

ANTISPASMODIC STUDIES ON LEAF EXTRACT OF *ERYTHRINA INDICA* LAM

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Received on: 02/07/2011 Revised on: 30/07/2011 Accepted on: 10/08/2011

ABSTRACT

The aim of the present investigation to evaluate the antispasmodic activity of *Erythrina indica* leaf, an indigenous plant used in ayurvedic medicine in India. The antispasmodic effect of hexane extracts of *Erythrina indica* Leaf were studied *in vitro* in guinea pig ileum against three spasmogens; acetylcholine, histamine and barium chloride. The hexane extract produces a significant antispasmodic effect on the contractions of the guinea pig ileum induced by acetylcholine, histamine and barium chloride. The inhibitory concentration for each was determined. These results show that hexane extract of *Erythrina indica* Leaf possesses antispasmodic properties.

KEYWORDS: Antispasmodic activity, *Erythrina indica* Lam, guinea pig, Ileum.

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INTRODUCTION

Antispasmodics are muscular relaxants that are used to relieve cramps or spasms of the stomach, intestines and bladder. They are commonly used for the treatment of different gastrointestinal disorders, including diarrhea and irritable bowel syndrome, which affect millions of people. Diarrhea continues to be one of the leading causes of mortality and morbidity especially in children in developing countries¹. Diarrhea results from an imbalance between the absorptive and secretory mechanisms in the intestinal tract, accompanied by intestinal hurry, and an excess loss of fluid in the faeces. In some diarrhoea the secretory component predominates, and other diarrhoeas are characterized by hypermotility². Many people nowadays turn to the use of natural product medicine for treatment of intestinal disorders. Natural products have served as a source of medicines for centuries, and about half of the pharmaceuticals in use today are derived from natural products³. Dependence on plants as the source of medicines is prevalent in developing countries where traditional medicine plays a major role in health care⁴. Specifically, the aim of this study was to test the antispasmodic activity of *Erythrina indica* leaf extract in guinea pig ileum induced by acetylcholine, histamine and barium chloride.

MATERIALS AND METHODS

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 hexane for 48 hours by maceration. Thus obtained hexane extract were filtered and vacuum dried using vacuum flash evaporator to yield the solid residue of 8.8% respectively to the starting dry powder. Then the extracts were sonicated before addition to the organ bath, acetylcholine, histamine and adrenaline were prepared by adding the substance directly to Tyrode solution.

Biological experimental procedures

Animal's male guinea pigs (250 - 400 g) were used in all experiments. The animals were housed in a cage under conditions of standard light (light on from 7.0 a.m. - 7.0 p.m.), temperature ($22 \pm 1^\circ\text{C}$) and room humidity ($60 \pm 10\%$) conditions for one week before the experimental sessions. All the animals were fed with standard animal feed (Hindustan Lever Limited) and allowed tap water *ad libitum*. The procedures involving animals and their care conformed to the international guidelines Principles of Laboratory Animals Care.

Tissue preparation

Male guinea pigs (250 - 400 g) were sacrificed by a blow to the base of the skull and cervical dislocation and 2 cm pieces of the ileum were dissected from the ileum segment 10 - 20 cm proximal to the ileocecal valve. Material was mounted for tension recording and allowed to equilibrate for 1 - 2 h in 10 ml chambers containing Tyrode solution [composition (mM): 136.0 NaCl, 5.0

KCl, 0.98 MgCl₂, 2.0 CaCl₂, = 0.36 NaH₂PO₄, 11.9 NaHCO₃, and 5.5 glucose], pH 7.4 maintained at 37 °C and bubbled with air (5% CO₂ and 95% oxygen). In solution with elevated [K]⁺, [Na]⁺ was simultaneously decreased to maintain isosmolarity⁵. Concentration-effect curves for extracts were performed by cumulative addition to the bath. In experiments examining the relaxation of the basal tonus of the ileum, paired segments of ileum were set up; one piece exposed to the extract and the other receiving no treatment. Relaxation was taken to be the difference between the tonus of the control and test segments for recording the contractions using force traducers connected to a polygraph (Grass D) as previously described⁶.

Measurement of contractile activity

After stabilization for 30 min, the test extracts were added to the bath. The extracts were dissolved in Dimethylsulfoxide (Merck). In control preparations of Dimethylsulfoxide, up to 100 mL were added to the organ bath to determine whether this vehicle alone was able to induced contractions. Next, the antispasmodic effect was investigated according to the following experimental schedule. (i) Hexane extract at concentrations of 50, 100, 200 and 300 µg/mL organ bath: 15 min contact period. (ii) When stable submaximal responses to standard agonist histamine 3 X 10⁻⁷ M, acetylcholine 10⁻⁸ M, and barium chloride 10⁻⁴ M were obtained, the extract were added into the bath⁷. Percentage inhibitions of histamine, acetylcholine or barium chloride-induced contraction, in the presence of extract, were calculated for each concentration⁸. (iii) The median inhibitory concentration (IC₅₀) was determined from the graph plotted of percent inhibition versus log dose.

Data analysis

The inhibition of ileal contractions by extracts were expressed as mean ± standard deviation (SD) of three replicates. Where applicable, the data were subjected to one way analysis of variance (ANOVA) and the differences between samples were determined by Duncan's multiple range test using the Statistical Analysis System (SAS, 1999) programmer. P values < 0.05 were regarded as significant.

RESULTS

The concentration of extracts which inhibited 50% of response (median inhibitory concentration) IC₅₀ was determined from the graph plotted of percent inhibition versus log dose. Addition of hexane extract of *Erythrina indica* Lam (50- 300 µg/mL) elicited a progressively increasing relaxation of the spontaneous tonus of the ileum with IC₅₀ = 83.75 µg/mL (c.l.: 79 – 93 µg/mL, n = 6). In a preliminary screening, the histamine induced

contraction in guinea pig ileum with IC₅₀ = 22 µg/mL (c.l.: 12-28 µg/mL, n = 6), acetylcholine with IC₅₀ = 27 µg/mL (c.l.: 15-30 µg/mL, n = 6), and barium chloride with IC₅₀ = 48 µg/mL (c.l.: 25-52 µg/mL, n = 6). The IC₅₀ for papaverine, used as a reference compound, were 3.4 µg/mL (c.l.: 1.2-4.5 µg/mL, n = 6), for histamine, 3.8 µg/mL (c.l.: 2.1-5.1 µg/mL, n = 6), for acetylcholine and 3.0 µg/mL (c.l.: 1.8 - 4.2 µg/mL, n = 6) for barium chloride-induced contractions respectively. The antispasmodic effects of *Erythrina indica* Lam (50-300 µg/mL) are shown in the **Figures 1 to 4**. The hexane extract of *Erythrina indica* Lam showed a concentration- dependent inhibition of tone and the amplitude of spontaneous contraction of ileum with IC₅₀ = 62.1 µg/mL (c.l.: 51-79 µg/mL, n = 7), acetylcholine with a IC₅₀ = 79.79 µg/mL (c.l.: 63-86 µg/mL, n = 7), for histamine and barium chloride with a IC₅₀ = 100.23 µg/mL (c.l.: 89-105 µg/mL, n = 7), IC₅₀ = 127.05 µg/mL. (c.l.: 118-132 µg/mL, n = 7) respectively. The IC₅₀ for hexane extracts are showed in **Table 1**. Hexane extracts were found to antagonize contractions of the guinea pig ileum, induced by acetylcholine, histamine and barium chloride in a concentration-dependent way.

DISCUSSION

The present study has shown that hexane extract from *Erythrina indica* Lam exerts reversible relaxant and antispasmodic effects on guinea-pig ileum. Our current data show that extracts are also capable of inhibiting the response of a wide range of contractile stimuli, such as neurotransmitters acetylcholine and histamine, barium chloride a release bound (Ca²⁺) although showing no Obvious selectivity between contractile agents. Fractionation of the hexane extracts is in progress to identify the active fractions, to isolate and to characterize the actives compounds and its mechanism.

ACKNOWLEDGEMENT

The author wish to acknowledge the management of Saastra college of pharmaceutical education and research, Nellore and Hospira Healthcare India Pvt, Ltd. for providing facilities

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Table 1: Inhibition of contraction of hexane extract of *Erythrina indica* Lam expressed as IC50.

Hexane extract	IC50 $\mu\text{g/ml}$ (<i>Erythrina indica</i> Lam)
Spontaneous Contraction	62.6
Contraction by Acetylcholine	79.7
Contraction by Histamine	100.2
Contraction by barium chloride	127

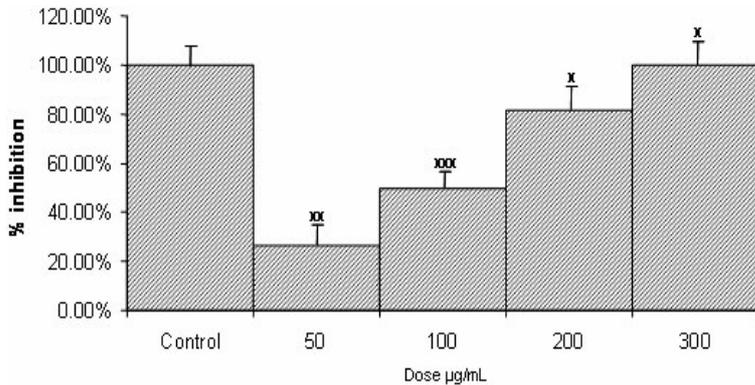


Fig 1 The inhibitory effects of the hexane extract of *Erythrina indica* Lam (50, 100, 200 and 300 $\mu\text{g/ml}$) on spontaneous contraction Of isolated guinea-pig ileum. Control correspond to responses to papaverine (100 μM), was set as 100% of maximum relaxation. Results are expressed as mean \pm S.E.M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (n=6)

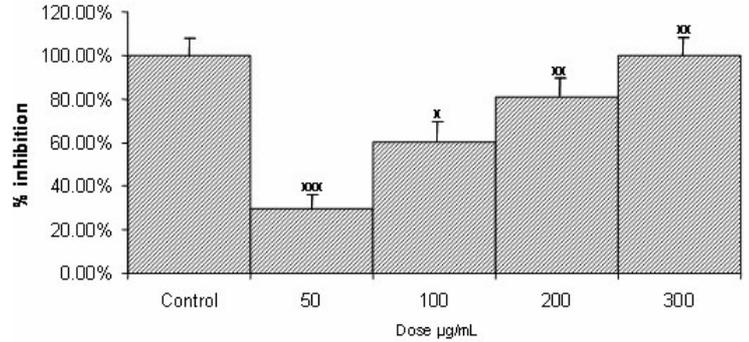


Fig 2 Concentration-response of the inhibitory effects of the hexane extract of *Erythrina indica* Lam (50, 100, 200 and 300 $\mu\text{g/ml}$) on contraction induced by acetylcholine. Control correspond to responses to papaverine (100 μM), was set as 100% of maximum relaxation. Each bar represents mean \pm S. E. M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (n = 6).

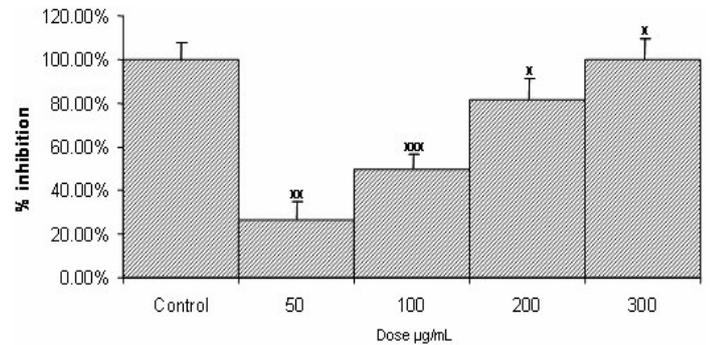


Fig 3: Effect of hexane extract of *Erythrina indica* Lam (50, 100, 200 and 300 $\mu\text{g/ml}$) on contraction induced by histamine in isolated guinea pig ileum. Contraction is expressed as a percentage against contraction induced by histamine in the absence of samples. Control correspond to responses to papaverine (100 μM), was set as 100% of maximum relaxation. Each value shows the mean \pm S.E. M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (n = 6).

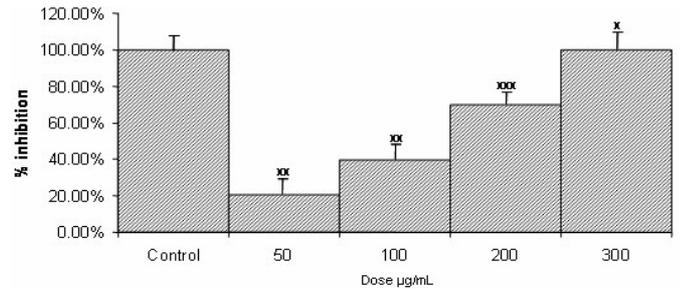


Fig 4: Effect of hexane extract of *Erythrina indica* Lam (50, 100, 200 and 300 $\mu\text{g/ml}$) on contraction induced by BaCl₂ in isolated guinea-pig ileum. Contraction is expressed in % of the maximal contraction obtained in the same tissue before the administration of antispasmodic. Control correspond to responses to papaverine (100 μM), was set as 100% of maximum relaxation. Results are expressed as mean \pm S. E. M. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ (n = 6).

Source of support: Nil, Conflict of interest: None Declared