

Anti-Inflammatory activity of *Erythrina stricta* Roxb. in Albino Rats

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Abstract:

Objectives: To study the anti-inflammatory activity of *Erythrina stricta* extract in albino rats.

Methods: Leaves of *Erythrina stricta* (ES) were extracted and subjected to Pharmacological studies. The Extract was screened for anti-inflammatory activity in albino rats using acute carrageenan paw oedema, formalin induced paw oedema and sub acute granuloma pouch model.

Results: In acute phase inflammation, a maximum inhibition of 78.79 % (P < 0.05), and 86.8 % (P < 0.05) was noted at the dose of 200 mg/kg after 3 hr of treatment with ethanol extract of ES in carrageenan and formalin induced paw oedema respectively. In the sub acute model (cotton pellet induced granuloma) the ethanol extract of ES (200 mg/kg) and standard drug (Indomethacin 10 mg/kg) showed decreased formation of granuloma tissue by 60.05 % (P < 0.05) and 65.6 % (P < 0.05), respectively. In ES the dose of 200 mg/kg is found to be more potent and efficacious towards the anti-inflammatory activity when compared with control and the activity is in dose dependent manner.

Conclusions: The extract exhibit its anti-inflammatory action by means of inhibiting the synthesis, release or action of inflammatory mediators viz, histamine, serotonin and prostaglandin might be involved in inflammation. From these results, it is suggested that anti oedematogenic effect of the ES on carrageenan and formalin induced oedema might be related to inhibition of inflammation mediator formation. So, our results strongly suggested that the extract of ES leaves showed anti-inflammatory activity in acute and sub acute administration in albino rats.

Key words: *Erythrina stricta*, albino rats, carrageenan, paw oedema.

Introduction

Inflammation or phlogosis is a pathophysiological response of living tissue to injuries that leads to the local accumulation of plasmatic fluid and blood cells. Although it is a defense mechanism, the complex events and mediators involved in the inflammatory reaction can be induced, maintained or aggravated to many diseases. However, studies are continuing on inflammatory diseases and the side effects of the

currently available anti-inflammatory drugs pose a major problem during their clinical use. Therefore, development of newer and more powerful anti-inflammatory drugs with lesser side effects is necessary¹.

The plants belonging to Rubiaceae family are found to be a rich source of substances of phytochemical interest². Number of plants from this family is used in traditional system of medicine. *Erythrina stricta* is one

member of this family which is traditionally used as rheumatism³. Literature survey indicates the presence of multiple chemical constituents in these leaves⁴. However, very few references about the evaluation of pharmacological activity of the extract are available indicating its cytotoxicity and CNS activity. The extract has indicated the presence of antibiotic principle with strong inhibitory activity on *Plasmodium falciparum* and *Mycobacterium tuberculosis*⁵. The leaves are used for the treatment of inflammatory conditions as a household remedy on empirical basis. However, no work has been reported on the anti-inflammatory effects on acute and chronic phases of inflammation using *E.stricta*. Keeping this in view, the present study has been undertaken to investigate the anti-inflammatory, potential of ethanol extract of *Erythrina stricta* in experimental animal models.

Materials and Methods

Plant material

Fresh leaves (5 kg) were collected directly from the CIMS PARK, Coonoor, Nilgiri district, Tamil nadu and were authenticated by Mr.G.V.S. Murthy, Joint Director, Botanical survey of India, Tamil nadu Agricultural University, Coimbatore,India. The voucher specimen (BSI/SC/5/23/06-07/Tech 641) of the same has been preserved in our department for future reference. The leaves was cut into pieces, dried in the shade to minimize the loss of volatile constituents and reduced to size with a pestle in a mortar.

Extraction

The small pieces were immersed into ethyl alcohol. Extracts were drawn at the intervals of 24 hours till the extract was almost dark green in colour. The combined extracts were concentrated under reduced pressure when the crude extract (34.3g, 0.686%) was obtained as a greenish thick paste.

Phytochemical analysis

Preliminary phytochemical screening of ethanol extracts of leaves were carried out for the detection of phytoconstituents, using standard chemical tests. Alkaloids, amino acids, flavonoids, carbohydrates, phenolics, steroids, and tannins were detected in the extracts⁶⁻⁸.

Pharmacological screening

Animals

Albino rats of 150-200 g of both sexes were used for anti-inflammatory studies, respectively. Animals were housed in polypropylene cages in an air-conditioned area at 25 ±2° C in 12 hr light dark cycle. They were provided with standard balanced feed and tap water *ad*

libitum. This study conformed to the guiding principles of Institutional Animal Ethics Committee (IAEC), Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Regn.No.817/04/ac/CPCSEA) and the Guide for the Care and Use of Laboratory Animals published by the National Institutes of Health (NIH Publication No. 85-23, revised 1996).

Acute toxicity test

The ethanol extract of *E.stricta* was devoid of any mortality or change in behaviour upto 2 g/kg orally in albino rats. Based on this observation maximum dose of 200 mg/kg orally was used for acute treatment in following experiments⁹.

Anti-inflammatory Activity

Carrageenan induced rat paw oedema: Twenty four rats were divided into 4 groups of rats. Each group of 6 animals. The first group of rats was treated with Vehicle alone. Second and third groups were treated with *Erythrina stricta* extract suspended in vehicle, administered orally at the dose of 100 and 200 mg/kg body weight. The fourth group of rats was treated with standard drug, indomethacin 10 mg/kg body weight, respectively. 0.1 ml of 1 % carrageenan was injected subcutaneously into the planter region of right hind paw to induce odema. The paw volume up to a fixed mark at the level of lateral malleolus, was measured by recording the volume displacement by plethysmometer, just before, and three hours after the injection of carrageenan. Percentage of activity was calculated, and compared with that of the control (saline) and Indomethacin groups^{10,12}.

In formalin-induced paw odema model, the same procedure was carried out, except that 0.05 ml of 1 % formalin was injected instead of carrageenan¹¹. The level of inhibition (%) of oedema was calculated using the relation:

$$\text{Inhibition (\%)} = 100[1 - (\text{Et}/\text{Ec})]$$

Where,

Et= Average oedema of the treated group

Ec= Average oedema of the control group

Cotton pellet granuloma: Cotton pellet granuloma was induced according to the method of D' Arcy et al¹³. Sterile cotton (10 ±1 mg) soaked in 0.2 ml of distilled water containing penicillin (0.1 mg) and streptomycin (0.13 mg) was implanted subcutaneously bilaterally in axilla under ether anaesthesia. The animals were treated with *Erythrina stricta* (100,200 mg/kg, orally) for consecutive six days. Saline (3 mg/kg, orally) treated animals served as control and

indomethacin (10 mg/kg, orally) was administered as standard drug. Subsequently, on 7th day all pellets were dissected out under ether anaesthesia and dried at 70^oC for 6 hours and weight of each granuloma was determined.

Statistical analysis:

The data were analyzed using one-way analysis of variance (ANOVA), followed by Dunnett's test. P <0.05 was considered as statistically significant. The data are expressed as mean \pm SEM. The results are shown in Table 2.

Table 1: Phytochemical constituents of extracts

Constituent	Ethanol extract
Carbohydrates	+
Reducing sugars	+
Glycosides	-
Flavonoids	+
Saponins	+
Tannins	-
Terpenoids	+
Alkaloids	+
Sterols	-
Resins	-

+ = presence of active constituents

- = absence of active constituents

Table 2. Acute anti-inflammatory activities of *Erythrina stricta* extract

Treatment	Carageenan-induced paw oedema		Formalin-induced paw oedema	
	Oedema volume (ml)	% Inhibition	Oedema volume (ml)	% Inhibition
Control	1.22 \pm 0.20	-	1.18 \pm 0.11	-
ES extracts: 100 mg/kg, p.o	0.45 \pm 0.09**	63.00	0.17 \pm 0.04**	85.61
200 mg/kg, p.o	0.26 \pm 0.13**	78.79	0.16 \pm 0.04**	86.86
Indomethacin (10mg/kg, p.o)	0.07 \pm 0.02**	93.9	0.10 \pm 0.05**	91.27

One way ANOVA	F	5.7	24.5
	df	3, 20	3, 20
	P	<0.05	<0.05

Values are mean \pm SEM; n = 6 animals in each group.

**P < 0.05 as compared to control (Dunnett's test).

Table 3. Sub-acute anti inflammatory activity of *Erythrina stricta* extract

Treatment	Weight of granuloma(mg)	Pair wise mean(mg) difference*
Control	35.331 +1.236	-
E.S Extracts;		
100 mg/kg, p.o	24.520 + 0.446	10.811 + 2.217
200 mg/kg, p.o	15.272 + 1.876	20.059 + 2.217
Indomethacin (10mg/kg, p.o)	10.160 + 1.212	25.171 + 2.217

Values are mean \pm SEM; n=6 animals in each group.

One way ANOVA P=0.05 indicating significant. df:3,20

**P < 0.05 as compared to control (Dunnett's test).

Results

The ethanol extracts showed (Table 1) following characteristic features in phytochemical studies.

Based on the Pharmacological studies

Carrageenan - induced rat paw oedema and formalin-induced paw odema:

The extract as well as Indomethacin showed antiphlogistic activity. This anti-inflammatory activity was dose-dependant and found to be statistically significant at the higher concentration, 200 mg/kg, (Table 2). The anti-inflammatory activity of Indomethacin, a standard reference drug, was also found to be significant.

Cotton pellet granuloma:

There was dose dependant reduction in granular tissue formation in extract and indomethacin treated rats as shown in Table 3. The activity was found to be statistically significant for the dose ranges used.

Discussion

The crude ethanolic extract was evaluated for its anti-inflammatory activity in acute and sub acute models. A significant ($p < 0.01$) anti inflammatory activity was observed for ES in carrageenan, formalin and cotton pellet induced granuloma models.

Carrageenan induced rat paw oedema was used as an inflammation model in order to investigate the anti-inflammatory effect of drug¹⁴. There was two phases

of carrageenan-induced inflammatory reaction: early or first phase and later or second phase. It was proposed that early phase results from histamine, serotonin and bradykinin liberation while late phase was associated with the release of prostaglandin¹⁵. In carrageenan induced paw oedema the ES showed maximum inhibition of 78.79 % at the dose of 200 mg/kg after 3 hr of drug treatment.

Formalin induced paw oedema was known to be mediated both by histamine and serotonin. Formalin is induced fluid accumulation. It contain little protein few neutrophils, whereas carrageenan induce protein rich exudation containing large number of neutrophil. The ES also exhibited significant anti-inflammatory activity in formalin induced paw oedema. This study showed that all the doses of ES effectively suppressed the oedema produced by the histamine, which indicated that the extract exhibited its anti-inflammatory action by means of either inhibiting the synthesis, release or action of inflammatory mediators viz. histamine, serotonin and prostaglandin might be involved in inflammation.

From, these results, it is suggested that anti oedematogenic effect of the ES on carrageenan, and formalin induced oedema may be related to inhibition of inflammation mediator formation. Sub acute inflammation is a reaction arising when the acute response is insufficient to eliminate proinflammatory agents. Sub acute inflammation includes a proliferation of fibroblasts and the infiltration of neutrophils and exudation^{16, 17}. Sub acute inflammation occurs by means of the development of proliferate cells. These cells can be either spread or granuloma form. Efficacy of anti-inflammatory agents in sub acute inflammatory

states is indicated by their ability to inhibit the increase in the number of fibroblasts and synthesis of collagen and mucopolysaccharides during granuloma tissue formation is recorded which is due to presence of higher amount of active constituents¹⁸. The *E.stricta* show significant ($P < 0.05$) anti-inflammatory activity in cotton pellet induced granuloma and thus it is found to be an effective in sub acute inflammatory condition.

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