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Note

Effect of *Delonix regia* (Boj. Ex Hook.) Raf. stem bark extract against experimentally induced ulcers in rats

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Delonix regia, commonly called Flame Tree or Flamboyant (locally, *Gul Mohor*) is a common tree traditionally used to treat various diseases like gastric problems, body pain, rheumatic pains of joints and wound healing. Here, we carried out biological profiling of *Delonix regia* as antiulcer agent. Antiulcer activity of the ethanol extract from stem bark was evaluated on pylorus ligation and indomethacin induced ulcer in Wistar albino rats. Ethanol extract from stem bark of *D. regia* was administered at the doses 100, 200 and 400 mg/kg/day, p.o. for 7 days. Ulcer index, gastric pH, volume, free acidity, total acidity, total carbohydrate (TC), protein (P), mucin content (TC/P) and gastric mucus were evaluated in pylorus ligation model, while ulcer index, malondialdehyde, GSH, PGE_2, and gastric mucus were estimated in the indomethacin induced ulcer model. *Ex vivo* assay for the activity of H^+/K^+-ATPase was also done. The results showed significant inhibition on H^+/K^+-ATPase in a dose dependent manner and comparable to their respective positive control group of rats demonstrating that ethanol extract of stem bark of *Delonix regia* possesses significant antiulcer properties.

Keywords: Antiulcer activity, Forest flame, *Gul Mohr*, Pylorus ligation, Stem bark

Human beings, since prehistoric times, have relied on the flora for their prophylactic and therapeutic properties. In recent times, due to adverse effects of modern medicines, there has been an increasing interest in plants for augmentation of health[1-3]. *Delonix regia* (Boj. Ex Hook.) Raf. (Fabaceae, subfamily: Caesalpiniaceae) commonly called Flame Tree or Flamboyant (*Gul Mohor* in Hindi) is an umbrella-shaped, medium-sized tree found in tropical countries grows up to 40 feet height and has large red-orange flowers (in April-May). *D. regia* is traditionally used to treat various diseases like gastric problems, body pain, rheumatic pains of joints, wound healing[4-6] and also as diuretic, antihelminthic, antileucorrhoea and anti-inflammatory agents[6]. The plant (flowers and stem bark) contains many bioactive molecules (*viz.* flavonoids, carotenoids and triterpenoids)[7].

Peptic ulcer is the one of most frequent causes of surgery in humans, with high morbidity and mortality rates. It is an excoriated area of the mucosa[8]. Peptic ulcer occurs when there is an imbalance between the aggressive factors such as gastric acid and pepsin; and the defence mechanisms *viz.* prostaglandins, mucus, bicarbonate and mucosal blood flow, cellular regeneration, and epidermal growth factors, which protect the gastric mucosa from these substances[8-12].

Researchers have recently demonstrated antiulcerative properties of *Aegle marmelos* (L.) Correa fruit extract[13] *Dillenia indica* L.[14] and *Pongamia pinnata* Linn. seeds[15]. Earlier, Goyal & Bhattacharya have also discussed the gastrointestinal mucosal defense and some mucosal protective agents[16]. Hydroalcoholic fruit extract of *Pithecellobium dulce* (Roxb.) Benth. has been shown to possess antioxidant and antiulcer property[10]. However, to the best of our knowledge, there is no report available on antiulcer potential of stem bark of *Delonix regia*. On the basis of literature survey, the aim of the present study was therefore undertaken to evaluate the antiulcer activity of ethanol extract from stem bark of *Delonix regia* (EDR) on different experimentally induced ulcer models in rats.

Materials and Methods

The stem barks of *Delonix regia* were collected from the Bazikhera garden, Unnao in the month of May-June 2011. Plant material was taxonomically identified and authenticated by Dr Anamika Tripathi, Hindu College, Moradabad as *Delonix regia* (Boj. Ex Hook.) Raf. (Fam.: Fabaceae; subfam.: Caesalpiniceae) with registration no. HC/Bot/PERL-26. The plant material to be used was dried under the shade and was later powdered finely. The powdered material was extracted using soxhlet apparatus (Borosil, India) successively using various solvents in an increasing order of polarity *viz*., petroleum ether, chloroform, ethyl acetate and ethanol. Yields for petroleum ether (40-60°C), chloroform, ethyl acetate

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and ethanol extracts were 0.76, 0.77, 0.80 and 1.63% (w/w), respectively. The ethanol extract of *Delonix regia* stem bark (EDR) obtained was subjected to preliminary qualitative tests for glycosides (Keller-Killiani and Legal's test), Phenolics & Tannins (Borntrager's test, modified Borntrager's test, 5% $FeCl_3$ solution, Lead Acetate solution and dilute Iodine solution test) and Flavonoids (Shinoda test, Sulfuric acid test and Lead Acetate solution)[17].

Wistar albino rats of either sex (6-8 month old) weighing 180-220 g were used for all the experiments. All the animals were housed in polypropylene cages in an air-conditioned area at 25±2°C with 12:12 h light:dark cycle, respectively. They were given food (Mona, Raman Dairy Vikash Udyog, Varanasi) and H_2O *ad libitum*. The Institutional Animal Ethics Committee affiliated to CPCSEA (837/ac/04/CPCSEA), India, approved the protocols used for animal experiments in this study.

According to Organization for Economic Cooperation Development guideline 423[18], EDR at a dose level of 2 g/kg [orally (p.o.)] was used for acute oral toxicity study on wistar albino rats. Three female rats, each sequentially dosed at intervals of 48 h (in four divided doses) were used for the test. Once-daily cage-side observations for changes in skin, fur, eyes, mucous membrane (nasal), autonomic nervous system (salivation, lacrimation, perspiration, piloerection, urinary incontinence and defaecation) and central nervous system (drowsiness, gait, tremors and convulsions) were recorded. Mortality, if any, was determined over a period of 2 weeks[18].

For the assessment of antiulcer activity in animal models, three dose levels of EDR were chosen in such a way that one dose was approximately one-tenth of the dose (i.e. 2 g/kg, p.o.) during acute toxicity studies. For low dose, which was 50% of the one tenth dose, and for a high dose, twice of one-tenth dose of acute toxicity study dose. Finally, the selected doses were 100, 200 and 400 mg/kg.

Pylorus ligated ulcers

Pyloric ligation was performed after 18 h of treatment with respective groups. Pre-treatment was done for 7 days with EDR (100, 200 and 400 mg/kg, body wt.) or lansoprazole (20 mg/kg, body wt.) or vehicle (0.5% Tween-80) to rats[19]. Animals starved for 18 h (but for water) were anaesthetized using diethyl ether. A small abdominal incision was made and the pylorus was ligated and closed. The animals were sacrificed after 4 h. The stomach was excised, opened along the greater curvature and the luminal contents were collected and centrifuged for 15 min to remove the residual debris. The gastric juice volume was measured and the free acid as well as total acid output was determined by titration against 0.01N NaOH, using Topfer's reagent and phenolphthalein as indicator, respectively[20]. Gastric wall mucus was determined according to the method of Corne *et al*.[21]. Pepsin concentration was determined by modification of the colorimetric method[22], involving digestion of 2% haemoglobin in 0.02N HCl (pH 2.0, 37°C, 15 min) followed by alkaline condensation with Folin Ciocalteu's reagent and spectrophotometric measurement at 578 nm. Pepsin output was expressed as mg of pepsin in 4 h. Also, ulcers were graded using the following arbitrary scale as: 0=normal mucosa, 0.5=blushing, 1=spot ulcers, 1.5=haemorrhage streaks, 2=3mm<ulcers<5mm and 2.5=ulcers>5mm[23].

For estimation of the total carbohydrate content, One mL of 5% phenol was pipetted out into the test tubes each containing 0.15 mL gastric juice or containing 0.15 mL distilled water and was thoroughly mixed. Five mL of 96% H_2SO_4 was added and mixed slowly. After 10 min, the test tubes were shaken, placed in water, and kept at 20°C for 20 min. The optical density of the developed yellow-orange chromophores was read at 482 nm using a spectrophotometer. A standard curve for glucose solution was drawn. Total liberated carbohydrates were expressed in terms of mg/mL of gastric juice[24]. The mucoadhesive activity was expressed as the total carbohydrate/ protein (TC/P) ratio. Estimation of the protein content was carried out as described by Lowry *et al*.[25].

Indomethacin induced gastric ulcer

Male Wistar rats (180-200 g) were deprived of food for 24 h with free access to water prior to the experiment. The animals received oral doses of EDR (100, 200 and 400 mg/kg), lansoprazole and vehicle 1 h before the administration of indomethacin (20 mg/kg; p.o.). The control group received only vehicle before 1 h of indomethacin administration. Six hour after indomethacin administration the animals were sacrificed, their stomach removed and examined for ulcer protection[20]. Malondialdehyde was estimated by the method of Fong *et al*.[26]. Prostaglandin was determined in mucosal tissue samples obtained from control, treatment and reference drug groups. Briefly, mucosa was scrapped and rapidly rinsed with ice-cold saline. The tissue was weighed and homogenized in

10 volumes of phosphate buffer (0.1 M, pH-7.4) containing 1 mM EDTA and 10 µM indomethacin. The homogenate was centrifuged (10000 rpm, 10 min, 4°C), and the supernatant was processed for PGE_2 estimation using enzyme immunosorbent assay kit, following the manufacturer's instructions. Results were expressed as PGE_2 (pg /mg protein). All groups of rats treated were utilised to estimate the reduced glutathione (GSH) content in stomach tissues as non-protein sulfhydryls according to the method described by Sedlak *et al*.[27].

In vitro assay of H^+ K^+-ATPase activity

Gastric microsomes were isolated from normal fasted rat stomach[28]. H^+/K^+-ATPase activity was assayed in gastric microsomes incubated with or without different concentrations of EDR as well as lansoprazole for 10 min at 37°C. Then each were added to an assay buffer containing (in mM) 150 KCl, 10 PIPES, 1 MgSO4, 5 Mg ATP, 1 EGTA and 0.1 ouabain, at pH 7.2 and 10 µg/mL valinomycin, 2.5 µg/mL oligomycin. The reaction was carried out at 37°C for 20 min and was stopped by adding 10% ice-cold trichloroacetic acid. After centrifugation at 2000 rpm for 1 min, inorganic phosphate release from the resulting supernatant was determined spectro photometrically at 310 nm wavelength and expressed as µM/h/mg protein.

Statistical analysis

The results were expressed as mean±SEM and were analyzed using one-way analysis of variance followed by Dunnett's test using GraphPad Prism 5.0 (Graph-Pad Software Inc., San Diego, California, USA). The value of P <0.05 was considered statistically significant.

Results and Discussion

Preliminary phytochemical analysis of ethanol extract of stem bark of *Delonix regia* revealed the presence of glycosides, flavonoids, phenolics and tannins. In the present study, EDR had no effects on clinical signs, body weight change or gross observation in rats. Also, no mortality was observed. Therefore, no acute toxicity was found in rats treated with EDR and LD_{50} might be higher than 2 g/kg.

Pylorus ligated ulcers

The pylorus ligation-induced ulcer model is a simple, reproducible, and highly predictable model for the screening of antiulcer drugs. It neither utilizes exogenous ulcerogens nor is induced by exogenous interfering factors. As it is assumed that ulcers are developed because of an excess of acid and pepsin for a given degree of mucosal defence[16,19]. Pylorus ligation-induced ulcers may be attributed by enhanced acid secretion leading to autodigestion of gastric mucosa[29], decreased mucosal blood flow, and breakdown of the mucosal barrier[30]. In our study, pylorus ligation produced marked gastric lesions and damaged the stomach mucosal layer in the experimental rats. The agents that decrease gastric acid secretion are effective in protecting against ulcers induced by using these methods[31]. The EDR reduced gastric acid secretion, proving its antisecretory effect.

In this study, stomachs of rats in the control group (saline treated) showed higher inductions of gastric ulcers. Group 2 (lansoprazole treated, 20 mg/kg) showed significant (P <0.001) protection (80%) against gastric ulceration caused by pylorus ligation compared to the ulcers produced in the control group. The EDR treatment (Groups 2-4) at different doses (100, 200 and 400 mg/kg) also showed significant (P <0.001) decreases in the ulcer index (UI) as compared to the control group (Table 1). The gastric content of the incised stomachs were analyzed for the gastric volume, pH, free acidity, and total acidity. The volumes of the gastric juice were significantly (P <0.01) decreased in a dose dependent manner (Fig. 1). The free acidity and the total acidity were significantly (P <0 .001) decreased (Fig. 1), and the pH value of the gastric content was significantly increased (P <0.01) by oral administration of Lansoprazole (20 mg/kg) and EDR (400 mg/kg) as compared to pylorus-ligated control group, but administration of EDR at 100 mg/kg showed a less significant result (P <0.05). The pepsin output decreased with increase in EDR dose (200 or 400 mg/kg bw) as compared to control group (Fig. 1). It significantly (P <0.001) decreased at dose level of 400 mg/kg and are comparable to lansoprazole These results indicate that the EDR is effective in reducing

Table 1—Effect of EDR on pylorus ligated induced ulcers in rats

Treatment	Ulcer Index	% Protection	Gastric wall mucus
Control	12.51±0.88	-	180.56±3.45
EDR 100	4.33 ± 0.63***	65.36	203.41±4.32*
EDR 200	3.50±0.48***	72.00	210.24±3.58**
EDR 400	2.75±0.51***	78.00	218.34±5.31***
Lansoprazole	2.50±0.35***	80.00	226.13±4.24***

[Values are expressed as mean±SEM (n = 6). ANOVA followed by dunnett's test with control group. Significance represented as *(P <0.05), **(P <0.01) and ***(P <0.001)]

gastric ulcers produced by hyperacidity in the stomach. Even the low dose (100 mg/ kg) has shown sufficient cytoprotective action against pylorus ligation-induced gastric ulcers and mucosal damage.

Mucus secretion is a critical factor in the protection of gastric mucosa from the gastric lesions and has been regarded as a vital defensive factor in the gastric mucus barrier. The increase in total carbohydrate/ protein (TC/P) ratio is the direct reflection of mucin activity. Fall off in protein content in the gastric juice also signifies decrease in leakage from the mucosal cells indicating mucosal opposition. The wide distribution of adherent mucus content in the gastrointestinal tract plays a pivotal role by forming a protective barrier preventing digestion of stomach wall and thereby repair the gastric mucosa[32].

In, the study, total carbohydrate content in the gastric content were increased in dose dependent order and statistically significant (P <0.001) (Fig. 2). Also, pre-treatment with different doses of EDR decreases the protein content of gastric content of the rats and increases the mucin content (Fig. 2). Gastric wall mucus was increased dose dependently and it was 218.34±5.31 at EDR (400 mg/kg) (Table 1). The results showed increased levels of adherent mucus content of gastric tissue pre-treated with EDR indicating its cytoprotective action on experimentally induced gastric ulcer.

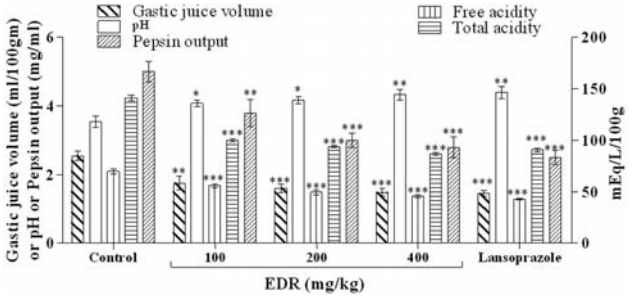

Fig. 1—Effect of EDR on the gastric juice volume, P^H, free acidity and total acidity in gastric secretion and on the level of pepsin output in gastric content of Pylorus ligated ulcer in rats. Bars are expressed as means±SEM (n=6.). Analysis of variance followed by dunnett's test with control group. * P <0.05, ** P <0.01 and *** P <0.001 were considered significant.

Indomethacin induced ulcers

The gastrointestinal irritant properties of nonsteroidal anti-inflammatory drugs (NSAIDs) such as indomethacin are the major impediment to their use as anti-inflammatory drugs[33]. Indomethacin is an NSAID that inhibits prostaglandin production that results in increased acid production and decreased cytoprotective mucus formation, which can lead to this gastrointestinal ulcer[34]. Indomethacin produces ulcers in the glandular region of the stomach of rat. Pre-treatment of EDR at different doses showed reduction in the ulcer score as compared to the control group 12.49±0.61 where only vehicle was given. EDR (400 mg/kg) showed a protective response up to 81.99% with a highly significant value (P <0.001). The doses of EDR at 100 and 200 mg/kg reduced the ulcer score up to 66.61 and 73.98%, respectively. Inhibitory effects of EDR are comparable to lansoprazole. Results are presented in Table 2. The malondialdehyde in the ulcer control group was 77.32±1.75 nmol/g wet tissue. The level of malondialdehyde in EDR treated group were found to

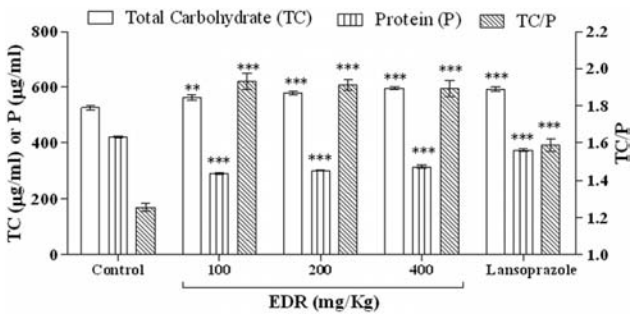

Fig. 2—Effect of EDR on the amount of total carbohydrate (TC), protein (P) and their ratio (TC/P) in gastric secretion of Pylorus ligated ulcer in rats. Bars are expressed as means±SEM (n=6.). Analysis of variance followed by dunnett's test with control group. ** P <0.01 and *** P <0.001 were considered significant.

Table 2—Effect of EDR on indomethacin induced ulcers in rats

Treatment	Ulcer Index	% Protection	Malondialdehyde (nmol/g wet tissue)	PGE_2 (pg/mg)	GSH (µg/mg protein)	Gastric wall mucus (µg/g wet glandular tissue)
Control	12.49±0.61	-	77.32±1.75	2223±325	1.12±0.04	194.35±3.56
EDR 100	4.17±0.56***	66.61	68.47±1.53***	3575±412**	1.15±0.07	201.24±4.56
EDR 200	3.25±0.31***	73.98	63.56±1.34***	4250±456***	1.63±0.05**	209.40±5.45**
EDR 400	2.25±0.51***	81.99	62.21±1.59***	4200±472***	1.77±0.08***	235.46±5.16***
Lansoprazole	1.83±0.46***	85.33	64.87±1.28***	3825±325***	1.72±0.04***	239.68±4.12***

Values are expressed as mean±SEM (n = 6). ANOVA followed by Dunnett's test with control group. Significance represented as **(P <0.01) and ***(P <0.001).

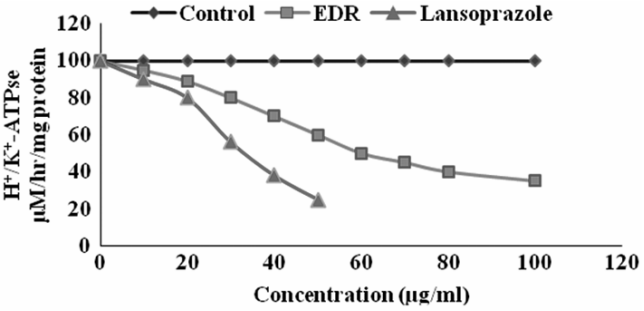

Fig. 3—Effect of EDR and Lansoprazole on H^+/K^+-ATPase activity in the rat gastric microsomes.

be decreased statistically significant (P <0.001) with the control and comparable to the lansoprazole (Table 2). Decrease in the value of malondialdehyde in EDR treated groups indicates its antioxidant activity.

Estimation of prostaglandin generation is of immense importance in context to NSAIDs induced ulcers. The PGE_2 generation in the ulcer control group was 2223±325 pg/mg tissue protein. The PGE_2 value of EDR in different doses and reference drug lansoprazole treated group were found to be comparable and statistically significant (Table 2). The results of gastric wall mucus are also presented in Table 2. In the experimental induction of gastric ulcers by indomethacin, EDR presented gastroprotection, in the doses of 100 and 200 mg/kg and 400 mg/kg. Given that the ulcerogenic properties of NSAIDs are due to the fact that they diminish the protective factors of the mucosa such as PG and mucus[35], it can be affirmed that the antiulcerogenic activity of EDR observed in this model must augment these mucosal protective factors.

Our results showed a significant reduction in nonprotein sulfhydryls content of gastric mucosa after indomethacin administration. Pre-treatment of rats with EDR significantly (P <0.001) prevented NP-SH depletion. The results are shown in Table 2. Decreased NP-SH level by noxious substance is in agreement with other reports which have demonstrated the important role of NP-SH in gastric mucosal damage by noxious substance[36]. These findings clearly showed the possible involvement of NP-SH in the cytoprotective and antioxidant activities of EDR.

Effects on H^+/K^+-ATPase activity

To ensure the gastroprotective activity of EDR, we investigated the effect of EDR on H^+/K^+ATPase inhibitory activity in isolated gastric microsomes from rat stomach. EDR inhibited the proton pump activity with an IC_{50} value 68.31 µg/mL comparable to reference drug lansoprazole with an IC_{50} value 34.32 µg/mL, and it was signifying the antisecretory activity of the EDR (Fig. 3).

Conclusions

The results of this study well substantiated the use of *Delonix regia* in the traditional management of gastric ulcers. The mode of action of the ethanol extract of *D. regia* may overlay the way for the establishment of a new gastric antisecretory and antiulcer therapy regimen that will obliterate the use of antacids and antisecretory agents. However, further studies to identify the dynamic constituent and elucidations of the mechanism of action are suggested.

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