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EVALUATION OF ANTI-INFLAMMATORY ACTIVITY OF LEAVES OF *ECLIPTA PROSTRATA* LINN. (ASTERACEAE)

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ABSTRACT

Eclipta prostrata Linn. (Asteraceae) popularly known as "Bhringaraj" is a very useful medicinal plant in India. The aim of the present study is to evaluate the anti-inflammatory activity of ethanolic extract of leaves of *Eclipta prostrata.* The anti-inflammatory effects were investigated by employing acute inflammatory model; Carrageenan induced paw edema and Sub-acute inflammatory model; formalin induced paw edema in rats. The ethanolic extracts at a dose of 200mg/kg and 400mg/kg body weight showed significant anti inflammatory activity in both the models in a dose dependent manner.

INTRODUCTION: Nature has provided a complete store house of remedies to cure all ailments of mankind. The use of natural products, especially plants, for healing is as ancient and universal as medicine itself. Natural products have been an integral part of the ancient traditional medicine systems, e.g., Chinese, Ayurvedic and Egyptian. Today these symbolize safety in contrast to the synthetics and so traditional systems of medicine continue to be widely practiced all over the world. This has led to increased emphasis on the use of plant materials as a source of medicines for a wide variety of human ailments [1].

Pain, inflammation and fever are very common complications in human beings. Inflammation is considered as a primary physiologic defence mechanism that helps body to protect itself against infection, burn, toxic chemicals, allergens or other noxious stimuli [2]. Several plants and their products are claimed and proved to posses anti inflammatory property. *Eclipta prostrata* (Asteraceae) is one from them. It is a herbaceous annual, 30-50 cm. high [3]. The plant has long been used in the treatment of hernias, eye diseases, "Kapha" and "Vata", bronchitis, asthma, leucoderma, anemia, diseases of the heart and skin, itching, night-blindness, syphilis [4].

MATERIALS AND METHODS:

Plant material: The plant material (leaves) of *Eclipta prostrata* were collected from healthy plants in PUSA institute (New Delhi) in the month of September 2010. It was dried under shade and then powdered to obtain a coarse powder. This powder was stored in air-tight container and used for further extraction.

Preparation of extract: The powder was macerated for 7 days using 850 ml of 95% Ethanol as menstrum. Maceration was repeated, twice (7 days each time) by adding fresh menstrum, equal to the volume of extract obtained. Total extract was combined and concentrated, under vacuum; to get a semi solid consistency [5]. The yield obtained was 8.2 % w/w.

Animal model: Wistar strain albino rats of either sex (150-200g) were approved by Institutional Animal Ethics Committee (Regn. No.-585/02/c/CPCSEA in 2011) and procured from Institutional Animal House, Hindu College of Pharmacy, Sonipat. The animals were provided with food (Golden feed) and water ad libitum and maintained at a temperature 22 to 25^{o}C.

Experimental Design: The anti-inflammatory activity of ethanolic extract of *Eclipta prostrata* leaves was assessed in acute and sub-acute inflammation.

Acute inflammation-Carrageenan induced paw edema [6, 7, 8]**:** The animals were divided into 4 groups having six animals in each group and placed in individual cages. These were maintained at standard diet and water *ad libitum*, 18 hrs prior to experiment food and water was withdrawn. Test and standard drug was administered orally. First group served as control and received 1% SCMC. Group II received standard (Diclofenac sodium; 5mg/kg body weight). Group III and IV received test drug at different doses. 30 min. later, the rats were challenged by a subcutaneous injection of 0.1 ml of 1% Carrageenan into the subplantar side of the left hind paw.

The paw was marked with ink at the level of the lateral malleolus and paw volume was measured by digital plethysmometer before and after 0.5, 1, 2, 3, 4 and 24 hr. of drug administration. The average paw volume in the test group animals of extracts treated were compared with control group and standard group animals. Mean increase in paw volume was determined and tabulated in **table 1.**

Sub-acute inflammation- Formalin induced paw edema [6, 7]**:** The animals were divided into 4 groups having six animals in each group and placed in individual cages. These were maintained at standard diet and water *ad libitum*, 18 hrs prior to experiment food and water was withdrawn. Test and standard drug was administered orally. First group served as control and received 1% SCMC. Group II received standard (Diclofenac sodium; 5mg/kg body weight). Group III and IV received test drug at different doses. 30 min. later, the rats were challenged by a subcutaneous injection of 0.1 ml of 1% Formalin into the subplantar side of the left hind paw. The paw was marked with ink at the level of the lateral malleolus and immersed in mercury upto this mark. The paw volume was measured plethysmographically before and after 0.5, 1, 2, 3, 4 and 24 hr. of drug administration. The average paw volume in the test group animals of extracts treated were compared with control group and standard group animals. Mean increase in paw volume was determined and tabulated in **table 2.**

RESULT AND DISCUSSION: Preliminary Phytochemical screening of crude extract revealed the presence of Carbohydrates, Amino acids, Steroids, Flavonoids, Alkaloids, Tannins and Phenolics [9, 10].

Acute toxicity study: No visible change was observed in any animal and all survived till 14 days; thus based on preliminary study, ethanolic extract of *E. prostrata* was found to be safe for further biological studies, as no lethality was observed at 2000 mg/kg (orally) in rats.

Acute inflammation:

Carrageenan induced paw edema: As shown in **table 1**, the ethanolic extract at a dose of 200 & 400 mg/kg body weight showed a significant and dose dependent inhibition in inflammation induced by carrageenan. The maximum percentage inhibition was 24.24 & 31.81% by ethanolic extract at 200 & 400 mg/kg body weight respectively at 3rd hr. However, standard drug (Diclofenac sodium, 5mg/kg body weight) showed highly significant inhibition ($p<0.001$) at the same time with the maximum percentage inhibition (48.48%) compared to the control group, shown in **fig. 1**.

TABLE 1: ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *E. PROSTRATA* BY CARRAGEENAN INDUCED PAW EDEMA

Drug/Extract	Group	Dose (mg/kg)	Mean paw volume (ml)						
			Before Dose	0.5 hr.	1 hr.	2 hr.	3 hr.	4 hr.	24 hr.
Control	I	-	0.65±0.00	0.83±0.01	1.00±0.01	1.16±0.01	1.31±0.01	0.85±0.00	0.80±0.01
Diclofenac Sodium	II	5	0.55±0.01***	0.65±0.00***	0.74±0.01***	0.82±0.01***	0.89±0.01***	0.67±0.01***	0.66±0.00**
EEL	III	200	0.60±0.02***	0.74±0.02***	0.87±0.00***	0.99±0.02**	1.10±0.01***	0.76±0.00**	0.73±0.01***
EEL	IV	400	0.62±0.01**	0.75±0.01**	0.86±0.01**	0.97±0.01***	1.07±0.01**	0.77±0.02**	0.75±0.01**

EEL: Ethanolic extract of leaves; Values are expressed in mean ± S.E.M.(n = 6), * Significant at $p < 0.05$, ** Significant at $p < 0.01$, ***Significant at $p<0.001$ Vs control Dunnet's test

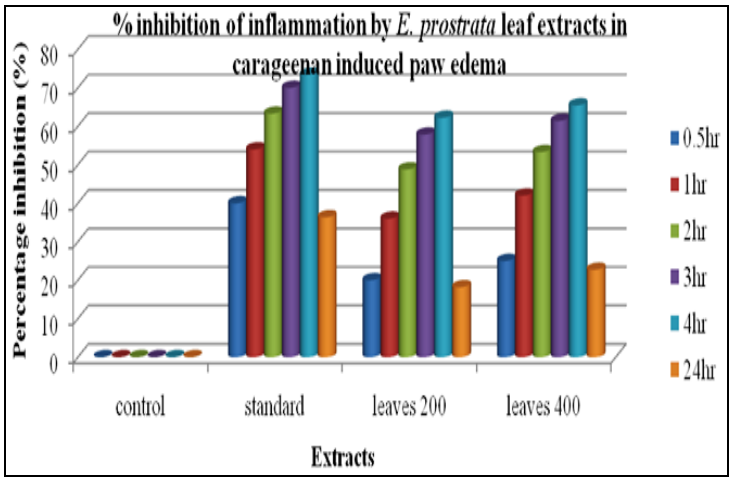

FIG. 1: ANTI-INFLAMMATORY ACTIVITY OF *ECLIPTA PROSTRATA* EXTRACT IN CARRAGEENAN-INDUCED RAT PAW EDEMA MODEL

Carrageenan induced inflammation is useful in detecting orally active anti-inflammatory agents. The development of carrageenan induced edema is biphasic, the 1st phase is attributed to release of histamine, 5-HT, Kinin, while 2nd Phase is related to the release of Prostaglandin. Carrageenan induces paw edema by inducing protein-rich exudates containing a large number of neutrophils.

Therefore, it is proposed that the anti inflammatory property of extracts is due to inhibition of one of the pain mediators like histamine, 5-HT, Kinin or Prostaglandin or both [7].

Sub-acute inflammation:

Formalin Induced paw edema: The data depicted in **table 2** showed that the ethanolic extract at a dose of 200 mg/kg had lesser activity than the ethanolic extract at a dose of 400 mg/kg, which exhibits more significant anti-inflammatory activity with decrease in paw edema when compared to control. The maximum percentage inhibition was 62.23 & 65.42% by ethanolic extract at 200 & 400 mg/kg body weight respectively at 4th hr. However, standard drug (Diclofenac sodium, 5mg/kg body weight) showed highly significant inhibition at the same time ($p<0.001$) with the maximum percentage inhibition (73.40%) compared to the control group, shown in **fig. 2**.

The nociceptive effect of formalin is also biphasic, an early neurogenic component followed by a later tissue mediated response [7].

TABLE 2: ANTI-INFLAMMATORY ACTIVITY OF ETHANOLIC LEAF EXTRACT OF *E. PROSTRATA* BY FORMALIN INDUCED PAW EDEMA

Drug/Extract	Group (mg/kg)	Dose	Mean paw volume (ml)						
			Before Dose	0.5 hr.	1 hr.	2 hr.	3 hr.	4 hr.	24 hr.
Control	I	-	0.68±0.01	0.88±0.00	1.18±0.01	1.58±0.01	2.08±0.01	2.56±0.01	0.90±0.00
Diclofenac Sodium	II	5	0.58±0.00***	0.70±0.00***	0.81±0.00***	0.91±0.01**	1.00±0.01***	1.08±0.01***	0.72±0.00***
EEL	III	200	0.63±0.01***	0.79±0.02***	0.95±0.00***	1.09±0.01**	1.22±0.01***	1.34±0.01***	0.81±0.00**
EEL	IV	400	0.65±0.00**	0.80±0.01**	0.94±0.02	1.07±0.00***	1.19±0.01**	1.3±0.02	0.82±0.00**

EEL: Ethanolic extract of leaves; Values are expressed in mean ± S.E.M.(n = 6), * Significant at $p < 0.05$, ** Significant at $p < 0.01$, ***Significant at $p<0.001$ Vs control Dunnet's test

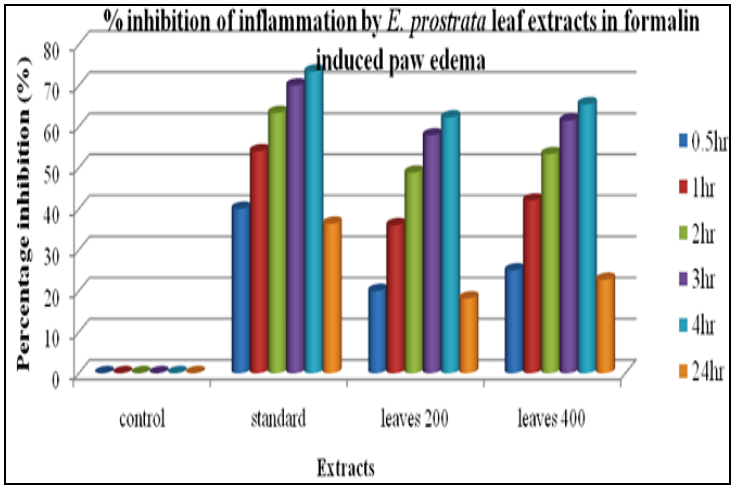

FIG. 2: ANTI-INFLAMMATORY ACTIVITY OF *ECLIPTA PROSTRATA* EXTRACT IN FORMALIN-INDUCED RAT PAW EDEMA MODEL

CONCLUSION: The present experimental protocols showed that the *Eclipta prostrata* (leaves) individually elicited a significant anti-inflammatory activity in carrageenan (acute) and Formalin (Sub-acute) rat models in a dose dependent manner. The activities of extract are comparable with Diclofenac sodium and hence it is useful in the treatment of inflammation associated disease like arthritis. The phytochemical investigation of the plant revealed the presence Of Carbohydrates, Amino acids, Steroids, Saponins, Flavonoids, Alkaloids, Tannins & Phenolic. The steroids, alkaloids and triterpenoids present in the extract may be responsible for this anti-oedematous effect [11].

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 ethanolic extract of leaves of *E. prostrata*.

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