, which is compared with Indomethacin.

Key words: *Eclipta alba*, anti-inflammatory effect, cotton pellet-induced granuloma.

INTRODUCTION

Eclipta alba (Family – Asteraceae) grows in tropical and subtropical countries at an altitude upto 2000 meters. It is commonly known as Karishalenganni in Tamil. The plant has a bitter, hot, sharp, dry taste and is used in Ayurveda for the treatment of 'Kapha' and 'Vata'. The fresh plant is used in treating enlargement of the liver and spleen and in various chronic skin diseases. The plant is used in many Ayurvedic formulation for the relief of pain, as anti-helmintic, antiflammation, in eye diseases, asthma and anaemia (1-4). The fresh juice of the leaves is rubbed on the shaven scalp for the purpose of promoting the growth of hair. The herb contains wedelolactone as coumestan derivatives, the first one has been responsible as a major anti-hepatotoxic compounds of this plant (5). It is also reported triterpenoidal saponin (Eclalbatin) is present in this plant (6). However, fewer reports are available with respect to the pharmacological properties of the plant. Keeping this in view, the present study has been undertaken to investigate the anti-inflammatory studies of chloroform extract of *Eclipta alba* in standard animal models.

Materials and Methods

The plant species of *Eclipta alba* were collected in Namakkal district of Tamilnadu, India. Dr. S. Jayaram, (Director, Medicinal plant research unit and plant anatomy

research center, Chennai, Tamilnadu, India) authenticated the plant and a specimen was preserved in the Department of pharmacognosy in our institutions for future references.

Chemicals and Reagents

The chemicals used in the present study were carrageenin (S.D. Fine Chemicals Limited, Bombay), histamine (Sigma, USA), 5-hydroxy tryptamine hydrochloride (serotonin) (Sigma, USA), and Indomethacin (IPCA, Bombay).

Preparation of Extract

The dried powdered plant material was extracted with chloroform in a Soxhlet extraction apparatus. The solvent was removed under reduced pressure and semi solid mass was obtained (Yield 16.7%). The extract showed positive test for alkaloids, steroids, and tannins. The extract at the different doses of 50,100 and 200 Mg/kg was suspended in aqueous Tween 80 solution (2%) and Indomethacin (10 mg/Kg) in saline were used for the present study.

Phytochemical profile

The phytochemcial profile was performed as described by Costa (19977). It was determined by identification reactions based on the chemical group to be determined by thin layer chromatography.

Animals

Albino Wistar rats of the either sex (180-120g) were used for the present study. They were maintained under standard environmental conditions and were fed with standard pellet diet with water as libitum.

Toxicity Study

An acute toxicity study relating to the determination of LD50 was performed (7).

Investigation of Anti-inflammatory effects:

Carragenin Induced Paw odema (8)

The rats were divided into 5 groups (n=6). The extract and the standard used for this study were prepared in the same manner as mentioned earlier. Animals were deprived of food and water for 18 hours before the experiment. On the day of the experiment they were assigned to 5 groups of six animals each. They were marked and numbered for identification. The test compounds and standard drugs were administered orally. After 60 mins of administration of extracts and standard drugs 0.9% saline was injected to the lateral mallelolus on the sub plantar region of the right hind paw of the tats and paw volume was measured plethys-mometrically, at 1h, 2h, 3h and 4h respectively. The percentage of inhibition of inflammation was calculated for comparison.

The ratio of the anti-inflammatory effect of CEEA was calculated by the following equation: anti-inflammatory activity(%) = (1-D/C)x 100, where D represents the percentage difference in paw volume after CEEA was administered to the rats, and C represents the percentage difference of volume in the control groups.

Mediator induced inflammation

The anti-inflammatory activity of the extract was measured with phlogistic agents (viz. histamine, 5-HT) which act as mediator of inflammation. The paw oedema was induced in rats by sub plantar injection of freshly prepared histamine (1mg/kg) and serotonin (1mg/kg) solutions respectively

and the paw oedema was measured as mentioned earlier (8). Cotton pellets-induced granuloma

The rats were divided into four groups (n=6). The cotton pellet granuloma model investigated the proliferation phase of inflammation (9). The extract of different doses (50,100, and 200 mg/kg) and Indomethacin at 10 mg/kg body weight was given to the animals orally. After shaving the fur, the rats were anaesthetized and 10 mg of sterile cotton pellets were inserted, one in each axilla. The extracts was administered daily for a period of seven days. The rats were sacrificed after a high dose of anesthesia on the eighth day and the pellets were removed surgically and made free form extraneous tissues. The pellets were incubated at 37o C for 24h and dried at 60o C to constant weight. Increment in the dry weight of the pellets was taken as measure of granuloma formation.

Statistical Analysis

The results are expressed as mean ± SEM. The statistical analysis was performed by ANOVA test.

Result

Anti-inflammatory studies

The anti-inflammatory potential of CEEA (50,100 and 200 mg/kg) against various experimental animal models exhibited significant ($P<0.05$) anti-inflammatory activity. The effects of CEEA and Indomethacin on the inflammation induced by carrageenin, histamine and cotton pellet induced granuloma are summarized in table 3-5.

As shown in table1, CEEA showed maximum inhibition of 55.85% at the dose of 200 mg/kg after 3h of treatment in carrageenin induced paw oedema, whereas the standard drug (Indomethacin 10 mg/kg) Showed 61.30% of inhibition ($P<0.05$). The CEEA showed 52.55 and 53.55 and 53.51% ($P<0.001$) of inhibition at the dose of 200 mg/kg whereas indomethacin showed 61.31, 59.10% of inhibition in histamine and serotonin induced paw oedema. In the chronic model (cotton pellet induced granuloma), the CEEA (50,100 and 200 mg/kg) and Indomethacin showed decreased formation of granuloma tissue at 46.7, 41.5 22.1% and 53.48% respectively.

Discussion

The present study establishes the anti-inflammatory activity of the *Eclipta alba* extract in standard experimental animal models. The effect of CEEA at the dose of 50,100 and 200 mg/kg showed significant anti-inflammatory activity. It is evident that carrageenin is commonly used to induce acute inflammation and is believed to be bi-phasic. Based on this, it could be argued that the suppression of the first phase may be due to inhibition of the release of early mediators, such as histamine and serotonin, and the action in the second phase may be explained by an inhibition of cyclo-oxygenase. These mediators take part in the inflammatory response and are able to stimulate nociceptor and thus induce pain (10). It has been reported that second phase of oedema is sensitive to most clinically effective anti-inflammatory drugs, which has been frequently used to access the anti-oedematous effect of natural products (10-11). Based on these reports, it can be inferred that the inhibitory effect of the extract of *Eclipta alba* on carrageenin-induced inflammation in rats may by due to inhibition of the mediators responsible for inflammation.

Histamine is the one the important inflammation mediators and it is potent vasodilators substance and increase the vascular permeability (12-13). The study showed that the dose CEEA effectively suppressed the odema produced by the histamine, which indicates that the extract exhibit the anti – inflammatory action by means of either inhibiting the syntheses, release or action of inflammatory mediators viz. histamine, serotonin and prostaglandin's might be involved in the inflammation. So, it is suggested that the anti-inflammatory activity is possibly backed by its anti-histaminic activity.

The extract exhibited significant anti-inflammatory activity on the cotton pellet test. The cotton pellet granuloma is widely used to evaluate the transudative and proliferative components of the chronic inflammation. The moist weight of pellets correlates with transude, the dry weight of the pellet of the correlates with the amount of granulomatous tissues (14-15). Chronic inflammation occurs by means of the development of proliferate cells. These cells can either spread or be in granuloma form. Non-steroidal anti-inflammatory drugs decrease the size of granuloma, which results from cellular reaction by inhibiting granulocyte infiltration/inflammation, preventing generation of collagen fibers and suppressing mucopolysaccharides (16 – 17). The *Eclipta alba* showed significant anti-inflammatory activity in cotton-pellet induced granuloma and thus found to be effective in chronic inflammatory conditions, which reflected its efficacy in inhibiting the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation.

So, we can conclude that the present study show that the chloroform extract of *Eclipta alba* exhibit significant anti-inflammatory activity against early phase (acute paw edema), late phase (cotton pellet granuloma) of inflammation models without any deleterious side effect. The anti-inflammatory activity could be attributed to the presence of phytopharamacophore and also it may be due to the synergistic effects of various active compounds in the plant.

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Table 1. Effect of the *Eclipta alba* extract on carrageenin induced pedal oedema

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition
Carrageenin control	0	0.734 ± 0.071	-
Indomethacin	10	0.284 ± 0.016	61.30
CEEA	50	0.457 ± 0.031	37.73
CEEA	100	0.396 ± 0.031	46.04
CEEA	200	0324 ± 0.026	55.85

Values are mean ± SEM (n=6). Experimental groups were compared with control $p<0.001$.

Table 2.
Effect of *Eclipta alba* extract on mediators like histamine and 5 – HT induced pedal oedema in rats

Treatment	Dose (mg/kg)	Paw volume (ml)	Percentage of inhibition
Histamine control	0	0.548 ± 0057	-
Indomethacin	10	0.212 ± 0.018	61.31
CEEA	50	0.348 ± 0.032	34.67
CEEA	100	0.321 ± 0.022	41.42
CEEA	200	0.260 ± 0.015	52.55
Serotonin control	0	0.626 ± 0.054	-
Indomethacin	10	0.256 ± 0.025	59.10
CEEA	50	0.472 ± 0.045	24.60
CEEA	100	0.325 ± 0.035	43.76
CEEA	200	0.291 ± 0.027	53.57

Values are mean ± SEM (n=6). Experimental groups were compared with control $p<0.001$.

Table 3. Effect of *Eclipta alba* extract on Cotton-pellets induced granuloma in rats

Treatment	Dose (mg/kg)	Weight of cotton pellet (ml)	Percentage of inhibition
Control	0	445 ± 4.5	-
Indomethacin	10	268 ± 2.2	53.48
CEEA	50	29.3 ± 2.4	27.41
CEEA	100	32.1 ± 2.6	38.42
CEEA	200	22.3 ± 1.5	55.23

Values are mean ± SEM (n=6). Experimental groups were compared with control $p<0.001$.

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