



Anti-diarrhoeal Potential of *Erythrina indica Lam*-leaf extracts in Laboratory Animals

R. Kamalraj.*

Bio Analytical Department, Hospira Healthcare India Pvt. Ltd, Irungattukottai-602 105, India

ABSTRACT

Erythrina indica Lam leaf has been used traditionally in ayurveda for the treatment of diarrhoea and dysentery. However, the claims of ayurveda need to be validated by a suitable experimental model. Therefore, the present study was undertaken to evaluate the effect of ethanol and aqueous extracts of *Erythrina indica* leaf for its anti-diarrhoeal potential against several experimental models of diarrhoea in albino Wistar rats. The anti-diarrhoeal activity of ethanol and aqueous extracts of *Erythrina indica lam* leaf at 500 mg/kg dose level was evaluated using castor oil-induced diarrhoea model in rats. Further, we evaluated the effect of ethanol and aqueous extracts on gastrointestinal tract motility after charcoal meal administration and PGE₂ induced intestinal fluid accumulation (Enteropooling) showed significant inhibitor activity. Loperamide was used as positive control. The results point out the possible anti-diarrhoeal effect of the leaf extracts and substantiate the use of this herbal remedy as a non-specific treatment for diarrhoea in folk medicine.

Keywords: Anti-diarrhoeal activity, *Erythrina indica lam*, castor oil-induced diarrhea, gastrointestinal tract motility.

INTRODUCTION

Diarrhoea has long been recognized as one of the most important health problems in the developing countries. [1] Secretory diarrhoea is the most dangerous symptom of gastrointestinal problems [2] and is associated with excessive defecation and stool outputs, the stools being of abnormally loose consistency. [3] *Erythrina indica Lam* is a common plant found in India. It is known as kalyana murukku [4] or mullu murukku in Tamil. In siddha system, it is being considered useful for treating antihelminthiasis, sedative, anti-inflammatory, nematocidal and worm infection. [5-7] The presence of active constituents viz. alkaloids, glycosides, phenyl coumarin, proteins, carbohydrates, amino acids, steroids, tannins [8-9] have been reported from root and seeds. Since no scientific proof about anti-diarrhoeal activity, in leaf extract of *Erythrina indica lam*, an attempt has been made to explore such activity for *Erythrina indica lam*. In the present work, vacuum dried aqueous and ethanol extracts were evaluated for anti-diarrhoeal activity.

MATERIALS AND METHODS

Plant material

The fresh leaves of *Erythrina indica Lam* were collected from in and around Chennai and identified by Dr. Sasikala,

Department of Pharmacognosy, Captain Srinivasamoorthy Drug Research Institute of Ayurveda, Chennai.

Extraction

Air dried coarsely powdered plant material was extracted with water and ethanol for 48 hours by maceration. Thus obtained water extract were filtered and vacuum dried using vacuum flash evaporator to yield the solid residue of 18.8±.45% and 17.1 ±.35% respectively to the starting dry powder.

Animals

Inbred Wister albino rats of either sex (180-250 g) were used for the evaluation of pharmacological activities. They were kept in colony cages at 25±2°C; relative humidity % and maintained under 12 hours light and dark cycle (0600-1800 h-light; 1800-0600 h-dark). All the animals were fed with standard animal feed (Hindustan Lever Limited) and water *ad libitum*. Acute toxicity study was performed for the extracts to ascertain the safe dose by acute oral toxic class method of organization of Economic Co-operation and Development, as per 423 guide lines (OECD). [8] The aqueous and ethanol extracts were at the dose level of 500 mg/kg.

Castor oil-induced method

Anti-diarrhoeal activities of the extracts were evaluated by Castor oil-induced diarrhoea. For that purposes following experimental procedure are used.

The animals were divided into four groups of six each, marked to individual identification and kept in their cages. They are as follows

*Corresponding author: Mr. R. Kamalraj, Bio Analytical Department, Hospira Healthcare India Pvt. Ltd, Irungattukottai-602 105, India;
E-mail: kamalrajkb@gmail.com

Group A: Aqueous extract of leaves of *Erythrina indica* Lam (500 mg/kg) suspended 1 % CMC

Group B: Ethanol extract of leaves of *Erythrina indica* Lam (500 mg/kg) suspended in 1 % CMC.

Group C: 1 ml of carboxyl methyl cellulose (1% CMC) control.

Group D: Loperamide 5 mg/kg (standard)

One hour after the above treatment, all the groups received 2 ml/kg b.w. Castor oil orally and each rat was then housed separately in the cages provided with a clean plastic sheet at the bottom and observed for the frequency of defecations and wet fecal droppings for 4 hours. After each hour the filter paper with stools was changed and frequency of defecation and total weight of stools for the total 4 hours was noted. [10]

Gastrointestinal motility Test

Rats were divided into four groups (n = 6) and fasted for 18 h before the experiment. Each animal was orally administered with 1 ml of charcoal meal (5% deactivated charcoal in 10% aqueous tragacanth) followed by oral administration of ethanol and aqueous leaf extracts *Erythrina indica* Lam to the first two groups of animals in the dose of 500 mg/kg. The third group was treated with 1 ml/kg of carboxyl methyl cellulose (1% CMC) served as a negative control. The fourth group received Loperamide 5 mg/kg (standard) as the positive control. Thirty minutes later, each animal was killed and the intestinal distance moved by the charcoal meal from the pylorus to caecum was measured and expressed as percentage of distance moved. [12]

PGE₂ induced Enteropooling

In this method rats were deprived of food and water for 18 h and placed in four cages, with six animals per cage. The first two groups were treated with 500 mg/kg dose of ethanol and aqueous leaf extracts *Erythrina indica* Lam. The third group was treated with 1 ml of a 5% v/v ethanol in normal saline (i.p.) and then it was treated with 0.5% Tween 80 suspension, which served as a negative control. Immediately after the extract administration PGE₂ (Astra Zeneca, India) was administered orally to each rat (100 µg/kg) in the first three groups. The fourth group was treated with PGE₂ (100µg/kg) as well as 0.5% Tween 80 suspension and served as the PGE₂ positive control group. After 30 min following administration of PGE₂, each rat was sacrificed and the whole length of the intestine from the pylorus to the caecum was dissected out, its content collected in a test tube, and the volume measured. [11]

Statistical analysis

All the grouped data were statically evaluated and the significant of various treatments was calculated using student's t-test data. All the results were expressed as mean ± S.D. from 6 rats in each group.

RESULTS AND DISCUSSION

Castor oil-induced Diarrhoea

Administration of castor oil produced characteristic semi-solid diarrhoea dropping in 18 h starved rats of the control group during the 4 h observation period (Table 1).

The ethanol and aqueous extracts at doses of 500 mg/kg showed significant ($p < 0.01$) reduction in the number of defecations over four hours when compared to that of untreated control rats; the activity was similar to that of Loperamide (5 mg/kg), the standard anti-diarrhoeal agent. Both ethanol and aqueous extracts delayed the onset of diarrhoea and rats were protected against castor oil-induced diarrhoea at four hour, respectively.

Small Intestinal Transit

The ethanol and aqueous extracts decreased propulsion of the charcoal meal through the gastrointestinal tract at the oral dose of 500 mg/kg; as compared with control group. A similar reduction in the gastrointestinal transit of charcoal meal in rat was achieved with the standard Loperamide (5 mg/kg) (Table 2).

PGE₂-induced Enteropooling

Both extracts significantly inhibited PGE₂ induced Enteropooling in rats at an oral dose of 500 mg/kg (Table 3). PGE₂ induced a significant increase in the fluid volume of the rate intestine when compared with control animals received ethanol in normal saline. The ethanol and aqueous leaf extract of *Erythrina indica* Lam exhibited significant anti-diarrhoeal activity against castor oil induced diarrhoea in rats. The extracts had a similar activity as Loperamide, when tested at 500 mg/kg and statistically significant reduction in the frequency of defecation and the wetness of the faecal droppings when compared to untreated control rats. It is widely known that castor oil or its active component Ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a Hypersecretory response and diarrhoea. The experimental studies in rats demonstrated a significant increase in the portal venous PGE₂ concentration following oral administration of castor oil. Ricinoleic acid markedly increased the PGE₂ content in the gut lumen and also caused an increase of the net secretion of the water and electrolytes into the small intestine. The liberation of Ricinoleic acid from castor oil results in irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion. [12-16] Inhibitors of prostaglandin biosynthesis delayed castor oil induced diarrhoea.

The extract appears to act on all parts of the intestine. Thus, it reduced the intestinal propulsive movement in the charcoal meal treated model at 500 mg/kg both extracts showed activity similar to that of Loperamide. The results show that the ethanol and aqueous extracts suppressed the propulsion of charcoal meal thereby increased the absorption water and electrolytes.

The extracts also significantly inhibited the PGE₂ induced intestinal fluid accumulation (Enteropooling). It has been shown that E type of prostaglandins cause diarrhoea in experimental animals as well as human beings. [17] These observations tend to suggest that both extracts at a dose of 500 mg/kg reduced diarrhoea by inhibiting PGE₂ induced intestinal accumulation of fluid.

Table 1: Effect of the aqueous and ethanol extracts of *Erythrina indica* Lam-leaf in castor oil induced diarrhoea

Group	Treatment	Mean /Defecation in 4 hours ± SEM	Mean Number of Wet Feces in 4 hours ± SEM
Group A	Aqueous extract 500 mg/kg	1.67±0.252**	1±0.154**
Group B	Ethanol Extract 500 mg/kg	1.41 ± 0.291**	0.83 ± 0.09**
Group C	1 % CMC 1mg/kg	4.5 ± 0.3342	4 ± 0.518
Group D	Loperamide 5mg/kg	0.33 ±0.211**	0

Statistical Significance ** P< 0.01 difference test (Vs) Control

The Values or expressed as mean ± SEM ** P<0.01 statistically significant from control

Table 2: Effect of the aqueous and ethanol extracts of *Erythrina indica* Lam- Leaf in gastro-intestinal motility using charcoal meal

Group	Treatment	Mean % movement of charcoal \pm SEM
Group A	Aqueous extract 500mg/kg	25.1 \pm 2.21
Group B	Ethanol Extract 500 mg/kg	35.45 \pm 064
Group C	1 % CMC 1mg/kg	66.05 \pm 2.03
Group D	Loperamide 5mg/kg	35.42 \pm 1.09**

Statistical Significance ** P< 0.01 difference test (Vs) Control

The Values or expressed as mean \pm SEM ** P<0.01 statistically significant from control

Table 3: Effect of the aqueous and ethanol extracts of *Erythrina indica* Lam- Leaf in PGE₂-induced Enteropooling.

Group	Treatment	Volume of intestinal fluid (ml)	P-values
Group A	Aqueous extract 500mg/kg	1.85 \pm 0.12	0.01 ^b
Group B	Ethanol Extract 500 mg/kg	2.02 \pm 0.05	0.01 ^b
Group C	Ethanol in saline	0.79 \pm 0.05	-
Group D	PGE ₂ in ethanol (100 μ g/kg)	3.05 \pm 0.01	0.001 ^a

Results are expressed as mean \pm S.E.M., n = 6. Statistical significance test with control was done by Anova test. aWith respect to ethanol in saline treatment. bWith respect to PGE₂ treatment.

The results indicate that the ethanol and aqueous extract of *Erythrina indica* Lam possesses significant anti-diarrhoeal activity due to its inhibitory effect both on gastrointestinal propulsion and fluid secretion. Further detailed investigations are underway to determine the exact phytoconstituents which are responsible for the anti-diarrhoeal activity.

ACKNOWLEDGEMENTS

The authors are grateful to M/s Astra Zeneca Limited, Bangalore, India for providing free samples of PGE₂ and wish to acknowledge the management of Hospira Healthcare India Pvt. Ltd. for providing facilities.

REFERENCE

1. Snyder JD, Merson MH. The magnitude of the global problem of acute diarrhoea disease: A review of active surveillance of data. Bull WHO.1982; 60:605-613.

2. Fontaine O. Diarrhoea and treatment. Lancet.1988; 28:1234-1235.
3. Aranda MJ, Gianella RA. Acute diarrhoea: A practical review. Am J Med.1999; 106:670-676.
4. Vaidyaratnam KS. Murugesu Mudaliyar Siddha Materia Medica. 1998, pp. 248.
5. S.Jayakumari, V.Ravichandiran, J.Karthik, N.Deepa, J.Anbu, A.Vijayalakshmi. Anti-Inflammatory Activity Of *Erythrina Indica* Linn. Leaves.2009; 5(2):56.
6. Ratna sooriya.W.D,Dharmasiri.G. Aqueous extract of srilankan *erythrina indica* leaves has sedative but not analgesic activity. Fitoterapia.1992; 70(3); 311-313.
7. Nihal Singh Verma , Paras Gupta. Investigation of anthelmintic activity of polyherbal preparation. IJPLS,2011;2(2):590-591.
8. Ecobichon DJ. The Basis of Toxicology Testing. CRC Press, New York 1997:43-86.
9. Lausell S. Serum triglyceride estimation. Scand J Clin Lab invest 1966; 18; pp. 668-70.
10. Gnanasekar N, Perianayagam JB. Influence of sodium curcuminat on castor oil induced diarrhoea in rats. Indian J Pharmacol. 2004; 36(3):177-178.
11. AM Mujumdar. Antidiarrhoeal activity of *Azadirachta indica* leaf extract. Indian Drugs, 1998; 35:417-20.
12. Ammon HV, Thomas PJ, Phillips S. Effect of oleic and ricinoleic acid on net jejunal water and electrolyte movement. J Clin Invest. 1974; 53:374-379.
13. Gaginella TS, Stewart JJ, Olson WA, Bass P. Actions of ricinoleic acid and structurally related fatty acid on the gastro-intestinal tract II. Effects on water and electrolyte absorption *in vitro*. J Pharmacol Exp Ther. 1975; 195:355-361.
14. Luderer JR, Dermers IM, Hayes AT. Advances in Prostaglandin and Thromboxane Research. Raven Press, New York, 1980, pp.1633-1638.
15. Beubler E, Juan H. Effect of ricinoleic acid and other laxatives on net water flux and prostaglandin E release by the rat colon. J Pharm Pharmacol.1979; 31:681-685.
16. Pierce NF, Carpenter CCJ, Elliott HZ, Greenough WB, Effects of prostaglandins, theophylline and cholera exotoxin upon transmucosal water and electrolyte movement in canine jejunum. Gastroenterology.1971; 60:22-32.
17. Eakins KE, Sanner JM. Prostaglandins Antagonists in Karim SMM. Wiley-Interscience, New York, 1972, pp. 263-264.