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IN VITRO ANTI-INFLAMMATORY ACTION OF *ERYTHRINA VARIEGATA* (L.) LEAVES BY HRBC MEMBRANE STABILIZATION

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ABSTRACT

The ethanolic extract of the leaves of Erythrina variegata Linn leaves (Fabaceae) were screened for its anti-inflammatory activity. The dried powder of leaves was extracted with ethanol (95%) for the present study. The prevention of hypotonicity induced HRBC membrane lysis was taken a measure of anti-inflammatory activity. The extract showed a significant activity comparable to the standard drug Diclofenac sodium.

Keywords: *Erythrina variegata, anti-inflammatory, HRBC, optical density.*

Introduction

Since time immemorial, our traditional system of medicine and folklore claims that medicinal plants as a whole or their parts are being used in all types of illness starting from infections to chronic ailments. Herbal medicines derived from plant extracts are being increasingly utilized to treat a variety of clinical diseases, though relatively little knowledge about their mode of action is available. There is growing interest in pharmacological evaluation of various plants used in Indian traditional system of medicine. The study of plant species with anti-inflammatory effects is still a fruitful research in search of new agents.

Erythrina variegata Linn. var. *orientalis* (Linn.) Merril (Fabaceae) (Syn: *E. indica*)is a medium sized tree with smooth yellowish or greyish, shining bark cultivated in many parts of India especially in Southern India often for ornamental purpose. The flowers are coral red and serve the purpose [1].The whole plant including the seeds is used for a variety of illness in traditional systems of medicine. The leaves were used to relieve pain and inflammation when crushed and applied to rheumatic joint. Fresh juice of the whole plant is used to cure long standing dysmenorrheal and remove sterility in fatty women by gradually reducing abdominal fat and producing natural menstrual flow [2]. In Siddha system, the entire plant and seed is used for the treatment of stomatitis, dysentery, sterility, diabetes and eye disorders [3]. Different parts of the plant are also used as nervine sedative, antiepileptic and as an antiseptic [1]. The plant and then seeds are reported to possess antihypertensive [4], anti-microbial [5], sedative [6], immunosuppressive [7]and anti-inflammatory properties [8].

Phytochemical investigations on the plant revealed the presence of alkaloids [1, 9], flavanoids and isoflavanoids [10-14]. Phenyl coumarins [15], lectins [16], flavones glycosides [17], steroids and fatty acids [18].

A survey of literature indicated no systemic approach has been made to evaluate the anti-inflammatory potential of *E. variegata* by *in vitro* method. The present study involves estimation anti-

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inflammatory activity of ethanolic extract of *E. variegata* by HRBC membrane stabilization.

MATERIALS AND METHOD

Plant material and preparation of extract

The leaves of *E. variegata* were collected from the villages around Chennai, TamilNadu, India. The identity of the leaves was confirmed by Dr. P. Jayaraman, Director, Plant Anatomy Research Centre. The fresh leaves were shade dried for two weeks and powered with an electric grinder. The coarse powder was extracted with ethanol (95%) and the residue was evaporated under reduced pressure in rotary vacum evaporator at 60°C to obtain a dark brown colored molten mass. The percentage yield was found to be 12.7% w/w.

Anti- inflammatory activity[19]

The HRBC membrane stabilization has been used as method to study the anti-inflammatory activity. Blood was collected from healthy volunteer. The collected blood was mixed with equal volume of sterilized Alsever solution (2% dextrose, 0.8% sodium citrate, 0.5% citric acid and 0.42% sodium chloride in water). The blood was centrifuged at 3000 rpm and packed cell were washed with isosaline (0.90%, pH 7.2) and a 10% (v/v) suspension was made with isosaline. The assay mixture contained the drug,1ml of phosphate buffer (0.15M, pH 7.4), 2ml of hyposaline (0.36%) and 0.5 ml of HRBC suspension. Diclofenac sodium was used as reference drug. Instead of hyposaline 2 ml of distilled water was used in the control. All the assay mixture were incubated at 37^0C for 30 min and centrifuged. The hemoglobin content in the supernatant solution was estimated using spectrophotometer at 560 nm.The percentage hemolysis was calculate by assuming the hemolysis produced in presence of distilled water of as 100%.The percentage of protection was calculated using the formula

$$\% \text{ Protection} = 100 - \frac{\text{Optical Density of drug treated sample}}{\text{Optical Density of control}} x100$$

RESULTS AND DSCUSSION

Inflammation is defined as the local response of living mammalian tissue to injury due to any agent. The body's defense mechanism acts to eliminate the spread of the injurious agent, which may be due to heat, cold, radiation, trauma, organic and inorganic poisons, bacteria, fungi, parasites, antigen-anti body reaction and cell mediated reactions. The lysosomal enzyme released during inflammatory condition produce a variety of disorders. The extra cellular activity of these enzymes is said to be related to acute or chronic inflammation. The anti-inflammatory agents act by either inhibiting the lysosomal enzymes or by stabilizing the lysosomal membranes. The lysosomal enzymes released during inflammation produces a variety of disorders. The standard drug Diclofenac sodium act either by inhibiting these lysosomal enzymes or by stabilizing the lysosomal membrane

Since the HRBC membrane are similar to lysosomal membrane components, the prevention of hypo tonicity induced HRBC membrane lysis is taken as a measure of anti-inflammatory activity of drug. The study results reveal that ethanolic extract of *E. variegata* produces above 90% protection of the HRBC membrane from lysis due to hyposaline at dose level of 1600 µg/ml, when compared to 100% lysis induced in control. The standard drug produced 95.26% protection at a concentration of 50 mg/100 ml (Table 1).

Table 1: In vitro Anti-inflammatory effect of *E. variegata* on HRBC membrane

Dose (µg)	Percentage Protection	Standard
	Ethanolic Extract of *E. variegata*	
50	66.59	
100	73.91	
200	77.28	Diclofenac Sodium
400	81.22	(50 mg)
800	86.03	
1600	90.45	95.26

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