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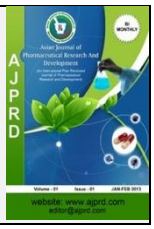

Open Access Research Article

Anti inflammatory potential of *Euphorbia hirta* L. leaves in Jaipur region

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A B S T R A C T

Introduction: Euphorbia hirta Linn. (*E.hirta* L.) is a medicinal herb belonging to the family Euphorbiaceae. It is commomly known as Asthma plant, Duddhi, Bara duddhi. It has been claimed for the various medicinal properties such as inflammation, diarrhoea, bronchitis, kidney stones, peptic ulcer, menstrual problems etc.

Objective: The goal of our research work is to assessing the anti inflammatory activity of *E.hirta* L. leaves in regards to phytochemical studies.

Methods: The method carrageenan induced paw edema in rats was used to assess the anti inflammatory activity of aqueous extract of *E.hirta* L. leaves. The extract was obtained by maceration method and the analysis of phytochemicals was carried out by performing different chemical test in laboratory. Crude aqueous extract was given orally at different doses of 100mg/kg, 300mg/kg and 500mg/kg in male wistar rats 30mins before carrageenan injection. Ibuprofen (50mg/kg) was taken as reference drug. The volume of paw edema was measured by using plethysmometer at 1, 2 and 3 hr after carrageenan administration.

Results: The preliminary phytochemical study reveals the availibility of flavonoids, terpenoids, sterols, glycosides, tannins etc in different solvents of crude drug extract. The anti inflammatory effect of crude drug extract was exhibited a remarkable dose dependent reduction in paw edema volume. The inhibitory effect was highest at 500mg/kg dose of drug. Extract and ibuprofen showed 53% and 64% inhibition of paw edema respectively, at 3hr after carragennan administration. **Conclusion**: *E.hirta* L. leaves aqueous extract has a strong anti inflammatory activity. These consequences help in the traditional uses of the plants and in the development of new treatments.

Keywords: Aqueous extract, flavonoids, Anti inflammatory activity.

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INTRODUCTION:

Inflammation is complex biological reaction to harmful agents such as microbes, pathogens, damaged cells that contains vascular responses, activation of leucocytes and various systemic reactions[1]. The inflammation response contains two main components, cellular reactions and vascular responses. These components are mediated by chemical factors that are acquired from plasma cells and these reponses are operated by the inflammatory stimulus. Inflammation is divided into three types: Acute, sub acute and chronic inflammation[2].

Acute inflammation is the basic mechanism of the body which is distinguished by five classical signs, Redness (Rubor), Increased heat (Calor), Swelling (Tumor), Pain (Dolor), Failure of fuction (Function laesa). This type of inflammation appears within few minutes or hours and it distinguish by increase in movement of leucocytes and basophils into the injured tissues[3].

Sub acute inflammation is longer process last upto 1 to 6 weeks or more. In this type of inflammation exudative and proliferative changes occurs[4,5].

Chronic inflammation is prolonged process. It leads to tissue destruction at the site of inflammation. It is divided into two types : specific and non specific inflammation[6].

E.hirta L. (Euphorbiaceae) is an annual hairy herb with various branches, spread upto 50cm in height. It is commonly known as Duddhi, Bara duddhi, Asthma weed. It is native to India, Australia and Africa[7]. It is used for the therapy of several ailments: GIT disorders (dysentery, bowel complaints, Diarrhoea), bronchitis, cancer, helminthic infestations, fever, cough, asthmatic problems, kidney stones, occular diseases (conjuctivitis)[8]. *E.hirta* L. contains different types of chemical compounds such as Phenols, flavonoids (quercitin, myricitrin, quercitrin, quercitrol), terpenoids (α- amyrin, β-amyrin), gallic, tannic, maleic, ellagic, tartaric acid, essential oil and other compounds[9]. Therefore the literature survey revealed that there are different compounds found in plant extract to comprehend the reason for anti inflammatory properties. These compounds are flavonoids (quercitin, myricitrin, quercitrin and sitosterols) show dose related anti inflammatory activity. The triterpene β-amyrin also exhibits a complementary anti inflammatory activity[10].

The objective of the present studies is to collect the drug that is leaf of Asthma plant from Rajasthan, authenticated and to carry out the extraction and isolation of the phytoconstituents from selected extract based on phytochemical screening. The different extracts or/and isolated compound would be studies for anti-inflammatory activity.

Figure: 1 Whole plant of *E.hirta* L.

Medicinal properties of *E.hirta* L.

- It has many medicinal properties like anticancer, anxiolytic, analgesic, antimalarial, antidiarrhoeal, antioxidant, antiamoebic, anti inflammatory etc[8].
- It is used in the management of hypertension, helminthic infestations, kidney stones and asthmatic problems etc[11].
- This plant is used in various skin diseases such as scabies, tinea, thrush, warts, wounds, sores and guinea worm[12].
- It is used as antidote and as pain reliever in scorpion stings and snake bites[12].

MATERIALS & METHODS

Plant material collection

The leaves of *E.hirta* L. was collected in the month of September 2020 from the campus of Arya college of pharmacy,Kukas, Jaipur, Rajasthan, India. The plant was botanically recognized and authenticated.

Chemicals

Ibuprofen was used as standard drug in this study and bought from local market. All chemicals and reagents used in this experiment were procured from Arya college of pharmacy, jaipur. These chemicals are highly pure and have good analytical grade.

Equipments & Instruments

Plethysmometer, weighing balance, Hot air oven, Dessicator, Heating mentle etc.

Glass wares

Glass Stopper Conical Flask, Petri Dish, Flat Bottom flask, Water Bath, Measuring Cylinder, Wide Mouth Conical Flask, Volumetric Flask, Volumetric Flask, Test Tube, Filter Paper, Tripod Stand etc.

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 3-5 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 through dry filter paper immediately. Transferred 100ml strain liquid to previously weighed petridish and evaporated on water bath to dryness. Dried in an oven at 105℃ for 6-7 hours. Cool in a deccicator for 30-40 minutes. Weigh the extract without delay. The crude extract percentage yield was 2.22% (w/w). The crude semi solid extract was protected in a refrigerator (below 10 ℃) for more investigation.

Animals

Healthy male wistar rats weighing about 100-150g were used in the experiment. Animals were managed under 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.

Preliminary phytochemical study

2gm of dried powder of *E.hirta* L. leaves were packed in separate flasks for sample extraction by using six indivisual solvents namely benzene, chloroform, petroleum ether, methanol, ethanol and water. The extraction was conducted with 20ml of each solvent for 24 hours. At the end of extraction the solvent were concentrated under low pressure and crude extracts were kept in refrigerator. The successive qualitative chemical test performed for analyzing several

phytochemicals existing in different extracts of leaf based on the protocol available in literature.

Tests for carbohydrates[13]:

Molisch's test – Add 0.1ml alcoholic α-napthol to the extracted solution. Add few drops of conc. sulphuric acid gradually through the sides of the test tube, purple color converted into violet coloured ring at the junction.

Benedict's test– Add few drops of Benedict's reagent (alkaline solution containing cupric citrate complex) to the extracted solution. Boiled it on water bath. If reducing sugar are present, reddish brown precipitate forms.

Test for proteins & amino acids[14]

Ninhydrin test: 0.2% Ninhydrin solution (Indane 1, 2, 3 trione hydrate) was added to the extracted solution and then boil it. Violet colour appears indicated the presence of proteins and amino acids.

Millon's Test *:* when extracted solution treated with Millon's reagent i.e 2ml gives white precipitate which converts red upon smooth heating.

Tests for sterols and triterpenoids[14]

Libermann-Burchard test:

Add 0.1ml of acetic anhydride to the extracted solution then boil and cooled it. Add few drops of concentrated sulphuric acid from the side of the test tube. At the junction of two layers brown ring appears and the upper layer converts green in color showed the presence of sterols and production of deep red colour showed the presence of triterpenoids.

Salkowski's test:

Chloroform is added to the extracted solution and add 0.1 ml of conc. Sulphuric acid with vigrously shaking and allow this solution to stand for few minutes, after that red colour appears in the lower layer which indicates the presence of sterols and the presence of triterpenoids is confirmed by the formation of yellow color in lower layer.

Tests for cardiac glycosides[15] **(keller-killani test)**

1ml of extracted solution was treated with 1ml glacial acetic acid and 0.1ml of 5% $Fecl_3$ solution. After that 0.5 ml of conc. Sulphuric acid was added to this mixture. Brown ring appeared at the interface that indicates the presence of cardenolides. A violet ring may appear below the ring while in the acetic acid layer, a greenish layer appeared.

Tests for alkaloid[14,16]

Mayer's test: (Potassium mercuric iodide solution).

0.1ml of Mayer's reagent was added to the test solution, a precipitate i.e. creamy white was obtained.

Dragendroff's test: (Potassium bismuth iodide solution).

0.1ml of Dragendroff's reagent was added to the test solution, a reddish brown precipitate obtained.

Tests for Phenolic compounds[17]

Ferric chloride test: Add 0.1ml of ferric chloride solution to the extracted solution, blue-green colour appeared.

Tests for flavonoids[17]

Alkaline reagent test:

0.1ml NaoH solution was added to the extracted solution, formation of an deep yellow colour which was converted to colorless after addition of few drops of dilute CH_3COOH that showed the presence of flavonoids.

Anti inflammatory activity

Carrageenan-Induced paw edema in Rats[18,19,20]

Animals are divided into five groups, six animals were kept in each group. According to the method, animals were treated with subplantar injection of 0.1 ml λ-carrageenan (1% in 0.9% saline) into the right hind paw for inducing paw swelling. The aqueous extracts of *E.hirta* L. at dose of 100, 300, and 500 mg/kg were given orally 30 min before the injection of carrageenan. Ibuprofen (50 mg/kg) was used as standard drug. Control group treated with 0.9% Nacl solution as vehicle (10 mL/kg) then 0.1 mL of 1% carrageenan solution was injected 30 minutes after drug or test compound (extracts) administration, into the subplantar region of right hind paws of all groups. On subsequent readings, a mark was put on the leg at the malleolus region to help in uniform dipping. The inflammation was quantified by measuring the volume displaced by the paw, using a plethysmometer at time 0 min, 30min, and 1, 2, and 3 h after the injection of carrageenan. The difference between subsequent hours readings was considered as actual volume of edema.

The five groups are as follows:

Group i: Treated as control and administration of saline solution (10ml/kg)

Group ii: Reference group, ibuprofen 50 mg/kg

Group iii: Aqueous extract of *E.hirta* L. leaves at 100 mg/kg

Group iv: Aqueous extract of *E.hirta* L. leaves at 300 mg/kg

Group v: Aqueous extract of *E.hirta* L. leaves at 500 mg/kg

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

$$\text{Percentage inhibition} = V_c - V_t / V_c \quad 100$$

Where, V_t = the mean volume of edema in the drug treated group

V_c = the mean volume of edema in the control group

RESULTS AND DISCUSSION

Table 1. Indicates the preliminary phytochemical constituents present in *E.hirta* L. leaves with various solvents namely petroleum ether, benzene, chloroform, ethanol, methanol and water. The phytochemical study of crude extract disclosed the existance of sterols, terpenoids, flavanoids, alkaloids, glycosides, saponins and tannins in methanol, ethanol and aqueous extract whereas the carbohydrates, proteins, mucilage and oil/fats were absent

in all the extracts. Resins were present in methanolic and ethanolic extract.

Table: 1 Preliminary phytochemical constituents of petroleum ether, benzene, chloroform, methanol, ethanol and aqueous extract of *E.hirta* L. leaves.

Test	Petroleum ether	Benzene	Chloroform	Methanol	Ethanol	Aqueous
Sterols	+	+	+	+	+	+
Terpenoids	-	-	-	+	+	+
Carbohydrates	-	-	-	-	-	-
Flavonoids	-	-	+	+	+	+
Proteins	-	-	-	-	-	-
Alkaloids	-	-	+	+	+	+
Cardiac glycosides	-	-	-	+	+	+
Saponins	-	-	-	+	+	+
Tannins	-	-	-	+	+	+
Mucliage	-	-	-	-	-	-
Resins	-	-	-	+	+	-
Oil/Fats	-	-	-	-	-	-

Positive + , Negative -

Table 2. shows the anti-inflammatory activity of the aqueous extracts on carrageenan-induced paw edema in rats. In control group, there was a slightly increase in volume of paw edema in rats. However, the extract showed a remarkable decrease in the volume of paw edema in the test groups. As specified in the oral administration of aqueous extracts at different doses of 100, 300, and 500 mg/kg p.o. 30 min before carrageenan injection, a dose-related inhibition of hind paw edema between 1 and 3 hr was exhibited. The inhibitory effect was highest with 500 mg/kg. Some remarkable effects were indicated by the different extract. Ibuprofen as standard drug (50 mg/kg p.o) composed a significant inhibitory effect comparable with the extract. Extract and ibuprofen showed 53% and 64% inhibition of volume of edema respectively, at 3 h after carrageenan administration.

Table: 2. 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	1hr	2hr	3hr	Average reading	% inhibition
1	**Carrageenan**	0.1mg 0.21% 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 along with dunnet's test). The comparison was made between control group versus standard group, low dose as well as high dose test drug.

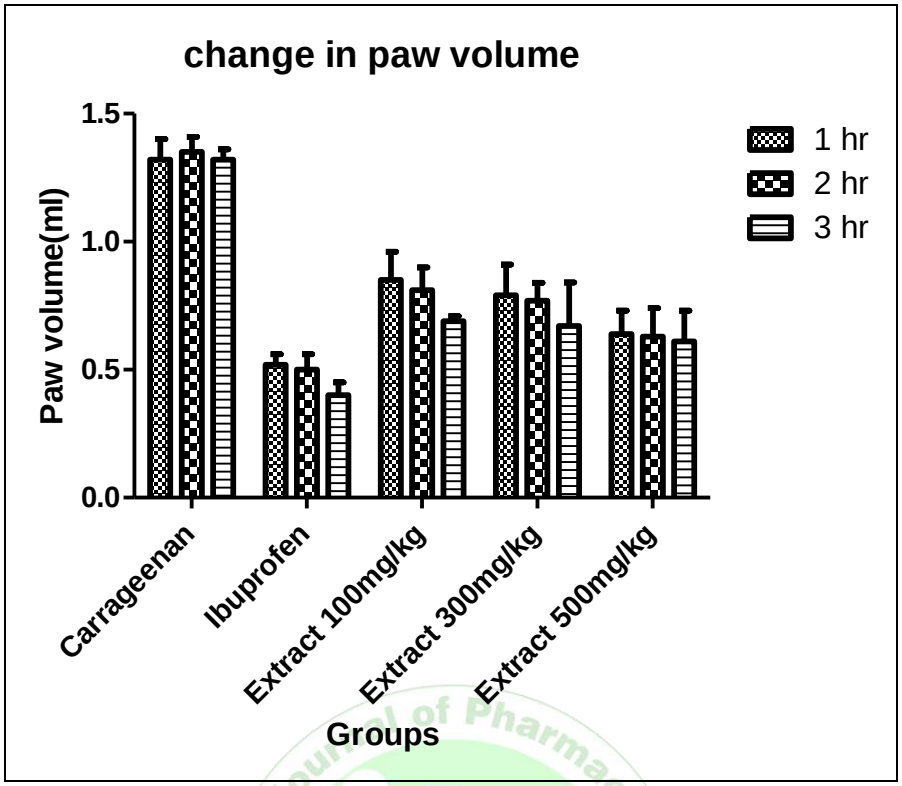

Figure: 2 The graph represents change in paw volume

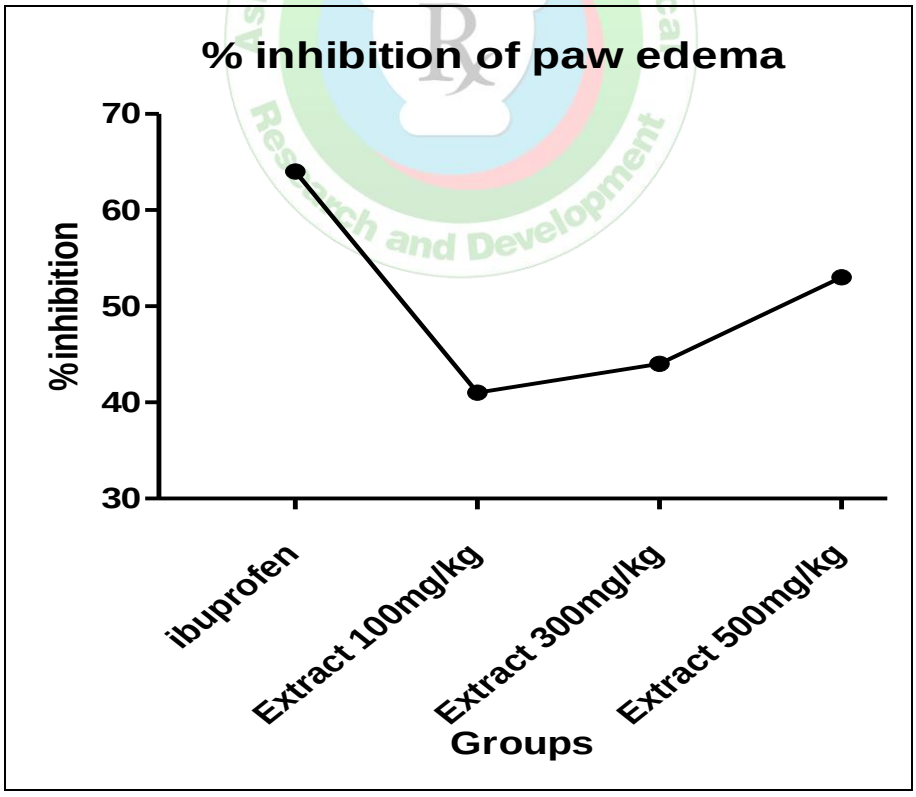

Figure: 3 The graph represents % inhibition of paw edema in rats

CONCLUSION:

Our study identified that *E.hirta* L. leaves contains various chemical constituents such as sterols, alkaloids, glycosides, saponins, tannins, flavonoids and terpenoids. The aqueous leaves extract of *E.hirta* L. was evaluated for anti inflammatory activity by carrageenan induced paw edema in rats. From this study it was concluded that the dose 300mg/kg and 500mg/kg of aqueous leaves extract of *E.hirta* L. was found to be effective in Anti inflammatory activity. These results allow us to conclude that *E.hirta* L. leaves have potential for the development in inflammatory conditions.

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