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Pharmacological evaluation of anti-inflammatory activity of *Euphorbia hirta* against carrageenan induced paw edema in Rats

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Abstract

Euphorbia hirta is a well known plant of the herbal world, which gains the popularity due to its various pharmacological activities. The roots of plant *Euphorbia hirta* used as purgative and leaves and stems are reported to be cooked with cooked with pork and used as tonic. The present study was carried out to evaluate the effect of *Euphorbia hirta* (ethanolic and aqueous extract p.o.) on carrageenan induced inflammation. The study was carried out on carrageenan induced inflammation model by carrageenan. Diclofenac Sodium 50 mg/kg was used as reference standard. It has been observed that the aqueous extract and ethanolic extract showed maximum percentage protection towards inflammation in comparison to other extracts. The plant *Euphorbia hirta* reduces inflammation and prevents the development of experimentally induced inflammation in rats.

Key Words: Carrageenan, Diclofenac Sodium, Prostaglandin, edematogenic, inflammation.

INTRODUCTION

Many medicinal plants used in ethno medical practices in India are unknown or little known to scientific world. The pharmacological activities of most of the plants remain to be studied. One such plant is "*Euphorbia hirta*", belonging to the family Euphorbiaceae. It is used against asthma, bronchitis, worm infestation, conjunctivitis and dysentery. It is used against asthma, bronchitis, worm infestation, conjunctivitis and dysentery. The latex of the plant is used for warts and cuts. The plant is also used as an Antiamoebic, Antispasmodic, Antidiarrhoeal, Anticancer, Antibacterial, Immunomodulatory, Antifungal, Aflatoxin inhibition, Galactogenic, Antifertility, Antiasthmatic. The plant contains Triterpenoids, Sterols, Alkaloid, Glycoside and Tannins[1-4].

The purpose of this investigation was to explore the potential of plant *Euphorbia hirta*. Its pharmacological effect was not studied previously, and no pharmacological literature is available.

MATERIALS AND METHODS

The whole plant of *Euphorbia hirta* was collected from Utkal University during the month of Nov-Dec and identified by the botanist of Department of Botany, Utkal University, Bhubaneswar by comparing with the voucher specimen present in the herbarium. After authentication fresh plant materials were collected in bulk, washed under running tap water to remove adhering dust, dried under shade and pulverized in a mechanical grinder. The coarse powder was used for further studies.

Experimental animals

Male albino Wistar rats weighing between 180 to 250gm were used. The experimental protocol is approved by the institutional Animal Ethics committee (I.A.E.C/U.D.P.S/990/2005-Vanivihar, Bhubaneswar).

Extraction of plant material and preparation of test dose

About 200 Gms of coarse dried powder of fruits of the plant *Euphorbia hirta* was taken in the soxhlet apparatus and first extracted with petroleum ether. The defatted material was then extracted with, petroleum ether, Chloroform, methanol, ethanol, and aqueous solvent successively. The extraction for each solvent was carried out for 18 to 20 hours and temperature during the extraction process was maintained with 35-37°C. At the end of each extraction, the plant materials were removed from the soxhlet apparatus and allowed to dry under shade so that the remaining parts of solvent get evaporated. After evaporation of solvent from the marc, it was again packed in the soxhlet apparatus and allowed to extract by successive solvents. The solvents of the extract so obtained were evaporated to dryness by applying slow heat treatment.

Acute toxicity studies

The adult Swiss albino mice 20-25gm were selected for the studies. Before the actual LD₅₀ determination a pilot study was made on a small group of mice, mainly to select close ranger for the subsequent study. The extract at various dose level (1000, 2000, 3000, 4000, 5000 mg/kg body wt.) suspended in 0.5% w/v Sodium-CMC were administered by oral route as single dose to a pair of mice per dose level.

The mortality was observed at a dose of 2500&3000 mg/Kg in ethanolic and aqueous extract respectively which were determined as LD₅₀.

Experimental model of Carrageenan induced inflammation: [5-10]

The anti-inflammatory activity was assessed as suggested by winter et. al. 1962 by using carrageenan as edematogenic agent on adult albino rats of either sex weighing b/w 150-200gm. The selected albino rats were housed in groups of six in each in acrylic cages under laboratory condition. They were fasted over night and during the experiment had free access to water. Diclofenac Sodium (50 mg/kg was used as reference standard.

The extracts were administered orally in the form of a suspension with 1% w/v CMC at dose levels of 100 and 200 mg/kg respectively. The control group received 1% w/v CMC (2ml/kg) in a similar manner. All the test samples were administered orally 30 min before injection of carrageenan (0.1 ml of 1% w/v solution in normal saline) in the sub planted region of left hind

paw of each rat. The conbilateral paw was injected with an equal volume of saline. The paw swelling was calculated by a plethysmograph as the difference between the volumes at mercury displaced by the two paws (mt). The observations are tabulated. The percentage inhibition of paw edema was calculated at the end of 3 hr.

Increase in paw thickness in control/ treatment

$$PC/PT = Pt - Po$$

$$\text{Percentage of inhibition} = \frac{PC - PT}{PC} \times 100$$

Where Pt = paw thickness at time t , Po = initial paw thickness, PC = Increase in paw thickness of control group and PT = Increase paw thickness of the treatment groups.

Statistical analysis

The results were subjected to statistical analysis by using student's t-test where applicable. p value less than 0.01 were considered significant.

Table 1: Anti-inflammatory effect of *Euphorbia hirta* Linn whole plant against Carrageenan induced paw edema in rats

Treatment	Dose mg/kg	Initial paw thickness	Paw thickness after 1hrs	% of inhibition	Paw thickness after 2 hrs	% of inhibition	Paw Thickness after 3 hrs	% of inhibition
Control	0.3ml	0.481±0.01	0.701±0.025	-----	0.911±0.035	-----	1.031±0.001	-----
Diclofenac Sod.	50mg/kg	0.481±0.002	0.480±0.001	31.5	0.476±0.001*	47.7	0.456±0.001**	55.7
Ethanollic ext.	100 mg/kg	0.591±0.002	0.479±0.035*	31.6	0.476±0.002	47.7	0.446±0.001*	56.7
Ethanollic ext.	200 mg/kg	0.594±0.001	0.591±0.002**	15.6	0.586±0.002*	35.6	0.442±0.035*	57.1
Aqueous ext.	100 mg/kg	0.481±0.001	0.591±0.002	15.6	0.481±0.002*	47.2	0.431±0.002	58.1
Aqueous ext.	200 mg/kg	0.482±0.001	0.581±0.002**	17.1	0.471±0.002*	48.2	0.471±0.002**	54.3

Results are express on mean ± SEM from our observations. P* :-< 0.05; P** :-< 0.001

RESULTS AND DISCUSSION

In carrageenan induced rat paw edema model Ethanollic and aqueous extract about 56.7 and 58.1% inhibition of increased paw thickness respectively at 100mg/kg body weight. However, at the dose of 200mg/kg body weight Ethanollic 57.1% and aqueous 54.3% inhibition of inflammatory increased paw thickness when compared to solvent control. At the same time Diclofenac sodium the standard drug produces 55.7% inhibition in inflammatory paw thickness. This study revealed that in carrageenan induced inflammation model Ethanollic and aqueous extracts are ranking in the following order: *Aqueous* > *Ethanollic*.

The percentage inhibition of inflammation of all extracts are dose dependent, as increase in dose level of extracts increases percentage of inhibition accordingly. The development of carrageenan induced edema is biphasic, the 1st phase is attributed to release of histamine, 5-HT, Kinin, while 2nd phase is related to the release of Prostaglandin.

Therefore, it is proposed that the anti-inflammatory property of extracts is due to inhibition of one of the pain mediators like histamine, 5-HT, Kinin or Prostaglandin. All extracts showed varying degrees of anti-inflammatory activities with statistical significance at all tested dose levels but the aqueous extract and ethanolic extract showed maximum-percentage protection towards inflammation at a dose level of 100 mg/kg compared to other derivatives at equivalent concentration.

CONCLUSION

In the present study ethanol and aqueous extracts were prepared from fruits of *Euphorbia hirta* and its Anti-inflammatory effect was studied in different established models in rats. Toxicological studies reveal that it was safe *Euphorbia hirta* and does not alter the normal physiological and behavioral process even at higher dose level. Administration of ethanol and aqueous extracts of fruits of *Euphorbia hirta* showed a remarkable Anti-inflammatory activity in Carrageenan induced paw oedema models. There is no doubt that, *Euphorbia hirta* possesses a significant, Anti-inflammatory activity which was confirmed in our study.

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