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Research Article

GASTROPROTECTIVE ACTIVITY OF ETHANOLIC EXTRACT OF *DELONIX REGIA* FLOWERS IN EXPERIMENTAL INDUCED ULCER IN WISTAR ALBINO RATS

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ABSTRACT
The ethanolic extract of flowers of *Delonix regia* (*leguminosae*) was obtained and investigated for its gastro protective activity in experimental induced ulcer model. 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 Antiulcer effect of ethanolic extract of *Delonix regia* flowers was studied in aspirin, alcohol and pylorus ligation induced gastric ulceration experimental models and the results were compared with that of lansoprazole (8mg/kg, p.o.) as reference standard drug. The various parameters like Ulcer index and percentage protection in all the models and gastric volume, pH of gastric juice, free acidity and total acidity in pylorus ligation induced gastric ulceration model were monitored. From the findings of our study, the ethanolic extract of flowers of *Delonix regia* showed gastroprotective effect of in a dose dependant manner.

KEY WORDS: *Delonix regia* flowers, Gastro protective, Gastric ulcer, pH of gastric juice, % protection and ulcer index.

INTRODUCTION

Gastric ulcer, most common disorder of GIT has multifunctional causes in its pathophysiology[1] Plants provide various traditional medicine known to posses' antiulcer properties. Borrelli and Izzo reveal the extensive variety of chemical compounds isolated from medicinal plants with antiulcer activity[2]. Literature search revealed that herbs rich in flavonoids show several biological activities including antiulcerogenic activity. This is an important reason to investigate antiulcer effects in medicinal plants with traditional use in gastric diseases[3]. The flowers of *Delonix regia* are commonly used in a dysmenorrhoea[4], antibacterial and analgesic anti-inflammatory[5], antimicrobial[6], broad spectrum antibacterial and antifungal activities[7]. However, there is no scientific proof justifying the traditional use of *Delonix regia* flowers in treatment of ulcer. Hence we aimed to evaluate its potential gastro protective efficacy in different experimental models of ulcer 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 until further 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

Albino Rats (wistar strain) weighing 120-200 g of either sex were used for experimental 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 allowed for acclimatization for ten days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C, relative humidity 65 ± 10% under 12 hrs. light / dark cycle. The animals were fed with rodent pellet diet (Gold Mohur Lipton India Ltd.) and water *ad libitum*. The study protocol was approved from the Institutional Animal Ethics Committee (IAEC).

Acute toxicity studies

The acute toxicity of 70% ethanolic extract of flowers of *Delonix regia* was determined in female albino mice (20-25 g). The animals were fasted overnight prior to the experiment. Fixed dose OECD guideline No. 420; (Annexure-2d) method of CPCSEA was adopted for toxicity studies[8]. $1/25^{th}$, $1/10^{th}$ and $1/5^{th}$ LD_{50} cutoff value of the extract was selected as screening dose.

Evaluation of In vivo antiulcer activity

General chemicals and instruments

All chemicals and solvents used in the study were of analytical grade. Acacia (Nice chemicals Cochin), Anesthetic ether (Sigma solvents Mumbai), Topfer's reagent and ethanol (S.D. Fine Chemical, Mumbai), Asprin (Intermed, Bangalore), pH Meter (Elico India), centrifuge (Remi RM12C, India), low deep freezer (Modern Industrial Corporation India) vacuum rotary evaporator (Shivam Instruments, India) and weighing balance (Sartorius, India) were used for the study.

Experimental design

Aspirin induced ulcer model

Albino rats of either sex weighing between 120-200 g were selected and divided into 5 groups of 6 animals each. Group I, Group II, Group III, Group IV and Group V. Aspirin (control)(200 mg/kg in 2% w/v gum acacia p.o.) to induce ulcer[9], Lansoprazole (standard) (8 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. Food was withdrawn for 24 hrs before the experiment and water was allowed *ad libitum*. The test drugs were administered orally 30 minutes prior to aspirin challenge. After 4 hrs animals were scarified, stomach dissected out for scoring ulcer index. The ulcer scoring done as follows.

0	=	Normal colored stomach.
0.5	=	Red coloration
1	=	Spot ulcers
1.5	=	Hemorrhagic streaks
2	=	Ulcers > 3 mm but < 5 mm
3	=	Ulcer > 5 mm

Mean ulcer score for each animal is expressed as Ulcer Index.

The percentage protection was calculated using the formula

$$\text{Percentage protection} = 100 \frac{Ut}{Uc} \times 100$$

Where,

Ut = Ulcer index of treated group

Uc = Ulcer index of control group.

Alcohol induced ulcer model

Albino rats of either sex weighing between 120-200 g were selected and divided into 5 groups of 6 animals each. Group I, Group II, Group III, Group IV and Group V. Alcohol(1 ml/200 g of 99.80% alcohol p.o.) to induce ulcer[9], Lansoprazole (8 mg/kg p.o.) (standard), 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. The animals were fasted for 24 hrs, with free access to water. The test drugs were administered orally 30 minutes prior to administration of 1ml/200 g of 99.80% alcohol by p.o. to each animal. Animals were sacrificed 1 hr. after alcohol administration, stomachs were isolated and cut open along the greater curvature and pinned on a soft board. The ulcer index and Percentage protection was measured as in above model.

Pylorus ligation induced ulcer model

a) Gastric ulcers in pylorus ligation

Albino rats of either sex weighing between 120-200 g were selected and divided into 5 groups of 6 animals each. Group I, Group II, Group III, Group IV and Group V. Aspirin (200 mg/kg in 2% w/v gum acacia p.o.) to induce ulcer[10], Lansoprazole (8 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. In this method albino rats were fasted in individual cages for 24 hrs. Care was being taken to avoid coprophagy. 70% ethanolic extracts of flowers of *Delonix regia* or standard drug were administered

30 minutes prior to pyloric ligation. The abdomen was then sutured. At the end of 4 hrs after ligation the animals were sacrificed with excess of anesthetic ether, and the stomachs were dissected out. Gastric juice was collected and drained into test tubes and then centrifuged at 1000 rpm for 10 min. and the volume was noted. The pH of the gastric juice was recorded by pH meter. Then the contents were subjected for the analysis of free and total acidity. The stomachs were then washed with running water to see for ulcers in the glandular portion of the stomach. The ulcer index and Percentage protection was measured as in above model.

b) Gastric secretion in pylorus ligation

1 ml of gastric juice was pipetted into 100 ml conical flask, added 2 –3 drops of Topfer's reagent and titrated with 0.01 N sodium hydroxide until all traces of red colour disappears and the colour of the solution turns to yellowish orange. The volume of the alkali added was noted. This volume corresponds to free acidity. Then 2 – 3 drops of phenolphthalein solution was added and titration was continued until a definite red tinge reappears. Again the total volume of alkali added was noted. Acidity was calculated by using the formula

$$\text{Acidity} = \frac{\text{Volume of NaOH x Normality of NaOH x 100}}{0.1} \text{ meq/L/100 gm}$$

Statistical analysis

The results were expressed as Mean ± SEM when compared with that of ulcer 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.

Determination of acute toxicity (LD_{50})

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 LD_{50} 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 ($1/25^{th}$ of 2500 mg/kg), 250 mg/kg ($1/10^{th}$ of 2500 mg/kg) 500 mg/kg ($1/5^{th}$ of 2500 mg/kg).

Evaluation of In vivo antiulcer activity

Aspirin induced ulcer model

The extract was found to possess remarkable ulcer protective property at a dose of 250 & 500 mg/kg. Where as the test extract at a dose of 500 mg/kg produced maximal effect (90.58 % Protection of ulceration) when compared to the standard drug (lansoprazole 8 mg/kg). The results were shown in Table 1.

Alcohol induced ulcer model

The extract (100, 250 and 500 mg/kg) significantly inhibited the ulcerogenic effect of ethanol in rats in a dose dependent manner. The percentage protection of extract at dose of 500 mg/kg was seems to be highly effective when compared to the reference standard, lansoprazole. The results were shown in Table No. 2.

Pylorus ligation induced ulcer model

a) Gastric ulcers in pylorus ligation

Gastric lesions induced by pylorus ligation were significantly reduced by pretreatment with test extract in dose related manner. The reduction of ulcer index in dose related manner and percentage of protection was 37.56%, 44.64% and 91.21% with 100, 250 and 500 mg/kg of the extract respectively. The ulcer protective action of 500 mg/kg dose of the extract was found to be closer to the reference standard drug, lansoprazole. The results were shown in Table No. 3.

b) Gastric secretion in pylorus ligation

The extract produced significant decrease in volume of gastric juice, free and total acidities and increase in pH of the gastric juice in dose dependent fashion compared to control group. The result of the test extract at higher dose (500 mg/kg) was found to be more effective when compared to the reference standard. The results were summarized in Table 4.

DISCUSSION

Qualitative phytochemical analysis

Preliminary phytochemical investigation revealed the presence of tannins and flavonoids, substance known to affect the integrity of mucus membrane[11]. Tannins, with their protein precipitating and vasoconstrictory effect, could prevent ulcer development[12]. Flavonoids have also been reported to offer some protection in ulcer development by increasing capillary resistance and improving microcirculation[13].

Aspirin induced ulcer model

Aspirin model was used considering its different mechanism by which it provokes gastric ulceration.

The reason being attributed principally to inhibition of biosynthesis of cytoprotective prostaglandins, by inhibition of cyclo-oxygenase pathway of arachidonic acid metabolism resulting in over production of leukotriene and other product of 5-lipoxygenase pathway[14]. Hence, the significant protective effect of test extract against aspirin challenged gastric ulcer may be due to its 5-lipoxygenase inhibitory action.

Alcohol induced ulcer model

Endogenous glutathione and prostaglandin levels are lowered by ethanol while the release of histamine, influx of calcium ions, and generation of free radicals and production of leukotrienes are all increased. The product of the 5-lipoxygenase pathway may also play a key role in the development of ulcer. The significant protection exhibited by the test extract against alcohol induced gastric ulceration may be due to inhibition of 5-lipoxygenase pathway or leukotriene antagonistic activity.

Pylorus ligation induced ulcer mode

In pylorous ligation, the digestive effect of accumulated gastric juice and interference of gastric blood circulation are responsible for induction of ulceration[16]. Antiulcer activity of test extract in pylorous ligation model was evident from its significant reduction in gastric volume, free and total acidities, ulcer index and increase in pH. Therefore it is suggested that the extract can suppress gastric damage induced by aggressive factors.

CONCLUSION

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

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Table 1: Effect of ethanolic extract of *Delonix regia* on aspirin induced gastric ulceration in rats.

Groups	Treatment	Dose	Mean ulcer index ± SEM	% protection
I	Control	Aspirin 200 mg/kg	3.05 ± 0.15	--
II	Standard (Lansoprazole)	8 mg/kg	0.716± 0.10***	95.42
III	EEDRF	100 mg/kg	2.33 ± 0.18	33.43
IV	EEDRF	250 mg/kg	1.5 ± 0.11*	57.15
V	EEDRF	500 mg/kg	0.33 ± 0.10***	90.58

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

Table 2: Effect of ethanolic extract of *Delonix regia flowers* on alcohol induced gastric ulceration in rats.

Groups	Treatment	Dose	Mean ulcer index ± SEM	% protection
I	Control	Alcohol 1ml/200 g	3.83 ± 0.21	--
II	Standard (Lansoprazole)	8 mg/kg	0.33 ± 0.10***	91.38
III	EEDRF	100 mg/kg	1.75 ± 0.11*	54.31
IV	EEDRF	250 mg/kg	0.16 ± 0.12**	69.62
V	EEDRF	500 mg/kg	0.5± 0.061***	86.95

The values are Mean ± SEM., n = 6. *p < 0.05 ** p < 0.01 and *** p < 0.01 vs control.

Table 3. Effect of ethanolic extract of *Delonix regia flowers* on pylorus ligation induced gastric ulceration in rats.

Groups	Treatment	Dose	Mean ulcer index ± SEM	% protection
I	Control	--	4.66± 0.15	--
II	Standard (Lansoprazole)	8 mg/kg	0.33 ± 0.06***	92.92
III	EEDRF	100 mg/kg	2.91 ± 0.07*	37.56
IV	EEDRF	250 mg/kg	2.58 ± 0.01**	44.64
V	EEDRF	500 mg/kg	0.41 ± 0.06***	91.21

The values are Mean ± SEM., n = 6. *p < 0.05 ** p < 0.01 and *** p < 0.01 vs control.

Table 4: Effect of ethanolic extract of *Delonix regia flowers* on gastric secretion following pylorus ligation in rats

Groups	Treatment	Mean vol. of Gastric Juice (ml) ± SEM	Mean pH ± SEM	Mean Free Acidity (mEq/L/ 100g)± SEM	Mean Total Acidity (mEq/L/ 100g) ± SEM
I	Control	7.08 ±0.21	2.46 ±0.04	99.91 ±2.15	114.84 ±2.80
II	Standard (lansoprazole)	2.23± 0.19***	4.9±0.11***	44 ±1.18***	52 ±1.95***
III	EEDRF	5.53± 0.19*	3.38 ± 0.09*	81.66±0.88*	98.16 ±1.13*
IV	EEDRF	4.98± 0.16**	3.58±0.05**	78.83 ±1.60**	93.83 ±1.10**
V	EEDRF	2.3± 0.12***	5.26±0.08***	51.33±1.17***	67 ±1.36***

The values are Mean ± SEM., n = 6. *p < 0.05 ** p < 0.01 and *** p < 0.01 vs control

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