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# EVALUATION OF ANTISECRETORY ACTIVITIES OF *ERYTHRINA VARIEGATA* IN ALBINO RATS

***B.Venkateswarlu and Y.Rama Rao**

Faculty of Biochemistry, Madawalabu University, Bale Robe, Ethiopia.

**ABSTRACT**

*Erythrina variegata* a member of the Fabaceae family, have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic, antiepileptic and antiulcer. The purpose of the present study is to investigate the acute oral toxicity and antiulcer activity of the ethanol extract of *Erythrina variegata* (EEEV) extract in albino rats. Study on acute toxicity of extract found to be safe at the doses 2000mg/kg body weight orally as per OECD guidelines No.423. PMP at the doses of 200 and 400 mg/kg body weight orally was administered to evaluate anti-ulcer activity by using pyloric ligation (PL), and cold-restraint stress induced gastric ulcer models in Albino rats. Ethanol extract of *Erythrina variegata* (EEEV) dose dependent inhibition in pyloric ligation (PL), and cold-restraint stress induced gastric lesions in rats. All the results are found to be statistically significant ($p \leq 0.01$). Hence we suggest that ethanol extract of *Erythrina variegata* (EEEV) possess antiulcer effect that may be due to cytoprotective mechanism. These results support the ethnomedical uses of the plant in the treatment of gastric ulcer.

**Keywords:** *Erythrina variegata*, Antiulcer, Pyloric Ligation (PL), Cold-restraint stress.

## INTRODUCTION

Peptic ulcer is the most common gastrointestinal disorder in clinical practice. Considering the several side effects (arrhythmia's, impotence, gynaecomastia and hematopoietic changes) of modern medicine [1], indigenous drugs possessing fewer side effects should be looked for as a better alternative for the treatment of peptic ulcer.

The genus *Erythrina* comprises of about 110 species of trees and shrubs. The name "coral tree" is used as a collective term for these plants. Coral tree is indigenous to the Old World tropics, possibly originally from India to Malaysia, but is native of ancient westward to Zanzibar and eastward to eastern Polynesia (the Marquesas). It is typically found on sandy soil in littoral forest, and sometimes in coastal forest up to 250m (800ft) in elevation. The coral tree is cultivated particularly as an ornamental tree and as a shade and soil improvement tree (it fixes nitrogen) for other tree crops such as coffee and cacao. The most attractive type, var. *variegata*, is grown for its variegated leaves, as well as its seasonal showy red flowers. This fast-growing, 50-60 feet tall and wide deciduous tree with green and yellow-variegated, 6-inch-long leaves creates a broad canopy but has spiny branches. In spring, before the leaves appear, coral tree is decorated with showy red blossoms, each flower 2.5 inches long and arranged in dense, six-inch-long racemes. These blooms are followed by 12-inch-long, red/brown seedpods which contain poisonous seeds.

Studies on phytochemical of *Erythrina variegata* species (Family: Fabaceae) have demonstrated alkaloids and flavonoids as major constituents. Different parts of *E. Variegata* have used in traditional medicine as nervine sedative, febrifuge, anti-asthmatic and antiepileptic. In the some experiments, it has potential effects for treatment of some diseases like convulsion, fever, inflammation, bacterial infection, insomnia, helminthiasis, cough, ulcer, cuts and wounds [2-7]. From the source of literature documentation and relevant traditional approaches on plant drugs, the present investigation was carried out to investigate the antisecretory effect of the ethanol extract of *Erythrina variegata* whole plant is being reported here.

## MATERIALS AND METHODS

### Plant material

The whole plant of *Erythrina variegata* was

Corresponding Author :- **B.Venkateswarlu** Email:- b_venki22@yahoo.co.in

collected from Tirumala hills, Tirupati, Andhra Pradesh. India. It was identified and authenticated by Prof. *Madhava Chetty, K.*, Taxonomist, S.V. University, Tirupati, Andhra Pradesh, India. A voucher specimen has been kept in our laboratory for future reference.

**Preparation of plant extract**

The collected plant was dried at room temperature, pulverized by a mechanical grinder, sieved through 40mesh. About 100g of powdered materials were extracted with ethanol (90%) using soxhlet apparatus. The extraction was carried out until the extractive becomes colourless. The extracts is then concentrated and dried under reduced pressure. The solvent free semisolid mass thus obtained is suspended in tween 80 and used for the experiment. The percentage yield of prepared extract was around 9.5%w/w.

**Animals Used**

Albino rats (180–200 g) of either sex were maintained in a 12 h light/dark cycle at a constant temperature 25 ◦C with free access to feed (Sai durga feeds and foods, Bangalore) and water. All animals were fasted prior to all assays and were allocated to different experimental groups each of 6 rats. Moreover the animals were kept in specially constructed cages to prevent coprophagia during the experiment. All experiments were carried out according to the guidelines for care and use of experimental animals and approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). Ethical committee clearance was obtained from IAEC (Institutional Animal Ethics Committee) of CPCSEA.

**Acute toxicity study**

The procedure was followed according to the OECD guidelines 423 (Acute toxic class method). The acute toxic class method is a step wise procedure with 3 animals of single sex per group. Depending on the mortality and or moribund status of the animals, on an average 2-4 steps may be necessary to allow judgment on the acute toxicity of the testing substance. According to this procedure minimum number of animals were to be used for acceptable data band scientific conclusion. The method uses defined doses (5, 50, 300, 2000 mg/kg body weight) and the results allow a substance to be ranked and classified according to the globally harmonized system (GHS) for the classification of chemical which causes acute toxicity.

Adult female wistar rats were used for this study. The starting dose of whole plant of *Erythrina variegata* extract (EEEV) was 2000 mg/kg body weight, as most of the crude extracts possess $LD_{50}$ value more than 2000 mg/kg body weight. The dose was administered to overnight fasted rats and food was withheld for a further 3-4 hours after administration of the drug and observed for signs of toxicity. Body weight of the rats before and after treatment were noted and any changes in skin, eye, and mucous membranes, salivation, nasal discharge, urination and behavioral (sedation, depression), neuromuscular (tremors, convulsions), cardiovascular, lethargy, sleep and coma were noted. The onset of toxicity was also noted. The animals were kept under observation for 14 days.

The acute toxicity of ethanol extract of whole plant of *Erythrina variegata* was determined as per the OECD guideline no. 423 (Acute Toxic Class Method). It was observed that the test extract was not lethal to the rats even at 2000mg/kg dose. Hence, $1/10^{th}$ (200mg/kg) and $1/5^{th}$ (400mg/kg) of this dose were selected for further study [8].

**ANTI-ULCER ACTIVITY**

**Pyloric ligation induced gastric ulcer**

Animals were divided into four groups each of six rats. Group I treated with 1% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with ethanol extract of whole plant of *Erythrina variegate suspended in* 1% v/v aqueous tween 80 (EEEV 200 & 400mg/kg p.o) respectively for 5 days and Group IV treated with Omeprazole (20 mg/kg p.o) were administered 30min prior to induction of gastric ulcer. On the $5^{th}$ day, all group rats were fasted 24 h prior to induction of gastric ulcer. Pyloric ligation was done by ligating the pyloric end of the stomach of rats 1 h after drug administration [9]. Animals were allowed to recover and stabilized in individual cage and were deprived of water during post-operative period. After 4 h of surgery, rats were sacrificed by cervical dislocation and ulcer index were examined on the dissected stomachs as described below.

**Cold-restraint stress-induced ulcers**

Animals were divided into four groups each of six rats. Group I treated with 1% v/v aqueous tween 80 (10 ml/kg p.o), Group II & III treated with ethanol extract of whole plant of *Erythrina variegate suspended in* 1% v/v aqueous tween 80 (EEEV 200 & 400mg/kg p.o) respectively for 5 days and Group IV treated with Omeprazole (20 mg/kg p.o). On the $5^{th}$ day, One hour after drug treatment, the experimental rats were immobilized by strapping the hind limbs on a wooden plank and kept for 1 h 30min, at temperature of 3–5°C [10]. One hour later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomachs as described below.

**Measurement of ulcer index**

The stomachs were excised and were examined for hemorrhagic lesions in glandular mucosa. Immediately after the animals were sacrificed, their stomachs were dissected out, cut along the greater curvature and the mucosa were rinsed with cold normal saline to remove blood contaminant, if any. The sum of the length (mm) of

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all lesions for each stomach was used as the ulcer index (UI), and the percentage of inhibition (%*I*) was calculated as described by Nguelefack et al. (2005) [11] using the following formula:

$$\%I = \frac{(USc - USt)}{USc} \times 100$$

Where USc = ulcer surface area in control and USt = ulcer surface area in treated animals.

**Statistical analysis**

The data were expressed as mean ± standard error mean (S.E.M). The Significance of differences among the group was assessed using one way and multiple way analysis of variance (ANOVA). The test followed by Dunnett's test p values less than 0.05 were considered as significance.

**RESULTS**

**Acute Oral Toxicity**

The acute oral toxicity study was done according to OECD guidelines 423 (acute toxic class method). A single dose of 2000 mg/kg body weight/po of the EEEV was administered to 6 female rats. Animals were observed for signs of toxicity for first 3 hours at 30 min time intervals. Thereafter animals were observed for 24 hours with continuous monitoring. The animals were observed for further 14 days period for all toxicity signs. There was no considerable change in body weight before and after treatment and no signs of toxicity were observed. $LD_{50}$ cut off dose per kilogram body weight was categorized as X (unclassified). The results are shown in Table -1.

**Effect of ethanol extract of whole plant of *Erythrina variegate* on gastric ulcer induced by pylorus ligation (PL)**

The ethanol extract of whole plant of *Erythrina variegate* showed significant anti-ulcer effect against ulcers induced by pylorus ligation in a dose dependent manner. In PL induced ulcer model, ethanol extract of whole plant of *Erythrina variegate* at a dose of 200 and 400 mg/kg body weight showed significant protective effect same like Omeprazole showed (Table 2).

**Effect of ethanol extract of whole plant of *Erythrina variegate* on gastric ulcer induced by Cold-restraint stress**

The ethanol extract of whole plant of *Erythrina variegate* showed significant anti-ulcer effect against ulcers induced by *Cold-restraint stress* in a dose dependent manner. In the gastric ulcer induced by *Cold-restraint stress*, ethanol extract of whole plant of *Erythrina variegate* at a dose of 200 and 400 mg/kg body weight showed again significant activity. Ethanol extract of whole plant of *Erythrina variegate* at a dose 200 and 400 mg/kg body weight showed dose-dependent significant protective effect same like Omeprazole showed protection. (Table 3).

**Figure 1. Effect of Ethanol Extract of *Erythrina variegate* (EEEV) in pylorus ligation Induced ulcer model**

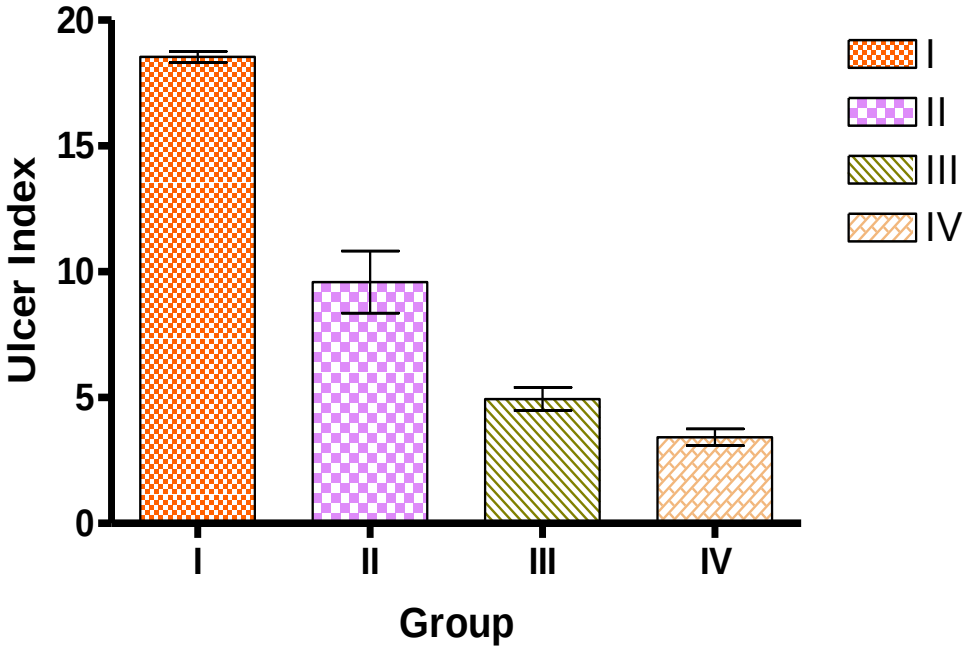

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**Figure 2. Percentage Inhibition of Ethanol Extract of *Erythrina variegate* (EEEV) in pylorus ligation Induced ulcer model**

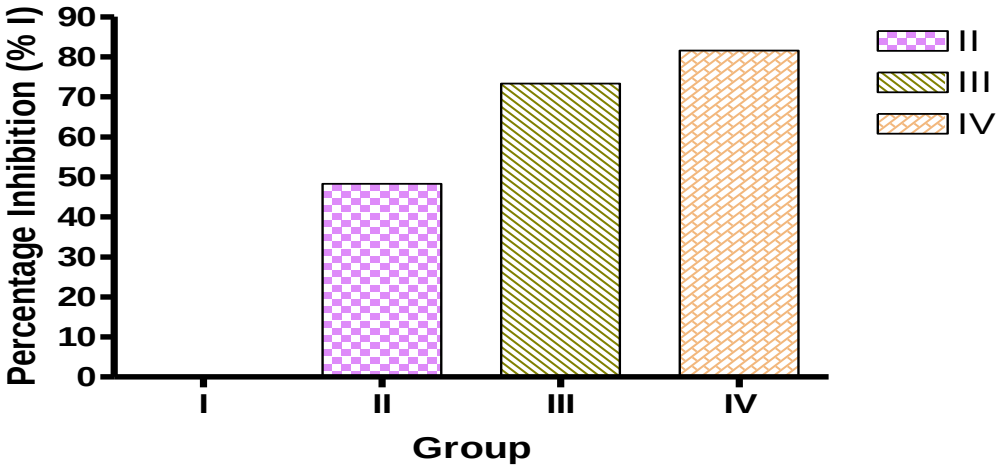


**Figure 3. Effect of Ethanol Extract of *Erythrina variegate* (EEEV) on Cold-restraint stress induced Gastric ulcer in Rats**

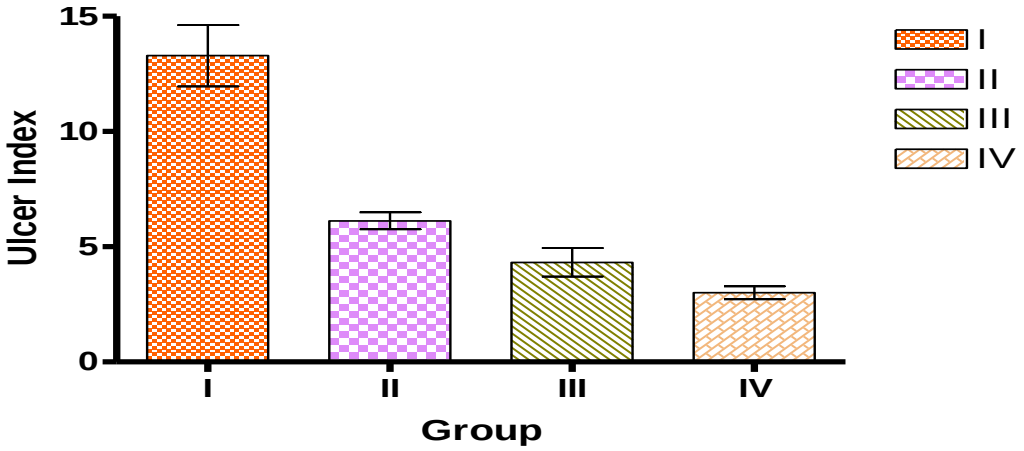


**Figure 4. Percentage Inhibition of Ethanol Extract of *Erythrina variegate* (EEEV) on Cold-restraint stress induced Gastric ulcer in Rats**

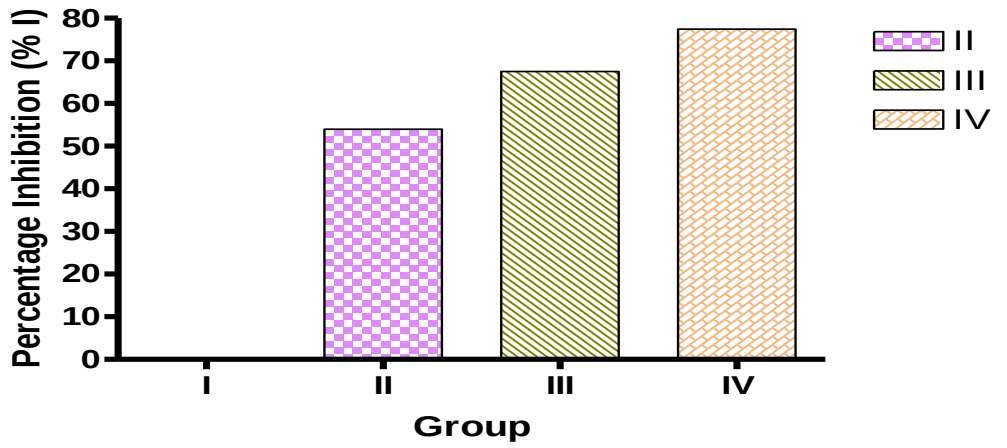

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**Table 1. Acute Oral Toxicity Study ethanol extract of *Erythrina variegate***

| S.No | Treatment | Dose | Weight of the animal in grams | | Signs of toxicity | Onset of toxicity | Reversible or Irreversible | Duration |
|---|---|---|---|---|---|---|---|---|
| | | | Before test (1st day) | After test (14th day) | | | | |
| **1** | EEEV | 2 g/kg | 190 | 195 | No signs of toxicity | Nil | Nil | 14 days |
| **2** | EEEV | 2 g/kg | 205 | 210 | No signs of toxicity | Nil | Nil | 14 days |
| **3** | EEEV | 2 g/kg | 200 | 210 | No signs of toxicity | Nil | Nil | 14 days |
| **4** | EEEV | 2 g/kg | 210 | 215 | No signs of toxicity | Nil | Nil | 14 days |
| **5** | EEEV | 2 g/kg | 210 | 220 | No signs of toxicity | Nil | Nil | 14 days |
| **6** | EEEV | 2 g/kg | 195 | 205 | No signs of toxicity | Nil | Nil | 14 days |

**Table 2. Effect of Ethanol Extract of *Erythrina variegate* (EEEV) in pylorus ligation Induced ulcer model**

| Group | Design of Treatment | Ulcer Index | Percentage Inhibition (% I) |
|---|---|---|---|
| I | Control (1% v/v aqueous tween 80, 10 ml/kg b.w ) p.o | 18.54 + 0.22 | - |
| II | EEEV (200mg/kg b.w) p.o | 9.59 + 1.24* | 48.27 |
| III | EEEV (400mg/kg b.w) p.o | 4.95 + 0.46** | 73.30 |
| IV | Omeprazole (20mg/kg b.w) p.o | 3.42 + 0.33** | 81.55 |

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. $^{*}P < 0.01$ and $^{**}P < 0.001$ as compared to control (n = 6 in each group).
EEEV = Ethanol Extract of *Erythrina variegate;*
B.W=Body weight.

**Table 3. Effect of Ethanol Extract of *Erythrina variegate* (EEEV) on Cold-restraint stress induced Gastric ulcer in Rats**

| Group | Design of Treatment | Ulcer Index | Percentage Inhibition (% I) |
|---|---|---|---|
| I | Control (4% v/v aqueous tween 80, 10 ml/kg b.w ) p.o | 13.29 + 1.33 | - |
| II | EEEV (200mg/kg b.w) p.o | 6.12 + 0.37* | 53.95 |
| III | EEEV (400mg/kg b.w) p.o | 4.32 + 0.62** | 67.49 |
| IV | Omeprazole (20mg/kg b.w) p.o | 3.00 + 0.28** | 77.43 |

Data are represented as mean ± S.E.M. Statistical analysis was done by one-way ANOVA followed by Dunnett's multiple comparison test. $^{*}P < 0.01$ and $^{**}P < 0.001$ as compared to control (n = 6 in each group). EEEV = Ethanol Extract of *Erythrina variegate*
B.W=Body weight.

## DISCUSSION & CONCLUSION

The results of this study show that the ethanol extract of whole plant of *Erythrina variegate* exert protective effects against pylorus ligation and cold restraint stress-induced gastric mucosal damage. In order to probe the effectiveness of ethanol extract of whole plant of *Erythrina variegate* in preventing gastric ulcer and also assess their antisecretory activity, they were tested against pylorus ligation- and cool stress induced ulcer. Pylorus ligation- [12] and cold restrained stress- induced ulcers are results of auto digestion of the gastric mucosal barrier probably due to excess production and accumulation of HCl in the stomach. Gastric acid is an important factor for the genesis of ulceration in pylorus-ligated rats. The activation of the vagus-vagal reflux by stimulation of pressure receptors in the antral gastric mucosa in the hyper secretion model of pylorus ligature is believed to increase gastric acid secretion [13]. The current data clearly demonstrated that, EEEV in a dose-dependent manner decreased hydrogenionic concentration suggesting that the pharmacological mechanism has a relationship to anti secretory activity (Table 2).

To further confirm its anti-ulcerogenic effect we have evaluated the efficacy of EEEV against Cold-restraint stress -induced ulcer model. Gastric ulceration induced by stress is probably mediated by the presence of acid, increase in gastric motility, [14] mast cell degranulation, decreased gastric mucosal blood flow [15], decreased prostaglandin synthesis [16] and augmented excretion of glycoproteins in the mucus. Moreover, stress-induced ulcer

can be prevented partially or entirely by vagotomy; vagal over activity has been suggested to be the principal factor in stress-induced ulceration [17]. Any of these factors could play a role in genesis of stress-induced ulcers. Oral administrations of the ethanol extract of whole plant of Erythrina variegate showed dose dependent inhibition of gastric ulceration induced by Cold-restraint stress (Table 3).

The ethanol extract of whole plant of *Erythrina variegate* at a dose of 400mg/kg showed similar activity to that of omeprazole (a proton pump inhibitor, which is used to heal stomach and duodenal ulcers). The gastro protective effect of omeprazole is mediated through block of acid secretion by inactivation of H+/K+-ATPase [18,19]. This study reveals that the ethanol extract of whole plant of *Erythrina variegate* are potent inhibitors of gastric mucosal lesions caused by pylorus ligation and cold-restraint stress in rats.

Further, our results fortify the ethano pharmacological importance of EEEV as an anti-ulcer agent. Etiology of ulcers produced in different ulcer models is diverse. Since EEEV has been found effective in various models depicting its antiulcer activity. EEEV and its active constituents may emerge as more effective therapeutic agent to counter gastric ulcer incidence. However more experimentation, detailed phytochemical and experimental analysis are required for a definitive conclusion.

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