Article

An Experimental Evaluation of Analgesic and Anti-inflammatory activity of Asthma plant leaves

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ABSTRACT

Inflammation is complex biological response against harmful stimuli such as microbes, bacteria or irritants and it is a protective response. Inflammation is divided into acute, sub-acute and chronic patterns. There are several mediators of inflammation which is originate from cells or plasma such as histamine, prostaglandins, leukotrienes, cytokines, bradykinin, Tachykinins and nitric oxide etc. *Euphorbia hirta*L. is a traditional medicine belonging to the family Euphorbiaceae. It is commonly known as duddhi, baradudhi and asthma weed. Traditionally, it is used in therapy of cancer, bronchitis, dysentery, asthmatic problems, helminthes infestations and kidney stones etc. Hence the objective of this research study is to conclude anti-inflammatory and analgesic activity of aqueous extract of *E.hirta*L. in wistar albino rats. Anti-inflammatory activity was studied by carrageenan induced paw edema method and analgesic activity was studied by Hot plate & Acetic acid induced writhing method.The leaves of *E.hirta* L. were shade dried, powdered and extracted with the help of water by cold maceration method. The phytochemical evaluation of plant leaves was done as per WHO guidelines. The phytochemical study confirms the existence of alkaloids, glycosides, terpenoids, flavonoids, saponins and tannins. For pharmacological evaluation of anti inflammatory and analgesic activity male wistar rats were used. Ibuprofen and Diclofenac sodium was used as standard drug. Crude aqueous extract was given orally at different doses of 100mg/kg, 300mg/kg and 500mg/kg in wistar rats 30mins before carrageenan injection. The volume of paw edema was measured by using plethysmometer at 1, 2 and 3 hr after carrageenan administration. Analgesic activity E.hirta L. was measured at different time intervals after the drug administration.The anti-inflammatory and analgesic activity of crude drug extract was exhibited a remarkable dose dependent reduction in inflammation and pain respectively. The inhibitory effect was highest ($p<0.01$) at 500mg/kg dose of drug. Extract and ibuprofen showed 53% and 64% inhibition of paw edema respectively, at 3hr after carrageenan administration. E.hirta L. showed significant analgesic activity ($p<0.05$) in all two models used to evaluate analgesic activity as compared to saline treated group.E.hirta L. leaves aqueous extract has a strong analgesic and anti inflammatory activity. These consequences help in the traditional uses of the plants and in the development of new treatments.

Key Words: Aqueous extract, flavonoids, Anti inflammatory activity, analgesic activity.

INTRODUCTION

Herbal medicines categorized into four main classes depending on their evaluation, source and the system of currently in use.

Class I Indigenous herbal remedies: historically used in a local community.

Class II Herbal remedies in systems: Since it has been used and reported with its related concepts for a long time, approaches that are approved by nations.

Class III Modified herbal remedies: These are natural drugs as depicted in classes 1 and2 aside from that they have been altered somehow or another either shape or structure including portion, measurement structure and strategies for the preparation of medicines.

Class IV Imported products with a medicinal herb base: This class includes all foreign medicinal herbs and raw materials. In various countries, herbal remedies must be licensed and promoted[1].

The Principles of Ayurvedic medication and the herbal plants employment of spices are contained in large number of graceful songs in the Rigveda. A medical Practitioner of traditional systems would collect the raw matter, process them and dispense them to own patients. During a good synchronization between the quality of raw material, processed materials and the finished products it is important to begin with regardless dependable, explicit and delicate quality control techniques utilizing a modern methods of instrumental analysis. The utilization of plants in conventional medication frameworks of numerous societies has been broadly reported. These plant-based frameworks keep on assuming a basic part in medical services and the World Health Organization assesses that 80% or the universes occupants keep on depending primarily on standard medications frameworks for their medical services[2].

Chemical constituents of herbal plants are caused by growing, processing, sale and supply conditions. Biological, hereditary and ecological factors influence biochemical and secondary development of metabolites in plants (photoperiods, temperature, Conditions of soil, accessibility of nutrients and moisture). The secondary metabolite content often varies depending on the time the material is harvested, stored, dried, extracted and finished. The production of herbal medicines requires a good understanding of plant systems, including natural, synthetic, genetic and industrial perspectives. The vital part of clinical preliminaries is the consistency confirmation of botanicals and herbal remedies. The declaration also rely upon different factors e.g. identity, moisture content, testing of chemical materials, impurities in metals, microbial parameters, carcinogens, insecticides andothers[3,4].

It is unpredictable response to harmful species for example, microorganisms and harmed necrotic cells that comprise of vascular reactions, relocation & initiation of leukocytes and systemic responses. The inflammation responses consist of two main parts, vascular responses and cellular reactions. It is classified in to acute, sub acute and chronic patterns. The vascular responses and cellular responses of acute, sub acute and chronic inflammations are moderated by synthetic factors that are obtained from plasma proteins or cell and are generated in response of inflammatory stimulus[5].

Sub-acute inflammationlasts for few weeks or more. It is longer as compared to acute inflammation.Exudative just as proliferative changes ofintense and ongoing inflammation are

available.Exudates chiefly comprise of plasma cells, histocytes, fibroblasts, eosinophils andlymphocytes[6]. Chronic inflammationis defined as longer process. In this process tissue damage and inflammation takes place at same time. It is divided into two types. Non specific and Specific inflammation[7].

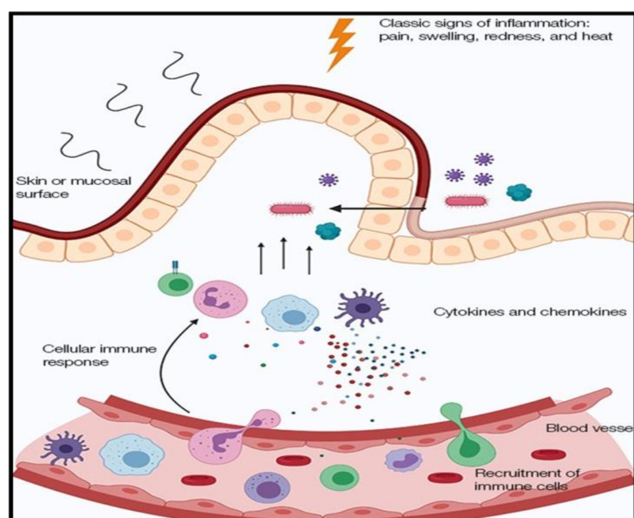

Fig. 1 Initiation of inflammation

*Euphorbia hirta*L. is a traditional medicine belonging to the family Euphorbiaceae. It is commonly known as duddhi, baradudhi and asthma weed. Traditionally, it is used in therapy of cancer, bronchitis, dysentery, asthmatic problems, helminthes infestations and kidney stones etc. Hence the objective of this research study is to conclude anti-inflammatory and analgesic activity of aqueous extract of *E.hirta*L. in wistar albino rats. Anti-inflammatory activity was studied by carrageenan induced paw edema method and analgesic activity was studied by Hot plate & Acetic acid induced writhing method.

MATERIALS AND METHODS

Plant material collection

The leaves of *E.hirta* L. was collected in September 2020 from the campus of Arya College of Pharmacy, Kukas, Jaipur, Rajasthan, India. The plant was authenticated from Rajasthan University, Jaipur. A voucher specimen of the plant was preserve in Rajasthan University, Jaipur for future references.

Chemicals used

Hydrochloric Acid, Molish Reagent, Benedict Reagent, Barford Reagent, Selwinoff's Reagent, Millon's Reagents , Ninhydrin Reagents, Ferric Chloride, 3,5-Dinitro Benzoic Acid, Sodium Hydroxide , Dil. Hcl, Picric Acid, Wagner's Reagent, Dragendroff's Reagent, Mayer's Reagent , acetic anhydride, concentrated sulphuric acid, Phosphate Buffer, Quinine Hydrochloride, Arsenic Standard Solution, Potassium Iodide(1M), Zinc Granules 10 gm, Ox Hide Powder, Ethanol 95%, Calcium Chloride, Methanol, Benzene, chloroform, Acetone.

Research equipments/facilities required

Equipments required- Microscope, Camera Lucida, Stage and Occular Micrometer, Cover Slip, Watch Glass, Digital Camera, Sieve No. 250 Sieve No. 100,Ash Less Filter Paper, silica Crucible, Single Pan Balance, Muffle Furnace, Desiccator , Reflux Condenser, Filter Paper, Hot Air Oven, Arsenic determination assembly, Test Tubes 20, UV chamber, Vacuum assembly , Auto clave, UV-spectrophotometer, Rota evaporator.

Glass ware and other materials used- Glass Stopper Conical Flask, Silica crucible, china dish, Petri dish, Flat Bottom Weighing bottle, Water Bath, Measuring Cylinder, Wide Mouth Conical Flask, Volumetric Flask 500ml, Volumetric Flask 100ml ,Test Tubes, Volumetric Flask 250ml, Filter Paper, Tripod Stand.

Preparation of plant extract

E.hirta L. plant leaves was collected and firstly cleaned with tap water 3-4 times and one time with distilled water to eliminate any type of contamination. The leaves were air dried under shade for 6-7 days then all dry leaves were pulverized into coarse powder and cold extraction method was enforced to extract active phytoconstituents. Weigh accurately about 100gm of coarsely powdered air-dried plant material. Macerate with 2500ml water at room temperature with frequent shaking in a glass-stoppered flask for 6 hours. Allow to stand for 18hours. Filtered it through dry filter paper immediately. After that the extract was filtered and concentrated under reduce pressure at low temp on rota evaporator. The extractive yield of*E.hirta* L. aqueous leaves extract was found to be 8.93% (w/w).

Animals

Healthy adult male wistar albino rats weigh about 150-200g were used in the experiment for the evaluation of analgesic and anti-inflammatory activity procured from the Dept. of Pharmacology, Arya College of Pharmacy, Jaipur. The research was approved by animal ethical committee Registration No – 1013/PO/Re/S/06/CPCSEA. Animals were managed below standard environmental situations (temp. 26±2°C and relative humidity 55-65% for 12 hours dark and 12 hours light cycle). Animals were maintained on the standard food diet and water ad libidum during the experiment. Animals were acclimatized for 8-10 days in our laboratory environmental conditions during the experiment.

Macroscopic and Microscopic examination[8,9]

Morphological highlights and organoleptic highlights e.g. colour, odour, taste, shape and sizes were noticed and assessed botanically. Following microscopic examination has been evaluated-

Transverse section of the leaf

Leaf Surface data

Cytomorphological characters play a crucial role in drug recognition. Currently, cytomorphological studies included determination of:

- *Stomatal Index*
- *Vein-Islet Number and Veinlet Termination Number*
- *Palisade Cell-Ratio.*

Chromatographic study of different extracts[10,11]

Three types of extracts, 95% ethanolic extract, hot water extract and cold maceration extract, were used for component separation of leaf extract in thin layer chromatography. Different solvent systems were designed and used for elution. Prepared plates of Silica gel were used. Developing chambers were saturated with solvent system for 30 minutes every time. Fine glass capillaries were used for applying the sample on the plates. Up to the previously marked height, the solvent was allowed to run. The plates were removed from developing chamber and air-dried. These plates were observed under normal daylight and UV light 254 nm.

Preliminary Phytochemical Study[12-14]

Different qualitative chemical tests were carried out on various leaf extracts to identify the different phytoconstituents present.

Analgesic activity[15,16]

Hot plate method

The temperatures that do not damage the paws of mice and rats are very susceptible to heat. The jumping, removal of the paws and the licking, main responses of the paws that should be observed. An electrically heated surface containing a hot plate, which is

commercially available. The temperature of 55 °C was controlled. This may be a heated glass surface or a copper plate. The animals to be placed on the hot plate must be observed and recorded by a stop-watch and the time until either licking or jumping takes place. Wistar albino rats weighing 150-200g are to be used for analgesic activity evaluation. Wistar albino rats of either sex are divided into five distinct groups containing six animals each, the animals being marked indivisually. Twelve hours prior to drug administration, food was withdrawn untill the experiment was completed. The animals should be properly weighed and numbered. The test drug and the standard drug to be given. The animals to be placed on the hot plate and the observation to be recorded after 30, 60 and 120 minutes time interval.

The five groups are as follows:

- Group I : Control, treated with saline(10ml/kg, p.o.)
- Group II : Standard group, Diclofenac sodium (10 mg/kg, p.o.)
- Group III :E.hirta L. aqueous leaves extract (100 mg/kg, p.o.)
- Group IV: E.hirta L. aqueous leaves extract (300 mg/kg, p.o.)
- Group V :E.hirta L. aqueous leaves extract (500 mg/kg, p.o.)

The Percentage inhibition of inflammatory reactions in different groups was estimated by using following formula:

% inhibition =

(Post treatment latency – Pre treatment latency) × 100/ (Cut off time – Pretreatment latency)

Acetic acid induced writhing method

This method was assessed on healthy male wistar rats (150-200gm). Animals were separated into five groups, each consisting of six animals. The animals were treated with normal saline (10ml/kg,p.o), Diclofenac sodium(10ml/kg,p.o) and E.hirta L. leaves extract (100, 300 and 500mg/kg bw., p.o.). After 1hr of above treatment, writhings was induced by the administration of 0.6% acetic acid solution in saline (v/v) at dose 1ml/100g (i.p.). Note the onset of wriths. Counting of writhing movements was carried out on 5, 10 and 15 mins after the acetic acid injection.

The five groups are as follow:

- Group I: Control, treated with saline (10ml/kg,p.o.)
- Group II : Standard group, Diclofenac sodium (10 mg/kg, p.o.)
- Group III :E.hirta L. aqueous leaves extract (100 mg/kg,p.o.)
- Group IV: E.hirta L. aqueous leaves extract (300 mg/kg,p.o.)
- Group V: E.hirta L. aqueous leaves extract (500 mg/kg,p.o.)

The % pain inhibition was determined by formula given below:

% inhibition = no. of wriths(control) - no. of wriths(test) × 100 / no. of wriths(control)

Anti inflammatory activity[17-19]

Carrageenan-Induced paw edema in Rats

Animals are divided into five groups and six animals in each group are housed. Animals were treated with a subplantar injection of 0.1 ml λ-carrageenan (1% in 0.9% saline) into the right hind paw for the induction

of paw swelling, according to the procedure. The aqueous E.hirta L. extracts was given oral at doses of 100, 300, and 500 mg/kg 30 minutes prior to injection of Carrageenan. The standard drug was ibuprofen (50 mg/kg). The control group was treated as a vehicle with 0.9 % Nacl solution (10 mL/kg) and then injected 0.1 ml of 1 percent carrageenan solution 30 minutes after administration of the drug or test compound (extracts) into the right hind paw subplantar region of all groups. A mark was placed on the leg in the region of malleolus on subsequent readings to assist in uniform dipping. Inflammation was quantified using a plethysmometer (Medicaid system) to calculate the volume displaced by the paw at 1, 2 and 3 h after injection of carrageenan. The difference between the subsequent hours of measurement was considered to be the actual edema volume.

The five groups are as follows:

- Group I: Control, treated with saline (10ml/kg,p.o.)
- Group II: Reference group, Ibuprofen (50 mg/kg,p.o.)
- Group III: E.hirta L. aqueous leaves extract (100 mg/kg, p.o.)
- Group IV: E.hirta L. aqueous leaves extract (300 mg/kg,p.o.)
- Group V: E.hirta L. aqueous leaves extract (500 mg/kg,p.o.)

The % inhibition of inflammatory reactions in different groups was estimated by using following formula:

$$\% \ inhibition = Vc - Vt \times 100 / Vc$$

Where,

Vt = the mean volume of edema in the drug treated group

Vc = the mean volume of edema in the control group

Statistical analysis

All data was expressed by mean±SD with n=6 group. Statistical significance was determined with the help of one way analysis of variance (ANOVA) followed by Dennett's test.

RESULTS

Macroscopy andMicroscopy

Given in Table 1 and Table No2.

Table 1: Macroscopy of leaf

S.NO.	LEAF PORTION	OBSERVATION
1	Margin	Leaves are simple, opposite, elliptic, oblong, oblong - lanceolate in shape, Faintly toothed margin
2	Apex	Acute
Lamina, leaf blade		
3	Shape	1-2 cm
4	Composition	Simple
5	Venation	venation is densely reticulate
6	Surface	Glabrous
Petiole		
7	Size	950µm wide and 750 µm thick

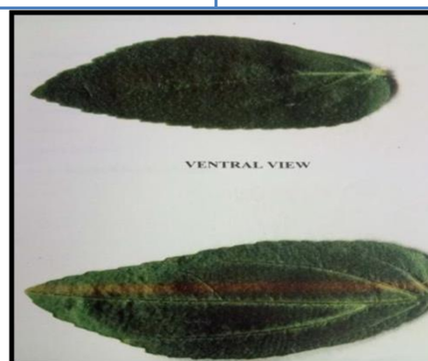

Fig. 2 Macroscopy of leaf

Table 2: Microscopy of leaf

Trichome	Glandular trichome
Stomata	occur only on the adaxial side of lamina (hypo stomatic). They are anomocytic type and have no subsidiary cells. The guard cells are small and elliptical in shape. They have wide stomatal pore.
epidermal layer	dorsi ventral with prominent adaxial midrib and uniformly thin lamina.The epidermal layer consists of fairly thick cylindrical, thin walled cells which are 10 µm thick. The cuticle is not prominent. The ground tissue is homogenous and parenchymatous.
Vascular bundles	Xylem and phloem
kranz-tissue	Along adaxial part of the xylem strand occurs on one of the dilated cells with dense chloroplasts. These cells are called ‘kranz-tissue

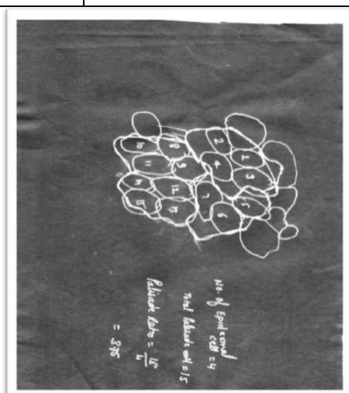

Fig. 3 Palisaderatio

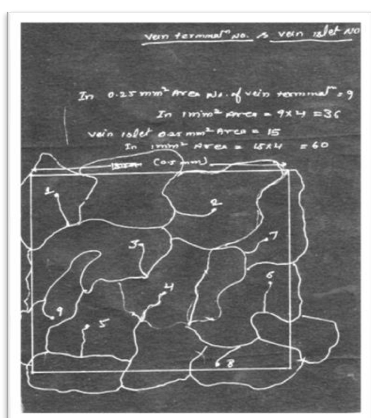

Fig.4 Vein islet and termination no.

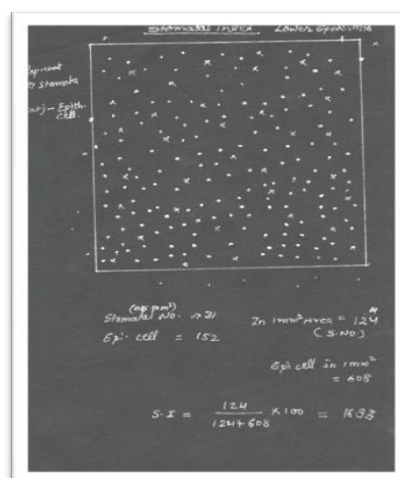

Fig. 5 Stomatal index(lowerepidermis)

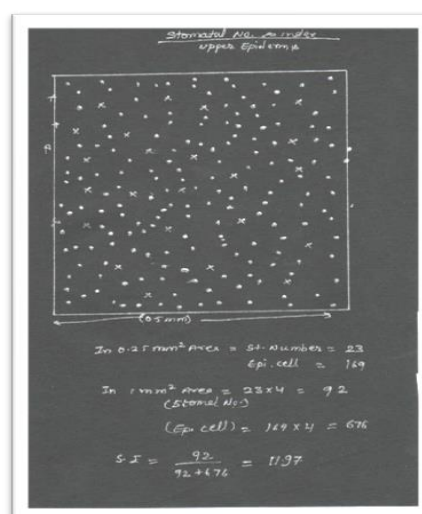

Fig. 6 Stomatal index (upperepidermis)

Table 3: Leaf surface data

S. NO.	LEAF SURFACE DATA	AVG.VALUE
1	Stomatal index of upper epidermis	**11 .97**
2	Stomatal index of lower epidermis	**16.98**
3	Vein islet no.	**60**
4	Vein termination no.	**36**
5	**Palisade ratio**	**1: 3.75**

Chromatographic study

From the different solvent systems tried it was found that in Cold water maceration extract single spot observed with Acetone and single spot remains slightly behind the solvent front

with Acetone: Methanol (50:10).

Table 4: Chromatographic study of cold water maceration extract

Solvent system	Observation under normal daylight	Observation under UV light
Acetone	Separation takes place	Separation takes place
Benzene	No separation takes place	No separation takes place
Chloroform	No separation takes place	No separation takes place
Methanol	No separation takes place	No separation takes place
Acetone: Methanol50:40	Moves with solvent front	Moves with solvent front
Acetone: Methanol50:30	Moves with solvent front	Moves with solvent front
Acetone: Methanol50:20	Moves with solvent front	Moves with solvent front
Acetone: Methanol50: 10	Single spot remains slightly behind the solvent front	Single spot remains slightly behind the solvent front
Acetone: Methanol16:4	Moves with solvent front	Moves with solvent front
Acetone: Methanol17:3	Moves with solvent front	Moves with solvent front
Acetone: Methanol18:2	Moves with solvent front	Moves with solvent front
Acetone : Methanol19:1	Moves with solvent front	Moves with solvent front

Preliminary phytochemical study

Table 5: Preliminary phytochemical study

Test	Pet ether	Benzene	Chloroform	Methanol	Ethanol	Aqueous
Sterols	+	+	+	+	+	+
Terpenoids	-	-	-	+	+	+
Carbohydrates	-	-	-	-	-	-
Flavonoids			+	+	+	+

Proteins	-	-	-	-	-	+
Alkaloids	-	-	+	+	+	+
Glycosides	-	-	-	+	+	+
Saponins	-	-	-	+	+	+
Tannins	-	-	-	+	+	+
Phenols	-	-	-	+	+	+

Analgesic Activity: Hot plate method

The hot plate was used to calculate response latencies as per the method described by Eddy and Leimbach (1953). The rats were placed on a Techno hot plate having a temperature 55°C and the time between the movement of the rat on the platform and the shaking or licking of the paws or jumping were reported as the latency of the hot plate. Rats were excluded from the analysis with baseline latencies greater than 10 s. Twenty-four hours later, 60 minutes before the test, the animals were treated with an aqueous extract of E.hirta L. (at doses of 100, 300 and 500 mg/kg p.o.) or with diclofenac sodium (10 mg/kg p.o.) The same amount of saline solution was obtained by control animals (10 ml/kg p.o.). 15 seconds was used as post treatment cut off time.

Table 6: Analgesic activity of *E.hirta*L. leaves extract by hot plate method

S. no.	Groups	Basal reaction time (sec)	Reaction-time(sec) after drug administration			Average mean	% inhibiti o n in pain
			30 min	**60 min**	**120min**		
1.	Control	3.11±0.406	3.36±0.258	3.52±0.492	3.49±0.375	3.45	-
2.	Diclofenac sodium	3.35±0.541	4.40±0.862	7.52±1.712*	10.02±1.625**	7.31	33.99
3.	*E.hirta*L. extract 100mg/kg	3.20±0.542	4.00±0.442	5.12±1.001	6.98±1.572*	5.36	18.30
4.	*E.hirta*L. extract 300mg/kg	3.42±0.123	4.42±0.894	5.68±1.022	7.25±1.562**	5.78	20.37
5.	*E.hirta*L. extract 500mg/kg	3.52±0.428	5.25±0.912	7.01±1.031*	8.99±1.362**	7.08	31

*(Values are mean ± SD (n = 6); represent number of animal in each group. **Represent statistically significant p< 0.01, *Represent statistically significant value <0.05 which are calculated with the help of one way analysis of variance (one way ANOVA). The comparison was made between control group versus standard group, low dose as well as high dose.)*

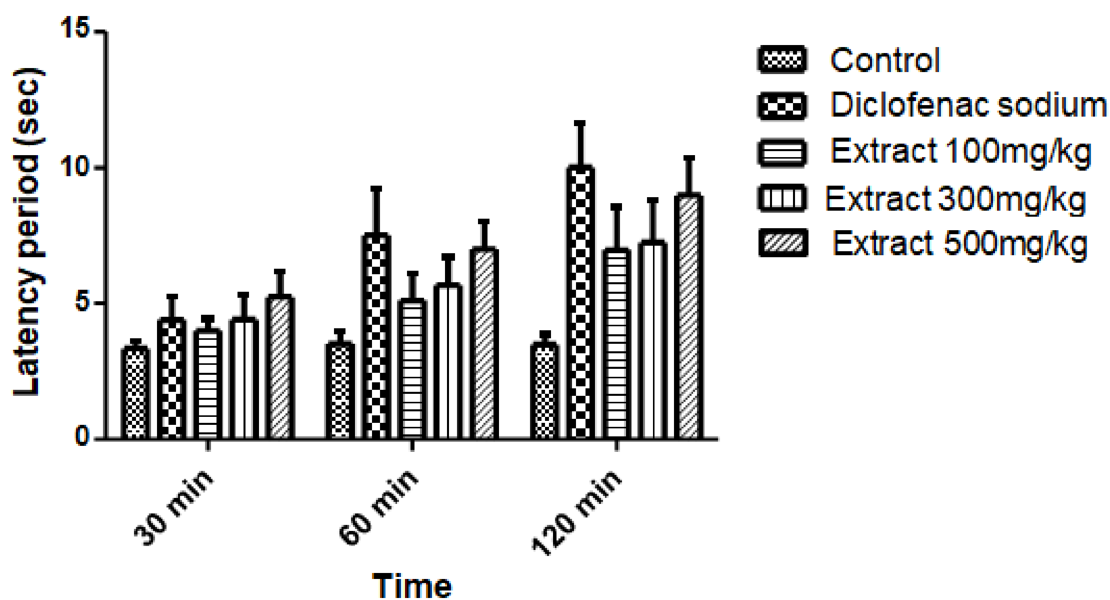

Fig.7 The graph represents average reaction time of aqueous extract of leaves of E.hirta L. at various time intervals

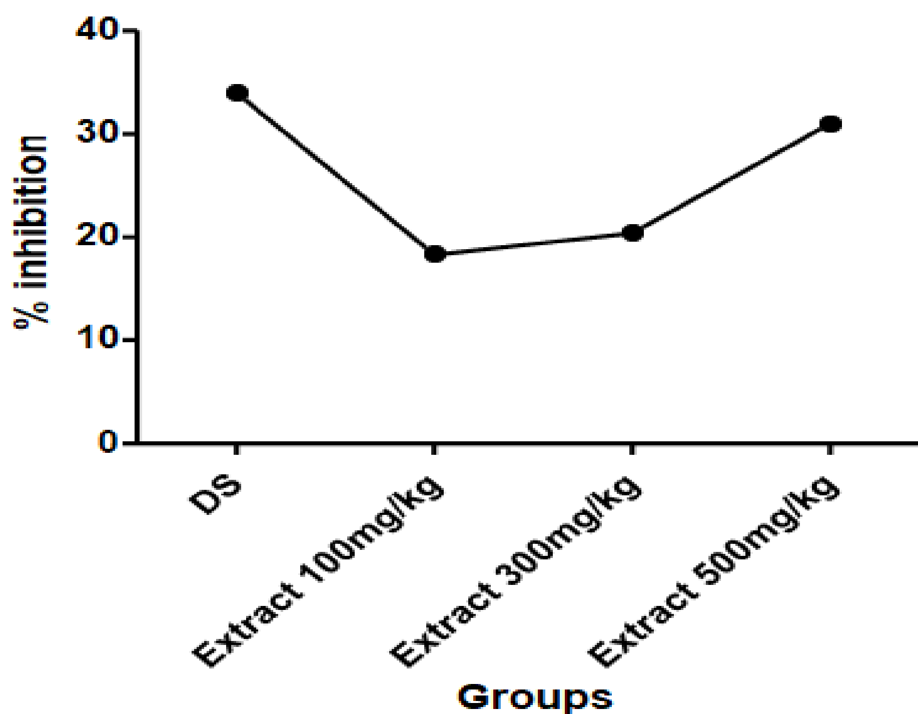

Fig.8 The graph represents % inhibition of pain of *E.hirta*L. leaves extract via hot plate method

Analgesic Activity: Acetic acid induced writhing method

The analgesic activity of E.hirta L leaves aqueous extract on the acetic acid induced writhing in rats was shown in Table 4.20. The no. of wriths was recorded at the time interval 5, 10 and 15 min after the administration of acetic acid. In the control group there was considerable increase in no. of wriths. In the test groups, the extract show significantly inhibition of no. of wriths in dose dependent manner. Writhing response was decreases significantly by standard drug as well as the different doses of E.hirta L. extract (100,300 and 500mg/kg). At the dose 100mg/kg, *E.hirta* L. showed 34.78% reduction in acetic acid induced writhings while at the doses 300mg/kg & 500mg/kg reduction was 50.57% and 57.20% respectively. Standard drug Diclofenac sodium shows 71.62% inhibitory effect.

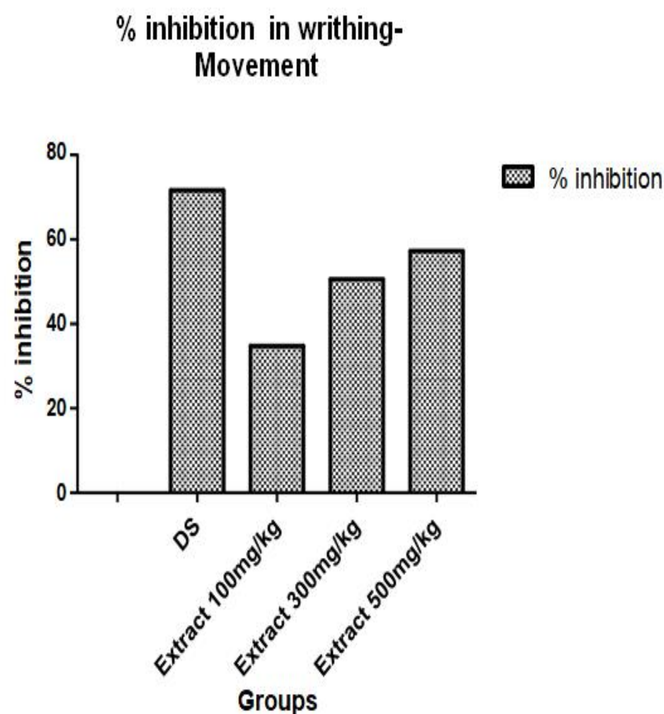

Fig.9 The graph represents % inhibition in writhing movement

Table 7: Analgesic activity of E.hirta L. leaves extract by Acetic acid induced writhing method

S.no	Groups	Dose	Mean number of Writhing ±SD	% inhibition in writhing- Movement
1.	**Control**	10ml/kg	43.7 ± 0.85	**-**
2.	**Diclofena sodium**	10mg/kg	12.4 ± 0.98**	**71.62**
3.	***E.hirta*L.extrac t**	100mg/kg	28.5± 0.56*	**34.78**
4.	***E.hirta*L.extrac t**	300mg/kg	21.6 ± 1.03*	**50.57**
5.	*E.hirta*L.extract	**500mg/kg**	**18.7± 0.96****	**57.20**

*Values are mean ± SD (n = 6); represent number of animal in each group.**Represent statistically significant p< 0.01, *P < 0.05, compared with control.*

Anti-inflammatory activity: Carrageenan-Induced Edema in Rats

Table 8 explains the anti-inflammatory effects of aqueous extracts on carrageenan-induced edema in the hind paws of rats. In the control group, the amount of the edema paw in rats has steadily increased. However, in the test groups, the aqueous extract showed a substantial decrease in edema paw volume as indicated in 100, 300 and 500 mg/kg oral doses, p.o. A dose related inhibition of hind paw edema between 1 and 3 h was observed 30 min before carrageenan. At 500 mg/kg, the inhibitory effect was the largest. The extract was shown to produce significant results.

Table 8: Anti-inflammatory effect of E.hirta L. leaves extract in carrageenan induced paw Edema in rats

Group no.	Mean change in paw volume (ml) by Carrageenan induce inflammation						
	Treatment	**Dose**	**1 hr**	**2 hr**	**3 hr**	**Average reading**	% inhibition
1	**Carrageenan**	0.1 mL 1% sol.	1.32±0.08	1.35±0.06	1.32±0.04	1.33	**-**
2	**Ibuprofen**	50 mg/kg	0.52±0.04	0.50±0.06*	0.40±0.05**	0.47	**64**
3	***E.hirta* L.extract**	100 mg/kg	0.85±0.11	0.81±0.09*	0.69±0.02**	0.78	**41**
4	***E.hirta* L.extract**	300 mg/kg	0.79±0.12	0.77±0.07*	0.67±0.17**	0.74	**44**
5	*E.hirta* L. extract	**500 mg/kg**	**0.64±0.09***	**0.63±0.11***	**0.61±0.12****	**0.62**	**53**

*All value are expressed in mean ±SD, (N=6, represent number of animal in each group)**Represent statistically significant p< 0.01, *Represent statistical significant value*<0.05 *which are calculated with the help of one way analysis of variance (one way ANOVA). The comparison was made between control group versus standard group, low dose as well as high dose test drug.*

DISCUSSION

In the current study, the aqueous leaves extract of E.hirta L. was assessed as analgesic and anti inflammatory activity. Leaves were shade dried, powdered and extracted with the help of water by using cold maceration process. Then the extract was evaluated phytochemically, pharmacognostically (as per WHO guidelines) and pharmacologically. The evaluation of drug was done by using different parameters such as macroscopic and microscopic examination, chromatographic study, determination of foreign matter in sample, ash value, Extractable matter, presence of tannins, water, swelling index, foaming index, presence of arsenic and bitterness value. A preliminary phytochemical analysis indicated the presence of glycosides, terpenoids, flavonoids, sterols, saponins, tannins and alkaloids are found in various solvents of crude drug. For pharmacological evaluation, Analgesic and anti inflammatory activity was carried out. The dose of crude drug extract was decided from review of literature, it was 100mg/kg, 300mg/kg and 500mg/kg. Wistar albino rats have been used for pharmacological assessment purposes. For the assessment of analgesic activity and anti-inflammatory activity, Eddy's hot plate method & Acetic acid writhing method and carrageenan-induced paw edema in rats method were used respectively[20].

Inflammation is a biological protective response of body which occurs by pathogens, microbes and irritants. The function of the inflammation is to remove necrotic cells or damaged tissues then start the repairing of tissues. It is divided into three types: Acute, Sub acute and chronic inflammation. There are some inflammatory signs i.e. redness, pain, swelling, heat and loss of function. In the onset of pain along with swelling of joints and limbs, bone deformities and joint impairment, the deliverance of many mediators of inflammation like cytokine, interferon's and platelet-derived growth factor play a key role in the production of pain. The literature survey reveals that the ibuprofen (Reference drug) inhibited inflammation induced by carrageenan induced paw edema.

The key parameter for testing the anti-inflammatory effects of different medications is the reduction in paw swelling. The level of inflammation and curative efficacy of anti-inflammatory agents can be easily measured by this factor. In the present study, The E.hirta L. leaves extract treated rats show remarkable dose dependent reduction in paw edema induced by carrageenan. This effect may occur due to inhibition of release of inflammatory mediator.

The inhibitory effect was highest with 500 mg/kg and 300mg/kg dose of crude drug extract. Extract and ibuprofen exhibited 53% and 64% inhibition of edema formation respectively at 3 hr after carrageenan administration. In Analgesic activity, Diclofenac sodium was used as reference drug. At various doses, the crude drug extract was given and note the reaction time of hot plate animals at 15, 30, 60 and 120 minutes after administration of drugs. In Acetic acid induced writhing method, % inhibition in writhing movement was 50.57% and 57.20% at dose of 300mg/kg and 500mg/kg. Standard drug, Diclofenac sodium show 71.62% inhibitory effect. Thus it was concluded that E.hirta L. leaves aqueous extract show potent anti inflammatory and analgesic activity. Further study was required to investigate different Phytoconstituents responsible for various Pharmacological activities.

CONCLUSION

This research has shown the analgesic and anti inflammatory effect of *E.hirta* L. leaves aqueous

extract in dose-dependent way. The dose 300mg/kg and 500mg/kg of aqueous extract of E.hirta L. leaves was highly significant. In the treatment of inflammation, it has been found to be more effective. To isolate the active compounds of the extract and to confirm the mode of action in the production of a strong analgesic and anti-inflammatory component, further investigations are required.

CONFLICT OF INTEREST

Authors don't have any conflict of interest

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