ejpmr, 2017,4(3), 391-399.

SJIF Impact Factor 4.161

EUROPEAN JOURNAL OF PHARMACEUTICAL AND MEDICAL RESEARCH

www.ejpmr.com

Research Article

ISSN 2394-3211

EJPMR

EVALUATION OF ANTI-INFLAMMATORY, ANALGESIC AND ANTIPYRETIC ACTIVITY OF *ECLIPTA ALBA* (LINN.) HASSK. IN EXPERIMENTAL ANIMALS.

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Article Received on 03/01/2017 **Article Revised on 24/01/2017** **Article Accepted on 14/01/2017**

ABSTRACT

Objective: Eclipta alba (L.) Hassk. (Sanskrit: Bhringaraj) found to contain phytochemicals wadelolactone, eclalbatin, ursolic acid, apigenin, ecliptalbine, verazine and α – amyrin having anti-inflammatory and analgesic properties. The aim of this study, therefore, was to evaluate anti-inflammatory, analgesic and antipyretic activity of single doses (250 mg/kg and 500 mg/kg) of ethanolic extract of leaves of *Eclipta alba* (L.) Hassk. using λ - carrageenan induced paw edema in *Wistar* albino rats, hot plate; acetic acid induced nociception in *Swiss* albino mice and gram -ve lipopolysaccharide induce fever in New Zealand white rabbits respectively. **Methods:** Decrease in the rat paw edema volume by herb compared to "disease control" group (0.1 ml of 1 % λ - carrageenan) was taken as the index of anti-inflammatory activity. Prolongation of reaction time before and after thermal nociception (55-56^{0}C) by herb in mice was taken as index of central analgesic activity. Reduction in number of abdominal constrictions produced by acetic acid (0.6% v/v; 10 ml/kg i.p.) in mice by herb was taken as index of peripheral anlagesic activity. Anti pyretic activity was assessed by decrease in gram -ve lipopolysaccharide (0.2 µg/kg i.v.) induced rectal temperature by herb in the pyretic rabbits. **Result:** Single dose of 500 mg/kg Eclipta alba produce statistically significant anti-inflammatory and almost similar central analgesic activity with disease control and morphine sulphate (5mg/kg s.c.) respectively. The extract did not shown peripheral analgesic and antipyretic activity. **Conclusion** – Ethanolic extract of leaves of *Eclipta alba* has anti-inflammatory and central antinociceptive activity.

KEYWORDS: Anti-inflammatory, analgesic, antipyretic, disease control, *Eclipta alba* (L.) Hassk.

INTRODUCTION

Inflammation is the local defensive response of tissue to injury evoked by noxious agents, infections, antibodies, physical injuries. The ability of a body to mount an inflammatory response is essential, as it is a protective attempt by the organism to remove the injurious stimuli as well as initiate the healing process for the tissue. Uncontrolled and persistent inflammation leads to many chronic diseases and destruction of the tissue. The classic signs of inflammation such as pain, redness, swelling and loss of function are produced by inflammatory mediators such as vasoactive amines (histamine, 5-hydroxytryptamine, neuropeptides), metabolites via COX pathway (prostaglandins, prostacyclin, resolvins), metabolites via lipo-oxygenase pathway (LTB_4, lipoxins), cytokines (IL-1β, IL-8, TNF-α, TNF-β, IFN-γ, chemokines), PAF, free radicals (Oxygen metabolites, NO). Inflammatory mediators - bradykinin, 5-HT, ATP, nerve growth factor, LTs and PGs, released from non-neuronal cells during tissue injury increase the sensitivity of nociceptors and potentiate pain perception. Moreover cytokines (IL-1, IL-6 and TNF- α) and PGE2 (Arachidonic acid pathway) elevates hypothalamic set point causing pyrexia. Hence, the screening methods for the evaluation of anti-inflammatory, and analgesic are aimed to counter these mediators.

Currently used analgesics and anti-inflammatory medicines carry potential risk of developing heart attack, stroke, severe gastritis, peptic ulcer, salt and water retention, blood dyscrasias, renal and hepatic dysfunction. Moreover all current antipyretics are liable to cause hepatic dysfunction in vulnerable patients. In recent years, many herbs and Phytopharmaceuticals are investigated which might serve as lead for the development of safer anti-inflammatory agent.

In the present study Eclipta alba (L.) Hassk. (Syn. Eclipta prostrate) was selected because of its medicinal values in alternative system of medicine, Ayurveda. It is a member of family Asteraceae, is a annual herb found as a common weed across waste places, marshy lands, roadsides and lakes, particularly in more tropical parts of the country. In India it is known as bhangra, bhrungraja and bhringraja etc. means 'King of Hair', clearly referring to its traditional reputation in Ayurveda as an herb supporting hair growth. Recently fix drug combination (FDC) - Tenofovir Disoproxil Fumarat 300 mg - Phyllanthus Urinaria 300mg - Adenosma Glutinosum 150mg - Eclipta Prostrata 150mg, Ascorbic acid 500 mg daily is in phase III clinical trial for the treatment of Chronic and Acute Hepatitis B in USA (Clinical trials.gov - NCT01198860). It is one of the active ingredient of many herbal formulations prescribed for liver ailments and hair vitalizer.[1,2] It contains bioactive compounds wedelolactone, stigmasterol, desmethylwedelolactone-7-glucoside, triterpene saponin, eclalbatin, ursolic acid, oleanolic acid[3], responsible for hepatoprotective, antihaemorrhagic[4], anticancer[5], antidiabetic[6], anti-arthritis[7] activities. Past studies showed that coumestan - wedelolactone present in *Eclipta alba* (L.) Hassk. found to inhibit 5-lipoxygenase[8] and protein expression levels of nitric oxide synthetase (iNOS) and COX-2 in lipopolysaccharide-stimulated cells, thus inhibiting production of NO and PGE_2.[9] Although few reports of anti-inflammatory, analgesic activities of *Eclipta alba* (L.) Hassk. have been reported.[10,11] Antipyretic activity was not studied in the past. Hence the present study aimed to evaluate the anti-inflammatory, analgesic and antipyretic effects of *Eclipta alba* (L.) Hassk. in selected animal models.

EXPERIMENTAL

Materials

Ethanolic powdered extract of the leaves of *Eclipta alba* (L.) Hassk. was procured from local market and identified by botanist. Morphine sulphate, diclofenac sodium injections and paracetamol drops (Troikaa and Hema laboratories, India, respectively) were purchased. λ-carrageenan (Sigma chemical Co. St Louis, USA), 100% acetic acid (Merck Specialties Private Limited, Mumbai) and lipopolysaccharide from Escherichia coli 0111:B4 (Sigma chemical Co. St Louis, USA) were of AR grade.

Animals

The study was conducted at the Department of Pharmacology, Government Medical College, Bhavnagar, Gujarat. The study protocol was approved by the Institutional Animal Ethics Committee of the same institute. All experiments were conducted according to CPCSEA (Committee for the Purpose of Control and Supervision of Experiments on Animals) guidelines for animal experiments.

Wistar rats (150-250 g), *Swiss* albino mice (20-40 g) and New Zealand white rabbits (1.5-2 kg) of either sex, were housed in stainless steel cages at room temperature (24 ± 2^0C) in 12 h light-dark cycle and provided standard laboratory food and water *ad libitum*. After one week acclimatization, animals were kept overnight fasting and weighed before the experiment. Animals were divided into four groups (two control and two test groups) with eight animals in each group.

Doses were selected according to the previous acute toxicity studies as per OECD guidelines. No adverse effect and mortality was detected in Wistar albino rats and *Swiss* albino mice up to 2gm/kg and 4 gm/kg p.o. of *Eclipta alba* at 1, 4 and 24 h after the treatment.[12] For rabbits, dose was calculated by applying the conversion factor 27.8.[13]

METHODS

Anti-inflammatory study

Carrageenan induced paw edema model: Acute anti-inflammatory activity of *Eclipta alba* 250 and 500 mg/kg was evaluated with slight modifications in the method, described by Winter *et al*. 1962.[14] Four groups of albino rats (n=8) were randomly distributed in disease control, active control and test (Eclipta *alba* 250 and 500 mg/kg) groups. Mark was made on both hind paws at lateral malleolus so that the paw could be dipped in the large diameter mercury column of the plethysmometer up to the mark to ensure constant paw volume measurement.

The initial paw volumes of each animal were measured by mercury plethysmometer. Disease and active control group received distilled water (10 ml/kg p.o.) and diclofenac sodium (10 mg/kg *i.p*.) respectively. Low and high dose test groups were given 250 and 500 mg/kg p.o. powdered extract of *Eclipta alba* respectively.

After 30 min of treatment, 0.1 ml of 1% freshly prepared a suspension of λ - carrageenan in distilled water was injected s.c. into right hind paw of each rat. The paw volume (ml) was measured up to a fixed mark on the lateral malleolus at 0 min (i.e. before carrageenan injection) 30, 60, 120 and 180 min using mercury filled plethysmometer. The difference between the initial and subsequent values gave the actual edema which was compared with control. Then percentage reduction in paw edema (anti-inflammatory activity) was calculated for each group at 180 min with respect to disease control group, according to the following formula.[15]

$$\% \text{ reduction in edema} = V_c - V_t / V_c \times 100$$

V_c = Mean edema in untreated disease control group.
V_t= Mean edema in drug treated group.

Decrease in the paw volume compared to disease control was taken as the index of anti-inflammatory activity of test drugs.

Analgesic studies

Central and peripheral analgesic activity was assessed in thermally and chemically induced pain using hot plate and acetic acid respectively.[16, 17]

Hot plate method

The method of was used to evaluate temperature withstanding power of prescreened *Swiss* albino mice. Mice with baseline latencies of more than 15 seconds were eliminated from the study. Four groups of selected mice (n=8) were randomly formed. The groups were treated as control (distilled water 10ml/kg, p.o.) and standard (morphine sulphate 5mg/kg i.p.) while test groups received 250 and 500 mg/kg *Eclipta alba* orally. Mice were placed in a glass beaker, placed on a thermostatically controlled hot plate (Inco. Ambala, India) maintained at 55-56^{0}C produces two behavioral components paw licking & jumping that was measured in terms of their reaction time, which was recorded before (0 min.) and after 20, 60, 90 and 120 min of morphine sulphate and *Eclipta alba* (L.) Hassk. administration by a stop watch. The cut-off time was set at 15 sec. to prevent damage to the paws. The mean reaction time for each treated group was calculated and compared with that obtained at 0 min.[18]

Percentage increase in reaction time (I %) was calculated, using the formula:

$$I\ \% = \{(R_t - R_{0)} / R_0\} \times 100$$

R_t = Mean reaction time at time t.
R_0 = Mean reaction time at time zero (0 hr).[19]

Acetic acid induced writhing method

The treatment and groupings of mice was done in the same manner as has been done in hot plate method except that standard group received morphine sulphate 5 mg/kg s.c. Acetic acid solution 0.6% v/v (10ml/kg) was injected by intraperitoneal route 30 min after treatment. Number of writhes (index of pain reaction against chemical which includes turning of trunk and extension of hind limbs, abdominal muscle contraction, so that ventral part of the mouse touches the floor) was counted after 5 min of administration of acetic acid for 20 min.[13, 20]

Analgesic activity was indicated by the reduction in the mean of the number of abdominal constrictions in the test groups compared with the control group (Table 3).

Percentage of inhibition of writhing is calculated by the following formula:

Percentage inhibition of writhing -

$$(W_c - W_t / W_c) \times 100$$

Where W_t represents the mean number of writhes in mice treated with test drug and W_c represents the mean number of writhes in a control group of mice.

Study of antipyretic activity

Gram −ve lipopolysaccharide induced pyrexia: The rabbits are allowed to adapt to the cages for 60 min. Rabbits have been found to be highly sensitive to the pyrexigenic effect of lipopolysaccharide obtained from gram −ve bacteria, E. coli. 0.2 µg/kg gram −ve lipopolysaccharide (LPS) administered intravenously, into the marginal ear vein of all rabbits.[21]

Study groups and method

New Zealand white rabbits were divided into four groups of eight each and allowed to adapt in suitable stainless steel cages for 60 min. The basal rectal temperature of all rabbits was measured by lubricated 12 probe telethermometer (Inco. Ambala, India) inserted at least 5 cm into the rectum. The rabbit was excluded from the study if the baseline temperature was not within the range of 38.4 – 39.6°C. Thereafter, 0.2 µg/kg gram –ve lipopolysaccharide (LPS) was administered intravenously, into the marginal ear vein of all rabbits. After 60 min vehicle and active control groups of rabbits received distill water and paracetamol (150 mg/kg p.o.) suspension, while test groups received 250 and 500 mg/kg *Eclipta alba* orally.

Rectal temperature was measured every 30 min. up to 180 min (Post Pyrogen Administration). A decrease of body temperature for at least 0.5°C for more than 30 min as compared with the temperature value before administration of the test compound is regarded as a positive effect. Decrease in body temperature after drug administration in the pyretic rabbits was taken as the index of anti pyretic activity.[22]

Statistical analysis

It was carried out using Graph Pad Prism 5.0 (Trial Version). All results were expressed as mean ± standard error of mean (SEM). All parametric data were analyzed by One-way ANOVA followed by Dunnet's Multiple Comparison Test and non-parametric data were analyzed by Kruskal-Wallis test followed by Dunn's Multiple Comparison Test to determine the significance of the difference between the control group and experimental animals treated with test drug for anti-inflammatory, analgesic and antipyretic groups. $P<0.05$ was considered statistically significant.

RESULTS

Anti-inflammatory activity

Table 1 shows single dose (500 mg/kg p.o.) of *Eclipta alba* (L.) Hassk produced delayed but significant inhibition of paw edema as compared to disease control at 180 min. similar to that produced by single dose of diclofenac sodium (10 mg/kg i.p.). Single low dose (250 mg/kg p.o.) of *Eclipta alba* (L.) Hassk do not show any anti-inflammatory effect.

Hot plate method

Table 2 shows that test herb in the dose of 500mg/kg p.o. showed maximum analgesic response at 90 min. and comparable to morphine sulphate (5 mg/kg s.c.), but it did not shown significant analgesic effect when compared to vehicle control group. Moreover lower dose (250 mg/kg p.o.) did not show any significant analgesic effect in this thermal nociception model.

Acetic acid induced writhing method

Table 3 shows single dose (5mg/kg s.c.) morphine sulphate significantly reduced a number of writhes due to acetic acid (10 ml/kg of 0.6% v/v). The degree of inhibition i.e. analgesic effect was found 81.58, 38.86 and 30.30% by morphine sulphate (5 mg/kg, s.c.), 250 mg/kg *Eclipta alba* (L.) Hassk. and 500 mg/kg *Eclipta alba* (L.) Hassk. respectively when compared with vehicle control group. Single doses (250 mg/kg and 500 mg/kg) of *Eclipta alba* (L.) Hassk. extract did not significantly reduce acetic acid induce writhing in *Swiss* albino mice.

Antipyretic activity

Table 4 shows intravenous injection of lipopolysaccharide (0.2 µg/kg) from *E. coli* increased the rectal temperature in vehicle control group upto 39.72°C at 180 min (i.e. rise of 1.08°C from baseline value). Single oral doses (250 mg/kg and 500 mg/kg) of *Eclipta alba* (L.) Hassk. does not show significant antipyretic effect upto 300 min when compared with vehicle and active control groups.

Table 1: Effect of *Eclipta alba* (L.) Hassk. in λ- carrageenan induced paw edema in *Wistar* albino rats (n = 8).

Groups	Dose / Route	λ- carrageenan-induced paw edema Paw volume (ml)					Percentage inhibition at 180 min
		0 Min	30 Min	60 Min	120 Min	180 Min	
Disease Control (λ- carrageenan 1 %)	0.1 ml s.c.	1.16 ± 0.03	1.31 ± 0.04	1.38 ± 0.06	1.38 ± 0.05	1.34 ± 0.05	00.00
Active Control (Diclofenac)	10 mg / kg i.p.	1.02 ± 0.05	1.03 ± 0.15	1.11 ± 0.06**	1.06 ± 0.05**	1.01 ± 0.04**	24.62
Low Dose *Eclipta alba*	250 mg / kg oral	1.03 ± 0.05	1.40 ± 0.27	1.41 ± 0.03	1.37 ± 0.03	1.33 ± 0.03	0.74
High Dose *Eclipta alba*	500 mg / kg oral	1.09 ± 0.04	1.26 ± 0.04	1.23 ± 0.04	1.20 ± 0.02*	1.11 ± 0.03**	17.16
One way ANOVA F (df)		2.47 (3,28)	3.34 (3,28)	7.76 (3,28)	14.48 (3,28)	17.22 (3,28)	
P value		0.0822	0.0331	0.0006	< 0.0001	< 0.0001	

Values are expressed as mean ± SEM.

*P< 0.05, **P < 0.01, ***P < 0.001 vs. disease control by One-way ANOVA followed by Dunnet's Multiple Comparison test.

Table 2: Effect of *Eclipta alba* (L.) Hassk. on prolongation of reaction time in *Swiss* albino mice by hot plate (55-56^{0}C) method (n = 8).

Groups	Dose / Route	Mean reaction time in seconds at baseline after drug administration (Mean ± SEM)					Prolongation of the reaction time (%)			
		0 min	20 min	60 min	90 min	120 min	20 min	60 min	90 min	120 min
Vehicle Control Dist. Water	10 ml/kg oral	1.25 ± 0.16	1.37 ± 0.3	1.62 ± 0.18	1.50 ± 0.19*	02 ± 0.27	9.6	29.6	20	60
Active Control (Morphine)	5 mg/kg s.c.	1.50 ± 0.19	03 ± 0.27**	3.62 ± 0.32 *	3.12 ± 0.30	2.12 ± 0.12	100	141.33	108	41.33
Low Dose *Eclipta alba*	250 mg/kg oral	1.62 ± 0.32	1.75 ± 0.49#	02 ± 0.87 @@	1.37 ± 0.42@@	1.12 ± 0.12#	8.02	23.45	15.43	44.64
High Dose *Eclipta alba*	500 mg/kg oral	1.29 ± 0.18	2.25 ± 0.56	2.88 ± 0.67	3.75 ± 0.77##	1.50 ± 0.27	74.41	123.25	190.69	72.41
P value		0.7159	0.0059	0.0036	0.006	0.0069				

Kruskal –Wallis Test followed by Dunn's Multiple Comparison Test (Non-parametric ANOVA) was applied.
At 20 min = **P < 0.01 vs. vehicle control; #P < 0.05 vs. morphine
At 60 min = *P < 0.05 vs. vehicle control; @@P < 0.01 vs. morphine
At 90 min = * P < 0.05 vs. morphine; *P < 0.05 vs. high dose *Eclipta alba* @@ P < 0.01 vs. morphine; ## P < 0.01 vs. low dose *Eclipta alba*
At 120 min = #P < 0.05 vs. morphine.

Table 3: Effect of *Eclipta alba* (Linn.) Hassk. in inhibiting 0.6% v/v acetic acid (10 ml/kg, i.p.) induced writhing in mice.

Groups	Dose / Route	Number of wriths in 20 min	Analgesic effect (% inhibition)
Vehicle control Distill Water	10 ml / kg Oral	24.75 ± 1.14	00.00
Active control Morphine sulphate	5 mg / kg Subcutaneous	13.63 ± 0.77**	81.58
Low dose *Eclipta alba*	250 mg / kg Oral	15.13 ± 1.38**	38.86
High dose *Eclipta alba*	500 mg / kg Oral	17.25 ± 0.94**	30.30

Values are expressed as mean ± SEM. **P < 0.01 vs. vehicle control.
One-way ANOVA followed by Dunnet's Multiple Comparison test.

Table 4: Effect of *Eclipta alba* (L.) Hassk. in reducing lipopolysaccharide (LPS; 0.2 µg/kg i.v.) induced pyrexia among the study groups of New Zealand white rabbits (n=8)

Groups	Dose Oral	Temperature in ^{0}C										
		0 min	30 min.	60 min.	90 min	120 min	150 min	180 min	210 min	240 min	270 min	300 min
Vehicle Control (D.W.)	0.2 ml/ kg	38.48 ± 0.10	38.78 ± 0.12	39.01 ± 0.10	39.20 ± 0.10	39.34 ± 0.10	39.49 ± 0.13	39.73 ± 0.16	39.59 ± 0.13	39.64 ± 0.14	39.63 ± 0.11	39.56 ± 0.09
Active Control (Paracetamol)	150 mg/kg	39.05± 0.15	39.01 ± 0.20	39.03 ± 0.14	39.16 ± 0.10	39.13 ± 0.13	39.53 ± 0.20	39.69 ± 0.19	39.45 ± 0.24	39.39 ± 0.24	39.49 ± 0.20	39.36 ± 0.15
Low Dose *Eclipta alba*	250 mg/kg	38.98 ± 0.17	39.41 ± 0.17	39.03 ± 0.14	39.58 ± 0.14	39.75 ± 0.16	39.84 ± 0.16	39.79 ± 0.15	40.03 ± 0.08*	39.94 ± 0.08	39.69 ± 0.08	39.56 ± 0.10
High Dose *Eclipta alba*	500 mg/kg	38.96 ± 0.12	39.09 ± 0.12	39.20 ± 0.15	39.25 ± 0.20	39.36 ± 0.12	39.44 ± 0.12	39.31 ± 0.10	39.56 ± 0.05	39.60 ± 0.08	39.43 ± 0.06	39.21 ± 0.07
One way ANOVA F (df)		1.721 (3,28)	1.958 (3,28)	0.390 (3,28)	Kruskal-Wallis Test	Kruskal-Wallis Test	Kruskal-Wallis Test	1.826 (3,28)	Kruskal-Wallis Test	2.232 (3,28)	0.8795 (3,28)	2.380 (3,28)
P value		0.1854	0.1432	0.7608	0.1197	0.0309	0.1333	0.1654	0.0239	0.1064	0.4636	0.0908

Value are expressed as mean ± SEM.
*P < 0.05 vs. paracetamol (active control) by Kruskal –Wallis test.
One-way ANOVA followed by Dunnet's Multiple Comparison test (Parametric data).
Kruskal-Wallis test followed by Dunn's Multiple Comparison test (Non-parametric data).

DISCUSSION

Anti-inflammatory activity

Though modern medicine gained a lot of pharmacologically active agents from plants, a potential of medicinal plants as a source of new medicines is still largely unexplored. Thus the search for novel anti-inflammatory, analgesic and antipyretic agents from medicinal plants resources is intensifying. Anti-inflammatory drugs are judged clinically by their effect on the pain, stiffness or swelling of the affected part, the action on swelling being the most objectively measured observation.[23]

In the present study anti-inflammatory activity of *Eclipta alba* (L.) Hassk. was studied using highly reproducible, standard in-vivo λ - carrageenan induced rat paw edema (measured by a manual plethysmometer) model.[24] λ – carrageenan induces a local inflammation by increased vascular permeability, edema, and neutrophil extravasation, similar to the pathology of inflammation.[25,26]

In this model early phase (0 - 1.5 hr) is mediated by histamine, serotonin and increase of prostaglandin synthesis in the surroundings of the damaged tissues.[27] Middle phase (1.5 – 2.5 hr) is due to kinin like substances followed by late phase (>2.5 hr) mediated by leukotrienes, bradykinin, TNF-α, IL-1β, IL-6, protease, lysosome, prostaglandins produced in tissue macrophages.[28,29] Past studies shows λ-carrageenan could cause the production of COX-2 in λ-carrageenan induced paw edema model.[30,31]
In the present study *Eclipta alba* (L.) Hassk., 500 mg/kg p.o. produce significant late phase anti-inflammatory activity, whereas reference drug 10 mg/kg diclofenac sodium i.p. produce middle and late phase anti-inflammatory activity (Table 1). It suggests that 500 mg/kg of extract containing wadelolactone possibly acts by inhibiting production of COX-2, iNOS and 5-LOX.Similar studies at the dose of 200 mg/kg and using Zeitlin's apparatus unable to show anti-inflammatory activity.

In the present study, carrageenan was administered in the right hind paw of vehicle control group to make it disease control group resembling pathology of inflammation. According to Gupta SK (2009) in control group animals only vehicle is injected, but in the present study we compare decrease in paw edema by *Eclipta alba* (L.) Hassk. with disease control group made by administering λ - carrageenan in right hind paw.[32,33]

Past phytochemical studies discovered the presence of coumestan - wedelolactone in leaves of *Eclipta alba* which inhibit 5 - lipoxygenase enzyme in, *in-vitro* porcine - leukocytes test.[8] Wedelolactone also found to inhibit protein expression levels of nitric oxide synthetase (iNOS) and COX-2 in lipopolysaccharide - stimulated cells, thus inhibiting production of NO and PGE_2.[9] Leaves of *Eclipta alba* also contains phytosterol – stigmasterol which is capable of inhibiting molecular targets of pro-inflammatory mediators in inflammatory responses.[34,35]

The volume of distribution of diclofenac sodium can be predicted after intravenous or oral administration of drugs in rats.[36,37] Pre-clinical pharmacokinetic data on diclofenac on oral administration shows that it is metabolized extensively in liver, resulting in 50 – 60% bioavailability and reach peak serum concentration 1 - 2 hr after oral dosing. With this background, in present study active control diclofenac is given intraperitoneally to increase bioavailability and rapid serum concentration. Since ethanolic extract of *Eclipta alba* is found to have cyclo-oxygenase, 5 – lipoxygenase and pro-inflammatory transcription factors inhibitory activity, it is possible that the anti-inflammatory and analgesic activity of the extract may involve, mainly, inhibition of PG synthesis.

Anti-nociceptive effect

The evaluation of the anti-nociceptive activity of *Eclipta alba* (L.) Hassk. is perform by using a hot plate (thermal heat) and writhing test (peritoneal test). The central anti-nociceptive effect of *Eclipta alba* (L.) Hassk. was evaluated by providing thermal heat, 55-56^{0}C (nociceptive stimulus) to the paws of mice which are highly sensitive to heat at temperatures which do not damage their skin. Hot plate test is commonly used to study the ability of experimental animals to tolerate brief, high-intensity noxious stimuli (supraspinal level) similar to clinical pain.[38] The time required for the onset of pain responses in mice is prolonged only by centrally acting analgesics.[39] Prolongation of reaction time is the usual measure to assess the anti-nociceptive effect.

Eclipta alba (L.) Hassk. do not shows central and peripheral analgesic activity than vehicle control group in thermal model. The peripheral antinociceptive activity of *Eclipta alba* was evaluated by intraperitoneal administration of 0.6% acetic acid.[40] Inhibition of "stretching" or decrease in no. of writhes is considered as an analgesic effect.[17] Compounds with less than 70% inhibition were considered to have minimal analgesic activity.[41]

Paste of leaves of eclipta alba (L.) Hassk. was applied to treat swelling in Asian sub-continent.[42] 200 mg/kg Eclipta alba previously shows central and peripheral analgesic effect by hot plate (54 $\pm$ 0.5°C) and writhing method respectively.[43] Present study 500 mg/kg extract shows equianalgesic effect like morphine at 90 min – suggest spinal or supraspinal mechanism.

Contradictory one more similar study at 1.5 and 2 gm/kg Eclipta *alba* (L.) Hassk. does not report any analgesic activity.[12]

The peritoneal test is sensitive to weak analgesics, at dose levels that may be inactive in the hot-plate test.[44,45] Phlogistic agents are thought to stimulate local peritoneal receptors found on the surface of the cells lining the peritoneal cavity[46] The abdominal constrictions (hyperalgesia) observed in this test is attributed to the generation of PGE_2 by a local inflammatory response of the peritoneal cavity caused by administration of the acetic acid. PGE_2 level reaches to peak at 15 min and equal to PGF2-α (antagonise pain produce by PGE_2) at 90 min.[47,48] The increase in prostaglandin levels within the peritoneal cavity then enhances inflammatory pain by increasing capillary permeability.[21]

Previous phytochemical studies discovered that anti-nociceptive activity of *Eclipta alba* (L.) Hassk. due to presence of triterpene saponin (Eclalbatin and α-amyrin) and alkaloid (Ecliptine, nicotine, verzine).[49.50] Leaves of *Eclipta alba* (L.) Hassk. did not shown to contain any of these phytochemicals responsible for analgesic effect.

The opioid agents exert their analgesic effects via supra spinal and spinal receptors. Narcotic analgesics are effective in all anti-nociceptive tests.[51] Morphine is administered here as reference standard drug as morphine is more effective in abolishing peritoneal nociception may be a dense distribution of opioid receptors in enteric neurons.[52]

When peritoneum is irritated rapidly or responses are counted for a shorter period (20 min) it lessens the inflammatory component of the reaction and aspirin is less active than usual more prolonged tests.[52] In the present study peritoneal pain is produced rapidly by 10 ml/kg (higher dose) of 0.6% acetic[53] instead of 0.1 ml of a 0.6% acetic acid in previous studies and writhes were counted for 20 min despite 30 min[54] or 45 min.[55]

In experimental nociception, drug is given before the noxious stimulus. Such tests measure the efficacy of the drug to increase the minimal stimulus required to elicit nociceptive response or pain. Increased permeability of blood vessels accompanies peritoneal irritation and so an antinociceptive effect cannot easily be distinguished from an anti-inflammatory action.[51] Hot plate method has the drawback that sedatives and muscle relaxants or psychotomimetics cause false positives, while mixed opiate agonists-antagonists provide unreliable results.[56] The validity of the test has been show even in the presence of substantial impairment of motor performance.[57]

Extract was unable to produce any peripheral anti-nociceptive activity, may be due to a higher dose (10 ml/kg of 0.6% acetic acid) of acetic acid used and absence of anti-nociceptive phytochemicals in the leaves of *Eclipta alba*.

Eclipta alba has high therapeutic and medicinal values as it contains wedelolactone, desmethylwedelolactone, 14-hepatocosanol, luteolin-7-0-glucoside, alkaloids and polypeptides as principle components. Because of its varied medicinal values, it has great commercial demand which calls for further investigation at the biomolecular level. Further work is required to identify molecular mechanisms underlying the anti-inflammatory and anti-nociceptive activity and also, a selectivity of COX-1 and COX-2 inhibitory activity of the active principles of the extract.

Antipyretic Activity

Most anti inflammatory and analgesic drugs usually possess antipyretic activity. NSAIDs produce antipyretic action through inhibition of PGE_2 in the hypothalamus. Antipyretic activity is a characteristic of drugs or compounds which have an inhibitory effect on prostaglandin biosynthesis.[58] To evaluate antipyretic activity in other medicinal plants, yeast was given intraperitoneally and LPS intravenously to induce fever in rabbits and rectal temperature was recorded every hr up to 15 to 18 hr after yeast injection and every 1 hr up to 4 hr after LPS injection respectively.

Lipopolysaccharide (LPS) from gram –ve bacteria e.g. E. coli induces fever in rabbits after i.v. injection. Measurement of body temperature in rabbits with polysaccharide induced fever a decisive test for the absence of pyrogens in parenteral drugs by several pharmacopoeias such as USP 23 (1955). In the rabbit two maxima of temperature increases are observed. The first maxima occur after 70 min and second after 3 hr.[21] In the present study anti-pyretic effect of ethanolic extract of *Eclipta alba* (L.) Hassk was investigated through LPS induced pyrexia test in rabbit. *Eclipta alba* (L.) Hassk. does not show the significant antipyretic effect up to 300 min as compared with vehicle and active control group (Table 4).

CONCLUSIONS

1. Ethanolic extract of leaves of *Eclipta* alba (L.) Hassk. in the oral dose of 500 mg/kg show anti-inflammatory and central anti-nociceptive activity.
2. Ethanolic extract of leaves of *Eclipta* alba (L.) Hassk in the oral dose of 250 mg/kg and 500 mg/kg did not shown peripheral anti-nociceptive and antipyretic activity.

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