

***In-Vivo* ANTIDIARRHOEAL ACTIVITY OF ETHANOLIC EXTRACT OF
DELONIX REGIA FLOWERS IN EXPERIMENTAL INDUCED DIARRHOEA IN
WISTAR ALBINO RATS**

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ABSTRACT

In the present study, *In Vivo* Antidiarrhoeal studies were performed on the flowers of *Delonix regia* (leguminosae). Ethanolic extract of the drug were evaluated for *in vivo* antidiarrhoeal activity, used experimental induced diarrhoea models are Castor-oil induced diarrhoea, Prostaglandin-E₂ induced enteropooling and Charcoal induced gastrointestinal motility test in rats. Pretreatment with 70% ethanolic extract of *Delonix regia* flowers at the doses (100, 250 and 500mg/kg.p.o) were administered through the oral route. The plant extracts exhibited dose dependent antidiarrhoeal effects in the all treated groups and the results were compared with that of loperamide (1mg/kg, p.o) as reference standard drug.

Keywords: *Delonix regia* flowers, diarrhoea, % inhibition, % protection, gastric motility.

INTRODUCTION

Diarrhoea is a major health problem especially for children under the age of 5 and up to 17% of children admitted in the pediatrics ward die of diarrhoea. According to WHO estimates for 1998, about 7.1 million deaths were caused by diarrhoea¹. It is therefore important to identify and evaluate available natural drugs as alternatives to currently used antidiarrhoeal drugs, which are not always free from adverse effects². A range of medicinal plants with antidiarrhoeal properties is widely used by traditional healers. *Delonix regia* traditional medicine as antidiarrhoeal drug. Reported studies on *Delonix regia* flowers include anti-inflammatory and analgesic³, antimicrobial and analgesic⁴, broad spectrum antibacterial and antifungal activities⁵. However, there is no scientific proof justifying the traditional use of *Delonix regia* flowers in the treatment of diarrhoea. Hence we undertake this study, to

evaluate its potential antidiarrhoeal efficacy in different experimental induced diarrhoea models in rats.

MATERIALS AND METHODS

Collection and identification of Crude drug

Flowers of *Delonix regia* were collected from the field of Harapanahalli in the month of May 2007. The plant was identified and authenticated by Prof. K. Prabhu, Department of Pharmacognosy, S.C.S. College of Pharmacy, Harapanahalli.

Preparation of extract

The flowers were shade dried at room temperature and pulverized. The powder obtained was subjected to successive soxhlet extraction with 70% ethanol (hydro-alcoholic extract), which was used for our studies after subjecting it to preliminary qualitative

photochemical studies. The extract were concentrated under reduced pressure and stored in desiccators for use.

Qualitative phytochemical analysis

The preliminary qualitative phytochemical studies were performed for testing the different chemical groups present in the ethanolic extract of the flowers of *Delonix regia*

Animal studies

Experimental animals

Albino Wistar strain rats weight range 120-200 g of either sex were used for study or female albino mice weighing 20-25 g were used in acute toxicity study. They were procured from Venkateswara Enterprises, Laboratory animals and preclinical services, Subramanyam nagar, Bangalore. The animals were fed with rodent pellet diet and water *ad libitum*. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

The animals were fasted overnight prior to the experiment. Fixed dose OECD guideline No. 420; (Annexure-2d) method of CPCSEA was adopted for toxicity studies⁶. 1/25th, 1/10th and 1/5th LD₅₀ cutoff value of the extract was selected as screening dose.

Evaluation of In Vivo Antidiarrhoeal activity

General chemicals and instruments

All chemicals and solvents used in the study were of analytical grade. Ethanol, Tragacanth,

Loperamide (Micro Labs, Bangalore), Prostaglandin E₂ (Astra zeneca Bangalore), Charcoal (E Merck India Ltd.), Castor oil (Microchem, Bangalore), Atropine sulphate (Ives Drugs (India) Pvt. Ltd., Indore), Anaesthetic ether (Sigma Mumbai), vacuum rotary evaporator (Shivam Instruments, India) and weighing balance (Sartorius, India) were used for the study.

Experimental design

Castor-oil induced diarrhoea model

Albino wistar strain rats weighing 150-200 g were used. They were divided into 5 groups of six animals each and fasted for 18 hrs before the test with free access to water. Group I, Group II, Group III, Group IV and Group V. Castor oil (control, 1 ml p.o.) to induce diarrhoea⁷, Loperamide (standard, 1 mg/kg p.o.), 70% ethanolic extract of flowers of *Delonix regia* (100 mg/kg p.o.), 70% ethanolic extract of flowers of *Delonix regia* (250 mg/kg p.o.) and 70% ethanolic extract of flowers of *Delonix regia* (500 mg/kg p.o.) respectively. After 30 minutes of this treatment, each rat received 1 ml of castor-oil orally. Each rat was then housed separately in perforated cage over a clean filter paper. Then diarrhoeal episodes were observed for a period of 4 hrs, during that period frequency of defecation, number of fecal drops and mean weight of stool were noted. Anti-diarrhoeal activity was determined in terms of percentage of protection, which was calculated by following formula

$$\text{Percentage of protection (\%)} = \frac{\text{Mean weight of stool of Control animals} - \text{Mean weight of stool of drug / extract treated animals}}{\text{Mean weight of stool of control animals.}} \times 100$$

Prostaglandin-E₂ induced enteropooling model

The wistar rats weighing 150 – 200 g were used. The animals were divided in to 6 groups of six animals each. These animals were placed in perforated cages and deprived of food and water for 18 hrs prior to the experiment. Group I, Group II, Group III, Group IV Group V and Group V1. control

(Negative, 1 ml of 5% v/v ethanol in normal saline p.o.), control (Positive, PGE₂ 100 µg/kg in 5% v/v ethanol in normal p.o.) to induce diarrhoea⁷, Loperamide (standard, 5 mg/kg p.o.), 70% ethanolic extract of flowers of *Delonix regia* (100 mg/kg p.o.), 70% ethanolic

extract of flowers of *Delonix regia* (250 mg/kg p.o.) and 70% ethanolic extract of flowers of *Delonix regia* (500 mg/kg p.o.) respectively. Immediately after the standard and extract treatment each rat was administered prostaglandin-E₂ in the Group III, IV, V and VI. All the rats were sacrificed after 30 minutes and the whole length of the intestine from the pylorus to the caecum was dissected out and its contents were collected in test tube and the volume was measured.

Gastro-intestinal motility test model

Albino rats of either sex weighing 150-200 g were used. The animals were divided into 5 groups of six rats each. They were fasted for 24 hrs before the test with free access to water. Group I, Group II, Group III, Group IV and Group V. Charcoal meal (Control, 3% of deactivated charcoal in 2% aqueous

tragacanth 1 ml p.o.) to induce diarrhoea⁸, Standard (Atropine sulphate 5 mg/kg, i.m.), 70% ethanolic extract of flowers of *Delonix regia* (100 mg/kg p.o.), 70% ethanolic extract of flowers of *Delonix regia* (250 mg/kg p.o.) and 70% ethanolic extract of flowers of *Delonix regia* (500 mg/kg p.o.) respectively. After 30 minutes of the above treatment, each rat was received orally 1 ml of charcoal meal thirty minutes after charcoal meal treatment, each rat was sacrificed and intestinal distance moved by the charcoal meal from pylorus to caecum was measured and expressed as a percentage of distance traveled from pylorus to caecum. The mean percentage movement of charcoal meal in ratio to the intestinal length, and percentage of inhibition was calculated by following the formula

$$\% \text{ travelled} = \frac{\text{Distance traveled by the charcoal meal}}{\text{Total length of small intestine}} \times 100$$

$$\% \text{ of inhibition} = \frac{\text{Mean length of the Small intestine} - \text{Distance traveled by the charcoal meal}}{\text{Mean length of small intestine}} \times 100$$

Statistical analysis

The results were expressed as Mean \pm SEM when compared with that of Diarrhea induced group and subjected to statistical analysis using ANOVA.

RESULTS

Qualitative phytochemical analysis

preliminary phytochemical investigation on 70 % ethanolic extract of *Delonix regia* flowers are showed the presence of Carbohydrates, Flavonoids, Saponin glycosides, Tannins and Steroids.

Acute toxicity studies (LD₅₀)

Ethanolic extract (70 %) of *Delonix regia* flowers was studied for acute toxicity at dose of 2000 mg/kg by i.p. route. The extract was found devoid of mortality of the animals.

Hence, 2500 mg/kg was considered as LD₅₀ cutoff value. So the doses selected for extract as per OECD guidelines No. 420; (Annexure-2d) fixed dose method are mentioned below. 100 mg/kg (1/25th of 2500 mg/kg), 250 mg/kg (1/10th of 2500 mg/kg) 500 mg/kg (1/5th of 2500 mg/kg).

In Vivo Anti-diarrhoeal activity studies Castor oil induced diarrhoea model

Ethanolic extract (70%) of *Delonix regia* flowers (EEDRF) exhibited significant antidiarrhoeal activity against castor-oil challenged diarrhea in rats. The extract showed marked reduction in the frequency of defecation, fecal droppings and mean weight of faeces when compared to control group. The extract at a dose of 500 mg/kg had shown more significant effect

when compared to the standard drug loperamide (Table No. 1).

PGE₂ induced enteropooling model

The extract (100, 250 and 500 mg/kg) dose dependently reduced the intestinal fluid accumulation by 43.85 to 71.49% in respect to PGE₂ control group. The effect of extract at dose of 500 mg/kg was found to be more potent than the reference standard, loperamide (5 mg/kg). There was significant increase in intestinal fluid accumulation was seen in PGE₂ control group when compared to negative control received only ethanol in normal saline (Table No. 2).

Charcoal meal test

In control animals, the charcoal meal traversed 80.93% of the total length (105.53 cm) of the small intestine. All the tested doses of the extract inhibited significantly the intestinal propulsion in dose dependent manner. The activity of test extract at dose of 500 mg/kg on charcoal meal test was found to be more effective when compared to atropine, an anticholinergic drug (Table No. 3).

DISCUSSION

Qualitative phytochemical analysis

Previous reports have demonstrated antidiarrhoeal activity of tannins⁹ and flavonoids¹⁰ containing plant extracts. Tannins can evoke an antidiarrhoeal effect since these substances may precipitate proteins of the electrolytes and reduce peristaltic movement and intestinal secretions^{11, 12}. The antidiarrhoeal activity of flavonoids has been ascribed to their ability to inhibit intestinal motility and hydroelectrolytic secretion^{13, 14} which are known to be altered in this intestinal condition. The preliminary phytochemical investigation of the 70% ethanolic extract of *Delonix regia* flowers showed the presence of tannins and flavonoids. These phytochemicals may be responsible for the significant antidiarrhoeal activity in this study.

Castor oil induced diarrhoea model

Castor oil, its active component ricinoleic acid induces permeability changes in mucosal fluid and electrolyte transport that results in a hypersecretory response and diarrhoea^{15, 16}. Ricinoleic acid also produces irritation and inflammation of the intestinal mucosa, leading to release of prostaglandins, which stimulate motility and secretion¹⁷. The antidiarrhoeal activity of the extract against experimentally induced diarrhoea may be attributed to an antielectrolyte permeability action. The significant reduction of frequency of defecation, number of fecal dropping and mean weight of stool demonstrates the efficacy of *Delonix regia* flowers as antidiarrhoeal agent.

PGE₂ induced enteropooling model

The extract significantly inhibited the PGE₂ induced intestinal fluid accumulation (enteropooling)¹⁸. PGE₂ also inhibit the absorption of glucose, a major stimulus to intestinal absorption of water and electrolytes¹⁹. These observations tend to suggest that extract reduced diarrhoea by inhibiting PGE₂ induced intestinal accumulation of fluid.

Charcoal meal test

The extract appears to act on all parts of intestine. Thus, it decreased the intestinal propulsive movement in charcoal meal test. The mechanism of this inhibition of motility may be due to the non-specific spasmolytic activity of the extract.

CONCLUSION

In Present study, the 70% ethanolic extract of *Delonix regia* flowers possessed significant antidiarrhoeal properties thus supports the traditional use of *Delonix regia* flowers in the treatment of diarrhoea. Further studies are required to identify and isolate the active principles to establish the exact mechanism of action of the test extract.

Table 1

Groups	Treatment	Dose mg/kg	Mean frequency of diarrhoea \pm SEM	Mean No. of fecal drops \pm SEM	Mean wt. of faeces \pm SEM after 4 hrs (gm)	% of protection
I	Control	–	6.16 \pm 0.15	6.16 \pm 0.14	2.99 \pm 0.26	–
II	Standard (loperamide)	1	0.16 \pm 0.08***	0.50 \pm 0.08***	0.47 \pm 0.16***	84.28
III	EEDRF	100	3.83 \pm 0.60	4.5 \pm 0.09	2.30 \pm 0.24	23.07
IV	EEDRF	250	3.33 \pm 0.07*	3.33 \pm 0.05*	2.10 \pm 0.09*	29.76
V	EEDRF	500	1.16 \pm 0.05***	0.66 \pm 0.14***	0.89 \pm 0.20***	70.23

The values are Mean \pm SEM, n = 6, *p < 0.05, and *** p < 0.001 vs control.

Table 2

Groups	Treatment	Dose	Mean volume of intestinal fluid (ml) \pm SEM	% of inhibition
I	-ve control	1 ml of 5% v/v ethanol and normal saline p.o.	0.88 \pm 0.07	--
II	+ve control	PGE ₂ 100 μ g/kg	2.23 \pm 0.06	--
III	Standard (loperamide)	5 mg/kg	0.93 \pm 0.05***	59.21
IV	EEDRF	100 mg/kg	1.28 \pm 0.04*	43.85
V	EEDRF	250 mg/kg	1.06 \pm 0.04**	53.50
VI	EEDRF	500 mg/kg	0.65 \pm 0.03***	71.49

The values are Mean \pm SEM, n = 6, *P < 0.05, ** p < 0.01 and *** p < 0.001 vs +ve control.

Table 3

Groups	Treatment	Dose mg/kg	Mean length of intestine \pm SEM (cm)	Mean distance traveled by charcoal meal (cm) \pm SEM	Mean % movement of charcoal (cm) \pm SEM	% of inhibition
I	Control	–	105.53 \pm 0.98	80.93 \pm 2.38	76.54 \pm 1.12	--
II	Standard (Atropine sulphate)	5	101.53 \pm 1.36	48.40 \pm 1.59	47.87 \pm 1.17***	52.32
III	EEDRF	100	106.35 \pm 1.60	73.11 \pm 2.11	63.77 \pm 0.79*	31.25
IV	EEDRF	250	98.75 \pm 1.99	66.48 \pm 1.80	59.76 \pm 0.75**	32.67
V	EEDRF	500	100.38 \pm 1.25	66.16 \pm 1.87	54.66 \pm 1.33***	34.09

The values are Mean \pm SEM, n = 6, *P < 0.05, ** P < 0.01 and *** p < 0.001 vs control.

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