

Evaluation of Analgesic and Anti-Inflammatory Activity of *Eclipta alba*

Rajesh Kumar, Y.S. Sarangdevot , Bhupendra Vyas

Abstract— *Eclipta alba* (Family – Asteraceae) commonly known as false daisy grows in tropical and subtropical countries at an altitude up to 2000 meters and is commonly found in waste places, marshy lands, hedges and roadsides. It is commonly known as ‘Bhringraja’ (Sanskrit) and ‘Bhangra’ (Hindi). The aim of the present study is to evaluate analgesic and anti-inflammatory activity of ethanolic extract of *Eclipta alba*. In addition the extract was also studied for its acute toxicity effects and preliminary phytochemical screening. The analgesic effect of the plant extract were investigated by hot plate and tail immersion test and the anti-inflammatory effects were investigated by employing acute inflammatory model (Carrageenan induced paw edema). The ethanolic extract at a dose of 200 mg/kg showed significant analgesic activity whereas the same extract at a dose of 400 mg/kg body weight exhibited significant anti-inflammatory activity in the given animal models in a dose dependent manner.

Index Terms— *Eclipta alba*, analgesic, inflammation, anti-inflammatory.

I. INTRODUCTION

Eclipta alba (Family – Asteraceae) commonly known as false daisy grows in tropical and subtropical countries at an altitude upto 2000 meters and is commonly found in waste places, marshy lands, hedges and roadsides. It is commonly known as ‘Bhringraja’ (Sanskrit) and ‘Bhangra’ (Hindi)¹. The plant has a sharp, bitter, hot, and dry taste and is used in Ayurveda for the treatment of ‘Kapha’ and ‘Vata’².

Eclipta alba (Linn.) Hassk of family Asteraceae also known as *Eclipta prostrata* (Linn.) has an important role in the traditional Ayurvedic, Siddha and Unani systems of medicine. is also known as *Eclipta prostrata* (Linn.) 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, antiinflammation, in eye diseases, asthma and

anaemia. The genus name *Eclipta* comes from the Greek word meaning "Deficient," with reference to the absence of the bristles and awns on the fruits and the specific *alba* means white which refers to the color of the flowers and is official in Indian Herbal Pharmacopoeia and The Ayurvedic Pharmacopoeia of India³⁻⁵.

Main active principles of *Eclipta alba* consist of coumestans like wedelolactone, desmethylwedelolactone alkaloids, flavonoids, glycosides, polyacetylenes, and triterpenoids. The leaves contain stigmaterol, a-terthienylmethanol, wedelolactone, demethylwedelolactone and demethylwedelolactone-7-glucoside⁶, furanocoumarins, oleanane & taraxastane glycosides⁷. The roots give hentriacontanol and heptacosanol. The roots contain polyacetylene substituted thiophenes. The aerial part is reported to contain a phytosterol, β -amyryn in the n-hexane extract and luteolin-7-glucoside, P-glucoside of phytosterol, a glucoside of a triterpenic acid and wedelolactone in polar solvent extract⁷. The polypeptides isolated from the plant yield cystine, glutamic acid, phenyl alanine, tyrosine and methionine on hydrolysis. Nicotine and nicotinic acid are reported to occur in this plant⁸. *E. alba* is also known to contain several active compounds such as phytosterols, flavones (luteolin), and triterpenes (ecalbatin, ursolic acid and echinocystic acid)⁹.

This plant is believed to have several important pharmacological activities such as antibacterial, antifungal, anthelmintic, antimalarial, hepatoprotective, neuroprotective, immunomodulatory, analgesic, diuretic, hypolipidemic, anti-inflammatory, antidiabetic, antioxidant, anticancer, hair growth promoting, memory enhancing and antivenom¹⁰.

E. alba has become a major concern for scientists to investigate its components related to the phytochemical and pharmacological activities of this plant. Although several

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reviews on *E. alba* have been reported, they do not encompass all facets of this plant¹¹⁻¹³. Drugs obtained from plants are easier to obtain, practical to use, require lower costs and only cause lower side effects compared to synthetic drugs¹⁴.

Pain is defined as “an unpleasant sensory and emotional experience associated with actual or potential tissue damage or described in terms of such damage.” It is fundamentally a protective response, the ultimate goal of which is to help the organism get rid of both, the initial cause of injury (e.g., microbes and toxins) and the consequences of such injury (e.g., necrotic cells and tissues).

Inflammation is defined as the reaction of living tissue to injury, and its cardinal signs include pain, swelling, redness, increased local warmth, and loss of function. Inflammation is of acute and chronic types. Acute inflammation is the immediate and early response to an injurious agent, while chronic inflammation is the inflammation of prolonged duration (weeks or months) in which there are active inflammation, tissue destruction, and attempts at repair, which proceed simultaneously. However, inflammation, if uncontrolled, can become a cause of suffering, leading to disabilities, contractures, disfiguring of body, and chronic pain. In such situations, the inflammation needs to be controlled or suppressed¹⁵. Inflammation is the first line of defensive mechanism of the body against invading microorganisms, tissue injury, or foreign substances. However, prolonged acute inflammation often leads to a chronic condition of tissue damage. Chronic inflammation results in various inflammation-mediated diseases, including rheumatoid arthritis¹⁶, atherosclerosis¹⁷, cancer¹⁸, and diabetics¹⁹.

Drugs used for the management of pain and inflammation are either narcotics (e.g., opioids) or non-narcotics (e.g., salicylates and corticosteroids), both of which are well known for their side effects, such as intestinal tract ulcers and erosions of the stomach linings²⁰. Nonsteroidal anti-inflammatory drugs used as medication for inflammatory diseases are associated with side effects, including gastrointestinal complications, high blood pressure, ulceration, and bleeding, but their long-term use leads to cardiovascular and renal complications²¹.

So there is greater interest in finding a safer and potent alternative, especially agents from natural sources. Hence, there is always a need for novel analgesic and anti-inflammatory compounds with lesser side effects, without respiratory depression, sickness, and addiction from natural sources²². It is a matter of great concern, to produce good analgesia with a drug or combination of drugs, the present experimental research work was undertaken to study the antinociceptive activity of total alcoholic extracts of Bhringaraja (*Eclipta alba*) in albino rats by using standard experimental models to find out the effective dose of the trial drug and to find out the side effects if any. Keeping this in view the need of the hour, the present experimental research work was undertaken to evaluate the analgesic and anti-inflammatory studies of alcoholic extract of *Eclipta alba* in standard animal models of pain and inflammation in rats. The extract was also studied for its preliminary phytochemical screening and acute toxicity effects.

II. MATERIALS AND METHODS

Plant material selection and collection

The plant species of *Eclipta alba* were collected from road sides situated on main roads of Sriganganagar (Rajasthan) and the plants was authenticated by Prof. (Dr.) Ajay Sharma, HOD, department of botany, Seth G.L. Bihani S.D. P.G. College Sriganganagar and a voucher (SDPG/2021019) specimen was deposited. The collected plant material was cleared, cleaned and dried in the shade and stored in airtight glass container for further studies²³⁻²⁵.

Drugs, Chemicals and reagents

All chemicals, reagents and solvents used were of AR and HPLC grade (Loba, Himedia, Qualigen). The standard drugs were procured from local market.

Preparation of plant extract:

A definite weight of dried and cleaned crude leafy drug was subjected to size reduction by dry grinder. The coarsely powdered leafy drug was taken in a soxhlet apparatus thimble and continuous hot soxhlet extraction was done by various solvents in a sequence of increasing polarity as Petroleum ether, chloroform, ethanol, and water. After completion of extraction, the extracts were concentrated using rota evaporator. The weight of the extract obtained was

determined, and the percentage of extractive value was calculated on dry weight basis. The concentrated extract was air dried and used for further study²³⁻²⁵. The suspension of hydro-alcoholic extracts was prepared by using 1% CMC in distilled water²⁶.

Phytochemical analysis/Preliminary phytochemical screening

Preliminary phytochemical screening of petroleum ether, chloroform, ethanol and water extract of plant were carried out by using standard procedures described by Kokate²³, Harborne²⁴ and Trease Evan²⁵ and Khandelwal²⁶.

Experimental animals

Healthy, 8-12 weeks young Albino rats (Wister strain) of either sex weighing 150 – 180 gms were used for present study. They were given a 7-day acclimatization period to the laboratory environment and were provided with food and water ad libitum. The animals were maintained as per CPCSEA regulations. The animals were housed in polypropylene cages individually at 22°C ($\pm 3^{\circ}\text{C}$), relative humidity 30%, lighting sequence being 12 hours light and 12 hours dark cycle. The animals were housed individually for 5 days in cages. For feeding, conventional rodent laboratory diets were used with unlimited supply of drinking water. Before conducting the experiment, ethical clearance was obtained from Institutional Animal Ethics Committee, Bhupal Nobles University, Udaipur, India, vide approval no. BNCP/IAEC/2023-2/1 dated June 06, 2023.

Test substances

Diclofenac Sodium, Carrageenan and carboxymethyl cellulose (CMC) were gifted by Lalit instruments and chemical industries, Sriganganagar, Rajasthan, India. Preparation of solution of test substances 0.1% solution of CMC was prepared and homogenized using a magnetic stirrer.

Experimental design

Acute toxicity study, analgesic activity and anti-inflammatory activity of ethanolic extract of *Eclipta alba* was assessed in animal models. For analgesic and anti-inflammatory experiments, 24 animals were randomized into four groups (control, standard, and two test groups of different dose level) of six animals each.

Grouping of animals

Twenty-four animals were used in all; six animals were used in each group.

Group I: Positive control group

Group II: Standard group

Group III: *Eclipta alba* low dose level of alcoholic extract

Group IV: *Eclipta alba* high dose level of alcoholic extract

Pharmacological studies

Methods used

Acute toxicity studies

The acute toxicity of the alcoholic extract obtained from *Eclipta alba* was determined by up and down procedure of OECD guideline 425²⁸. One animal was dosed with 2000mg/kg of test drug solution. The animal survived, therefore main test was conducted to determine LD50. Then four additional animals were dosed sequentially and total five animals were tested. After dosing, animals were observed for 48 hours, no animal was died. The animals were observed for seven days as animals had not shown any sign of toxicity, behavioural changes and mortality. Then animals were observed up to 7 days for toxicity, behavioural changes and mortality.

Analgesic activity

a. Hot plate method

Wistar albino rats of either sex ($n=6$), weighing 150-180 g, were used for the study. The analgesic properties of alcoholic extract of *Eclipta alba* were assessed using the hot plate method developed by Eddy and Leimbach. The temperature of the hot plate was maintained at $55\pm 0.2^{\circ}\text{C}$, a level that is uncomfortable but does not cause skin burns. Animals displayed discomfort by licking or leaping.

The rats were administered the following treatments:

The control group received normal saline (0.9% v/w).

The test groups received oral doses of 100 mg/kg and 200 mg/kg of *Eclipta alba* alcoholic extract.

The standard group received (10 mg/kg, i.p.) diclofenac sodium.

Rats were placed on hot plate and recorded the reaction time in second for licking of hind paw or jumping, with cut-off time of 20 sec. The reaction time following the administration of the test extracts, reference standard drug, and control vehicle were measured at 0, 30, 60 and 120 min.

The latency time, defined as the duration until the animal licked its front or rear paw or leaped off the plate, was recorded to assess analgesic response²⁹⁻³¹. 15 second was used as post-treatment cut-off time. The percentage inhibition was obtained as follows:

$$\% \text{ Inhibition} = \frac{\text{Post treatment latency} - \text{Pre-treatment latency}}{\text{Cut off time} - \text{Pre-treatment latency}} \times 100$$

b. Tail flick method

Tail flick method Radiant heat was applied to a single spot on the proximal third of the tail using an analgesiometer. The time taken for the animal to withdraw (flick) its tail was recorded as the reaction time. The standard drug or test substance was administered after measuring the baseline reaction times at intervals of 0, 30, 60, and 120 minutes^{32, 33}.

After placing the rat in the analgesiometer, the middle part of the tail was exposed to heated nichrome wire that served as a radiant heat source with the strength of the electric current passing through the wire kept at 6 Amps. The endpoint was a "tail-flick response" which is characterized by a sharp withdrawal of the tail or a high-pitched noise called a squeak. The time to this response was recorded using a stopped clock as the "reaction time/latency." A cutoff time of 10 s was set as "maximum latency" to rule out thermal injury. The test was performed at baseline (basal latency) and at the end of 30, 60 and 120 min after drug administration (test latency)³⁴. Percentage maximum possible effect (%MPE; analgesia) was calculated by using the formula:

$$\% \text{ MPE} = \frac{\text{Test latency} - \text{Basal latency}}{\text{Maximum latency} - \text{Basal latency}} \times 100$$

Anti-inflammatory tests

Anti-inflammatory activity was analyzed by the carrageenan-induced rat paw edema method³⁵⁻³⁷. Diclofenac Sodium (10 mg/kg per oral) was taken as standard drug.

a. Carrageenan-induced paw oedema

The method was used to determine the anti-inflammatory activity of the extract. Animals were divided into four groups to evaluate the anti-inflammatory activity against acute inflammation. Group A (carrageenan control) did not receive any oral treatment. Group B (standard group) received 10 mg/kg of diclofenac sodium. Group C was administered 200 mg/kg of alcoholic extract of *Eclipta alba*, while Group D received 400 mg/kg of *Eclipta alba* extract. Inflammation was induced by injecting 0.1 ml of carrageenan (prepared as a 1% suspension in sterile normal saline) into the rat's left hind paws to cause edema. All treatments were given orally 1 hr prior to the carrageenan injection. Paw volume was measured using a plethysmometer before injection (0 hr) and at 2, 4 and 6 hr post-injection.³⁸⁻⁴⁰

The percentage inhibition was obtained as follows:

$$\% \text{ Inhibition} = \frac{\text{Increase in paw edema (control)} - \text{Increase in paw edema (test)}}{\text{Increase in paw edema (control)}} \times 100$$

III. STATISTICAL ANALYSIS

All data were expressed as mean $\pm$ SEM, and the test dose was compared with control and the results were analysed using the statistical program Graph Pad, with one-way analysis of variance (ANOVA) employed for group comparisons. $P < 0.05$ was considered significant, $P < 0.01$ more significant, and $P < 0.001$ highly significant.

RESULTS

Acute toxicity studies

According to oral toxicity study as per OECD 423 guidelines, the dose of 2000 mg/kg of *Eclipta alba* ethanol extract, was the non-toxic dose for rats. The behaviour of the animals was closely observed for the first 3 h then at an interval of every 4 h during the next 48 h. All extract did not cause mortality in mice and rats during 48 h observation but little behavioural changes, locomotor ataxia, diarrhoea and weight loss were observed. Food and water intake had no significant difference among the group studied.

Preliminary phytochemical screening

Extraction process:- The percentage yield and physical appearance of various extracts of *Eclipta alba* is shown in

Table 1. The successive solvent extraction showed maximum yield with ethanol.

Phytochemical screening: The phytochemical analysis of all extracts for presence of various chemical constituents is shown in Table 2. Preliminary phytochemical study of the *Eclipta alba* ethanol extract, showed the presence of alkaloids, carbohydrates, flavonoids, phenols and tannins. The ethanol extract contained flavonoids protect from allergies, carcinogens and other harmful toxic substances⁴¹.

Table 1: Percentage yield and physical characteristics of various extracts of *Eclipta alba*

Extract	% yield w/w	Color
Petroleum ether	1.28	Yellowish green
Chloroform	2.86	Dark green
Ethanol	5.93	Greenish yellow
Water	5.23	Dark brown

Table 2: Phytochemical screening of *Eclipta alba*

Compound	Petroleum ether extrat	Chloroform extract	Ethanol extract	Water extract
Alkaloids	-	+	+	-
Carbohydrates	-	-	+	+
Flavonoids	-	-	+	-
Saponins	-	-	+	-
Steroids	-	-	+	+
Proteins	-	-	+	+
Tannins	-	+	+	-
Glycosides	-	-	+	+
Coumarins	-	-	+	-

Analgesic studies

Hot plate test

The results of the analgesic activity of the ethanolic extract of *Eclipta alba* are presented in Table 3 and Figure 1. Throughout the 120 min observation period, Wistar albino rats treated with normal saline (control) did not show any significant changes in their reaction times in Eddy's hot plate test. However, a significant improvement in reaction times was observed after administering two different doses of the ethanolic extract of *Eclipta alba*. Animals treated with diclofenac sodium and *Eclipta alba* extract had significantly

longer reaction times compared to the saline-treated group. At the 120-min mark, the reaction time for diclofenac sodium was 13.28 sec, 5.51 sec for the saline group, 8.31 sec for the ethanolic extract of *Eclipta alba* (100 mg/kg) group, and 11.62 sec for the ethanolic extract of *Eclipta alba* (200 mg/kg) group. The percentage inhibition of ethanolic extract of *Eclipta alba* (100 mg/ kg) group, (200 mg/kg) group, and diclofenac sodium, respectively, was 49.62%, 75.09% and 83.85% at 120 min. The increase in reaction time for diclofenac sodium also varied significantly at different time points compared to baseline values within the same treatment group.

Table 3: Effect of *Eclipta alba* ethanol extract by hot plate test, all values are expressed in second.

Extract and	Dose	Reaction Time
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compounds	mg/kg	0 min	30 min	60 min	120 min
Control (Saline)	0.9%	4.34±0.3	5.9±0.41	4.55±0.23	5.51±0.47
Standard (Diclofenac)	10 mg/kg	4.34±0.3	6.53±0.31	7.72±0.20**	13.28±0.41**
Ethanollic Extract	100 mg/kg	1.72±0.23	3.67±0.49*	5.07±0.22*	8.31±0.43**
Ethanollic Extract	200 mg/kg	1.43±0.07	5.04±0.16**	6.55±0.49*	11.62±0.58**

Values are mean ± S.E.M. * $p < 0.05$ versus control, ** $p < 0.01$ versus control.

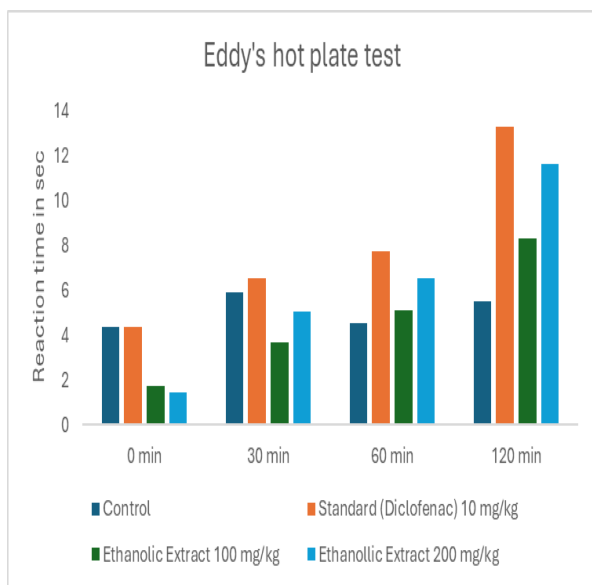


Figure 1: Graph Representation of effect of ethanolic extract (doses 100 & 200 mg/kg) of *Eclipta alba* using Hot Plate. Diclofenac (10 mg/kg) used as a standard. All values are expressed as mean± SEM.

Table 4 and Figure 2 presents the results of the ethanolic extract of *Eclipta alba* evaluated using the tail flick method. Wistar albino rats administered normal saline (control) exhibited no significant changes in their tail flick reaction times throughout the 120 min observation period. However, a marked improvement in reaction times was observed after administering two different doses of the ethanolic extract of *Eclipta alba*. Reaction times were significantly longer in animals treated with diclofenac sodium and *Eclipta alba* extract compared to those treated with saline. At the 120 min mark, the reaction time for diclofenac sodium was 5.83 sec, while it was 2.23 sec for the saline group, 5.35 sec for the *Eclipta alba* ethanolic extract (200 mg/kg) group, and 5.63 sec for the *Eclipta alba* extract (400 mg/kg) group.

Tail flick method

Table 4: Effect of *Eclipta alba* ethanol extract by tail flick test, all values are expressed in second.

Extract and compounds	Dose mg/kg	Reaction Time			
		0 min	30 min	60 min	120 min
Control (Saline)	0.9%	1.58±0.07	2.19±0.22	3.05±0.20	2.23±0.34
Standard (Diclofenac)	10 mg/kg	2.63± 0.37	5.10±0.22**	6.09±0.38**	5.83±0.42**
Ethanollic Extract	100 mg/kg	0.93±0.34	2.49±0.08*	4.39±0.05*	5.35±0.31*
Ethanollic Extract	200 mg/kg	1.07±0.23	4.42±0.14**	5.28±0.13**	5.63±0.02**

Values are mean ± S.E.M, * $p < 0.05$ Versus control and Standard.

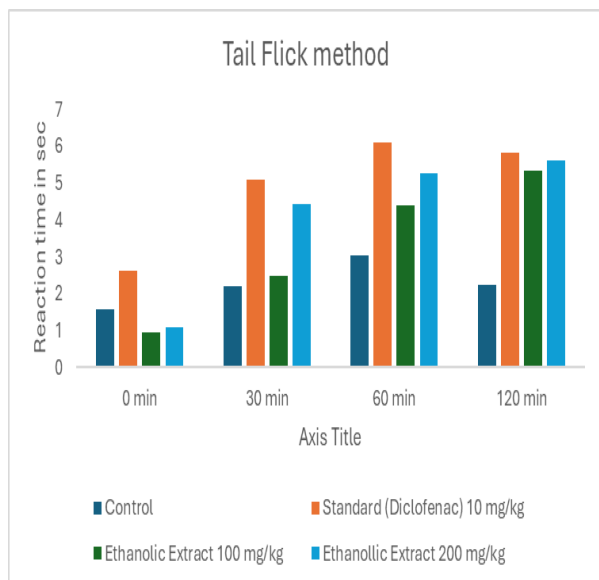


Figure 2: Graph Representation of effect of ethanolic extract (doses 100 & 200 mg/kg) of *Eclipta alba* using Tail Flick Method. Diclofenac (10 mg/kg) used as a standard. All values are expressed as mean $\pm$ SEM.

Anti-inflammatory studies

Anti-inflammatory activity-Carrageenan-induced paw oedema

The anti-inflammatory effects of the ethanolic extract of *Eclipta alba* on carrageenan-induced oedema in rat's hind paws are presented in Table 5 and Figure 3. The edema developed rapidly and was visibly apparent. It was observed that the edema peaked approximately 6 hr after Carrageenan induction in the control group. The extract demonstrated a dose-dependent reduction in edema. The 400 mg/kg dosage significantly reduced edema compared to the 200 mg/kg dosage and was comparable to the standard diclofenac sodium treatment.

Table 5: Effect of *Eclipta alba* ethanol extract on carrageenan-induced hind paw oedema model of anti-inflammatory activity by plethysmometer. All values are in ml (Paw volume).

Extracts and compounds	Dose mg/kg	Reaction Time				Mean	% Inhibition
		0 hr	2 hr	4hr	6 hr		
Positive Control	1%v/w carrageenan	0.5 $\pm$ 0.45	82 $\pm$ 0.56	1.28 $\pm$ 0.66	1.59 $\pm$ 0.69	1.04 $\pm$ 0.59*	
Standard (Diclofenac)	10 mg/kg	0.28 $\pm$ 0.36*	0.35 $\pm$ 0.28*	0.47 $\pm$ 0.34**	0.27 $\pm$ 0.65**	0.34 $\pm$ 0.40**	67%
Ethanolic Extract	200 mg/kg	0.35 $\pm$ 0.48	0.41 $\pm$ 0.53*	0.59 $\pm$ 0.57*	0.46 $\pm$ 0.38*	0.45 $\pm$ 0.49*	56%
Ethanolic Extract	400 mg/kg	0.29 $\pm$ 0.56	0.36 $\pm$ 0.51*	0.47 $\pm$ 0.21**	0.28 $\pm$ 0.3**	0.35 $\pm$ 0.39**	66%

Values are mean $\pm$ S.E.M, * p <0.05 Versus control and Standard.

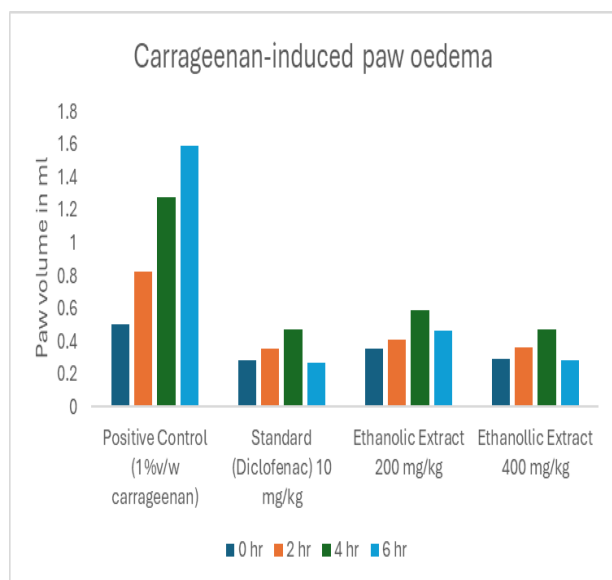


Figure 3: Graphical representation of effect of ethanolic extract (doses 200 and 400 mg/kg) of *Eclipta alba* in carrageenan induced hind paw oedema. Diclofenac sodium (10 mg/kg) was used as a standard drug. All values are expressed as mean $\pm$ SEM.

IV. DISCUSSION

In this study, ethanolic extract of *Eclipta alba* showed positive results. Dose 200 mg/kg was found to be highly significantly effective as analgesic and dose 400 mg/kg as significantly effective anti-inflammatory drug compared to effects of low dose. The effect of high dose was increasing with time, but the maximum time limit for its effects could not be elucidated because of the limited time of the study. The bioactive compounds present in herbs aid in treating and preventing various illnesses, boosting the body's defence mechanisms against infections, and promoting overall health. The aim of the current study was to evaluate analgesic and anti-inflammatory properties of *Eclipta alba*, a promising herb.

The complex process of pain is mediated by numerous physiological mediators, including prostaglandins, substance P, bradykinins, and others. The current study used the hot plate and tail flick tests to investigate the analgesic properties of ethanolic extract of *Eclipta alba*. The results showed that *Eclipta alba* extracts at doses of 100 and 200 mg/kg increased the reaction latency time in both tests, indicating a significant antinociceptive effect.

The anti-inflammatory properties of ethanolic extract of *Eclipta alba* were evaluated using a rat model of carrageenan-induced paw edema. The results demonstrated

that ethanolic extract of *Eclipta alba* significantly inhibited edema, with the highest inhibition observed at 400 mg/kg of the extract. The inflammatory response, measured by paw swelling, was reduced following a single carrageenan injection. According to numerous studies, carrageenan-induced paw edema occurs in two stages: Stage I (0-2 hr after stimulation), associated with the release of histamine and 5-HT, and Stage II (2-6 hr after stimulation), which involves the production of inflammatory mediators such as bradykinin, COX-2, and prostaglandins⁴².

Therefore, it is believed that ethanolic extract of *Eclipta alba* prevents carrageenan-induced inflammation by inhibiting cyclooxygenases, histamine, and 5-HT, which subsequently reduces prostaglandin formation.

The co-existence of analgesic and anti-inflammatory effects seen with this extract is well-defined for various non-steroidal anti-inflammatory drugs (NSAIDs) particularly salicylates and their derivatives. It's therefore interesting that the extract behaved like NSAIDs in this study which correlates well with the traditional application of the plant. The ethanolic extract of the *Eclipta alba* does possess significant analgesic and anti-inflammatory effects in laboratory animals at the doses investigated. The results support the traditional use of this plant in some painful and inflammatory conditions and also suggest the presence of biologically active principals i.e. flavonoid glycosides, saponins, proteins and carbohydrates. Several flavonoids and saponin glycosides isolated from medicinal plants have been discovered to possess significant analgesic and/or anti-inflammatory effects⁴³. It is, therefore, possible that both the analgesic and anti-inflammatory effects observed with ethanolic extract of the *Eclipta alba* may be attributed to its flavonoids and saponin glycoside components, shown to be present during phytochemical screening. The oral and intraperitoneal LD₅₀ obtained with this plant extract also suggest that it may have a reasonable safety margin with regards to acute toxicity further justifying its wide application in various communities and lack of any reported side effects with the traditional use of this plant.

CONCLUSION

In conclusion, the ethanolic extract of the *Eclipta alba* possesses analgesic and anti-inflammatory properties,

providing a scientific basis for its ethnobotanical use in alleviating pain and inflammatory conditions. The results obtained may indicate the ethnopharmacological basis of the use of *Eclipta alba* in traditional medicine for treating many diseases. This knowledge could be tapped to formulate new agents to treat inflammatory and pain. Still, as this is only preliminary study, further studies are necessary to identify the phytochemicals responsible for the different pharmacological effects.

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Nil

CONFLICTS OF INTEREST

The authors declare that there is no conflict of interest.

ABBREVIATIONS

NSAID: Non-Steroidal Anti-Inflammatory Drugs; **OECD:** Organization for Economic Co-operation and Development; **COX-2:** Cyclooxygenase-2; **5-HT:** 5-Hydroxytryptamine; **IAEC:** Institutional Animal Ethics Committee; **CPCSEA:** Committee for the Purpose of Control and Supervision of Experiments on Animals; **ANOVA:** Analysis of Variance; **SEM:** Standard Error of the Mean.

REFERENCES

- [1] The Wealth of India – Raw Materials Publications and Information Directorate, CSIR, New Delhi, India. 1952; pp.127-128.
- [2] Kritkar, K.R. and Basu, B.D. Indian Medicinal Plants, 2nd edn, Vol II. Bishen Singh Mahendra Pal Singh, Dehradun. 1975; pp. 1361-63.
- [3] Indian Herbal pharmacopoeia Indian Drugs Manufactures Association, Worli, Mumbai, vol-I. 1998; pp. 81-88.
- [4] Chopra R.N. Glossary of Indian Medicinal Plant, Council Of Scientific and Industrial Research, New Delhi, India. 1956; pp.104.
- [5] Roy RK, Thakur M and Dixit VK: Hair growth promoting the activity of *Eclipta alba* in male albino rats. Archives of Dermatological Research 2008; 300: 357-64.
- [6] Wagner H. et al. Coumestans as the main active principles of the liver drugs *Eclipta alba* and *Wedelia calendulaceae*. Planta Med. 1986; 5: 370-74.
- [7] Amritpal Singh, Sanjiv Duggal, Asish Suttee, Jaswinder Singh, Shankar Katekhaye. *Eclipta Alba* Linn. - Ancient remedy with therapeutic potential. 2010; 1(2): 57-63.
- [8] (Jadhav VM, Thorat RM, Kadam VJ, Salaskar KP. Chemical composition, pharmacological activities of *Eclipta alba*. Journal of Pharmacy Research. 2009; 2(8): 1129-1231.
- [9] Upadhyay R.K., Pandey M.B., Jha R.N. and Pandey V.B., *Eclalbatin*, a triterpene saponin from *Eclipta alba*, J. Asian Nat. Prod. Res., 2001; 3(3) 2001; 213-217.
- [10] Jahan R., Al-Nahain A., Majumder S. and Rahmatullah M., Ethnopharmacological Significance of *Eclipta alba* (L.) Hassk. (Asteraceae), Int. Sch. Res. Not., 2014; 1-22.
- [11] Chung I.M., Rajakumar G., Lee J.H., Kim S.H. and Thiruvengadam M., Ethnopharmacological uses, phytochemistry, biological activities and biotechnological applications of *Eclipta prostrata*, Appl. Microbiol. Biotechnol., 2017; 101(13), 5247-5257.
- [12] Jahan R., Al-Nahain A., Majumder S. and Rahmatullah M., Ethnopharmacological Significance of *Eclipta alba* (L.) Hassk. (Asteraceae), Int. Sch. Res. Not., 2014, 11-22.
- [13] Neeraja P.V. and Margaret E., *Eclipta alba* (L.) Hassk: a valuable medicinal herb, Int. J. Curr. Pharm. Rev. Res., 2012; 2(4), 188-197.
- [14] Khairullah A.R., Solikhah T.I., Ansori A.N.M., Hidayatullah A.R., Hartadi E.B., Ramandinianto S.C. and Fadholly A., Review on the Pharmacological and Health Aspects of *Apium Graveolens* or *Celery*: An Update, Syst. Rev. Pharm., 2021; 12(1), 606-612.
- [15] Robbins SL, Cotron RS. Acute and chronic inflammation. In: Kumar V, Abbas AK, Fausto N, editors. Pathologic Basis of Disease. 9th ed. Philadelphia: Elsevier Publications; 2013. p. 29-72.
- [16] Chimenti MS, Triggianese P, Conigliaro P, Candi E, Melino G, Perricone R. The interplay between inflammation and metabolism in rheumatoid arthritis. Cell Death Dis 2015; 6:e1887.
- [17] Siegel D, Devaraj S, Mitra A, Raychaudhuri SP, Raychaudhuri SK, Jialal I. Inflammation, atherosclerosis, and psoriasis. Clin Rev Allergy Immunol 2013; 44:194-204.
- [18] Cruz SM, Balkwill FR. Inflammation and cancer: Advances and new agents. Nat Rev Clin Oncol 2015; 12:584-96.
- [19] Lontchi-Yimagou E, Sobngwi E, Matsha TE, Kengne AP. Diabetes mellitus and inflammation. Curr Diab Rep 2013; 13:435-44.
- [20] Hayliyar J, Macpherson A, Bjarnason I. Gastro protection and nonsteroidal anti-inflammatory drugs. Drug Saf 1992; 4:1323-6.
- [21] Thiagarajan P, Jankowski JA. Aspirin and NSAIDs; benefits and harms for the gut. Best Pract Res Clin Gastroenterol 2012; 26:197-206.
- [22] Das S, Mandal SK. Current developments on anti-inflammatory natural medicines. Neurodegener Dis 2018; 23:24.
- [23] Kokate CK. Practical pharmacognosy. Vallabh Prakashan, Delhi. 1986; pp. 111.
- [24] Harborne JB. Phytochemical methods In: A guide to modern techniques of plant analysis. 3rd ed. UK: Chapman and Hall, ICMR; 1998; p. 89-131.
- [25] Evans WC. Trease and Evans' Pharmacognosy E-Book. United Kingdom: Elsevier Health Sciences; 2009.
- [26] Dora MB, Isabel CG, Julieta V, Fernando A, Andrea G, Jose AF. Neuropharmacological effects of the ethanolic extract of *Sida acuta*. Brazilian Journal of Pharmacognosy. 2016; 26:209- 15
- [27] Khandelwal KR. Practical Pharmacognosy. Nirali Prakashan, Pune 2004; 149-156
- [28] OECD guideline for testing of chemicals. Acute Oral Toxicity Acute Toxic Class Method (425); OECD 2008.
- [29] Ben Ali AA, Chouikh A, Haddad L. *Citrullus colocynthis* fruit resin and *Cyperus rotundus* tuber resin: assessing their analgesic effects with hot plate and writhing tests. Acta Period Technol. 2024;(55):1-12.
- [30] Tadege G, Sirak B, Abebe D, Nureye D. Antinociceptive and anti-inflammatory activities of crude leaf extract and solvent fractions of *Commelina latifolia* Hochst. Ex CB Clarke (Commelinaceae) leaves in murine model. Front Pharmacol. 2023; 14:1284087.
- [31] Isah AO, Idu M, Amaechina FC. Antinociceptive properties of leaf aqueous extract of *Crossopteryx febrifuga*. Fudma J Sci. 2022;6(5):255-8.
- [32] Aburas K, Misbah A, Fehelbum H, Abukhdir A. Analgesic effect by using hot Plat and tail flick test in rats models for Aqueuos *Moringa oleifer* extract. Libyan J Med Res. 2023; 17(1):107-19.
- [33] Kim HY, Lee HJ, Zuo G, Hwang SH, Park JS, Hong JS, et al. Antinociceptive activity of the *Caesalpinia eriostachys* Benth. Ethanolic extract, fractions, and isolated compounds in mice. Food Sci Nutr. 2022; 10(7):2381-9.
- [34] Vogel HG. Discovery and Evaluation Pharmacological Assays. 2nd ed. Germany: Springe; 2008; 1120-64.
- [35] Okpo SO, Fatokun F, Adeyemi OO. Analgesic and anti-inflammatory activity of *Crinum glaucum* aqueous extract. J Ethnopharmacol 2001;78:207-11.
- [36] Handunnetti SM, Kumara RR, Deraniyagala SA, Ratnasooriya WD. Anti-inflammatory activity of *Ixora coccinea* methanolic leaf extract. Pharmacognosy Res 2009; 1:80-90.

- [37] Dutta S, Das S. A study of the anti-inflammatory effect of the leaves of *Psidium guajava* Linn. on experimental animal models. *Pharmacognosy Res* 2010; 2:313-7.
- [38] Xiang L, Huang Q, Chen T, He Q, Yao H, Gao Y. Ethanol extract of *Paridis rhizoma* attenuates carrageenan-induced paw swelling in rats by inhibiting the production of inflammatory factors. *BMC Complement Med Ther*. 2023; 23(1):437.
- [39] Almasarwah SY, Oran SA, Darwish RM. Efficacy of *Nitraria retusa* L. Fruits aqueous and methanol extracts as antioxidant and anti-inflammatory activities on carrageenan-induced paw edema in rats. *Trop J Nat Prod Res*. 2023; 7(4):2725-9.
- [40] Reysov M, Pavlov D, Novakovic M, Tesevic V, Georgieva A, Eftimov M, et al. The flavonoid fustin exerts anti-inflammatory effect in a model of carrageenan-induced paw oedema. *Acta Aliment*. 2023; 52(1):155-62.
- [41] Bimakr M, Rahman RA, Taip FS, Ganjloo A, Salleh LM, Selamat J, et al. Comparison of different extraction methods for the extraction of major bioactive flavonoid compounds from spearmint (*Mentha spicata* L.) leaves. *Food Bioprod Process*. 2011; 89(1):67-72.
- [42] Xu Q, Wang Y, Guo S, Shen Z, Wang Y, Yang L. Anti-inflammatory and analgesic activity of aqueous extract of *Flos populi*. *J Ethnopharmacol*. 2014 ; 152(3):540-5.
- [43] J.A. Duke. *Handbook of Biologically Active Phytochemicals and their Activities*, (CRC Press, Boca, Raton, FL, 1992).