

ANTIULCER ACTIVITY OF THE SEEDS OF ENTADA PHASEOLOIDES

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Summary

To evaluate the antiulcer activity of ethanol extract of seeds of Entada Phaseoloides. The ethanol extract of the seeds of Entada phaseoloides was assessed for its antiulcer activity against aspirin plus pylorus ligation induced gastric ulcers in rats, HCl-ethanol induced ulcer in mice and water immersion stress induced ulcers in rats. A significant ($P < 0.001$) antiulcer activity was observed in all the models. The parameters taken to assess antiulcer activity were volume of gastric secretion, free acidity, total acidity and ulcer index. Preliminary phytochemical screening of the Entada Phaseoloides gave positive test for steroids, saponins and alkaloids. The results indicate Entada phaseoloides possess antiulcer activity.

Key words: Ethanol extract of Phaseoloides, Aspirin pylorous ligation method, HCl-ethanol induced ulcer, stress induced ulcers.

Introduction

Nature always stands as a gold mark to exemplify the out standing phenomenon of symbiosis. In traditional medicines various herbal preparations are used for duodenal ulcers (1). Entada phaseoloides is a very large woody climber belonging to family Mimosidae, subfamily of Leguminosae, in India, the species is widely distributed in eastern himalyas, East Bengal, Tirupati (Andhra Pradesh) (2). The main chemical constituents present are sulphur containing amide entadamide A isolated from seeds and

characterized, in addition entadamide B isolated from seeds (3) and its structure determined. Two new entada saponins – ES II and ES IV isolated from bark and characterized as 3-O-[β -D-xylopyranosyl(1 \rightarrow 2) α -L-arabinopyranosyl(1 \rightarrow 6)] [β -D-glucopyranosyl(1 \rightarrow 4)]-2-acetamido-2-deoxy- β -D-glucopyranosyl-28-O-[β -D-apiofuranosyl(1 \rightarrow 3)- β -D-xylopyranosyl(1 \rightarrow 2)][2-O-acetyl]- β -D-glucopyranosyl(1 \rightarrow 4)]-6-O-[6(R)6-hydroxy-2,6-dimethyl-2(E)2,7-octadienoyl]- β -D-glucopyranosyl-oleanolic acid and entagenic acid respectively(4). Entada saponin ES III isolated from bark and characterized as echinocystic acid bearing same carbohydrate moieties as in case of ES II and ES IV (5), isolation and structure determination of a homogentisic acid glucoside-phaseoloidin from seeds: a new amide entadamide C (6) isolated from leaves along with entadamide A: its structure and absolute configuration determined and confirmed by synthesis (7). The plant has been used in traditional system of medicine, the seeds are fish poison, considered tonic, emetic, antiperiodic and anthelmintic(8). A paste of the seeds is applied to relieve inflammatory glandular swellings in the axilla known as Khaka bilari. The present study was undertaken to evaluate the antiulcer activity of ethanol extract of entada phaseoloides against aspirin induced plus pylorus ligation induced gastric ulcer, HCl-ethanol induced ulcer and water immersion test induced ulcers in rats.

Materials and Methods

Collection and extraction of Seeds

Entada phaseoloides seeds were collected from Thalkona forests of the southern region of Chittoor district of Andhrapradesh, India and authenticated by Dr. Najmunnisa Begum, Botanist, Ghulam Ahmed College of Education, Hyderabad. A voucher specimen has been deposited at the museum of our college. The seeds of Entada phaseoloides were air-dried. The plant material was extracted by soxhlet extraction with ethanol for 96 h. The extract was concentrated using rotary vacuum to get the solid mass. The yield was 7.51%. The extract ranitidine, sucralfate and omeprazole were suspended in 1% sodium carboxy methyl cellulose and used for antiulcer studies.

Animals

Wistar Albino rats of either sex and Swiss albino mice were obtained from sainath agencies, authorized agency Hyderabad. The animals were housed in polypropylene cages at 24±2°C and fed with commercial pellet diet and water ad libitum. All animal experiments were carried out in accordance with guidelines of CPCSEA and the study was approved by the Institutional Animal Ethical committee (IAEC/SUCP/2007/07).

Aspirin plus pylorus ligation induced gastric ulcer in rats

The wistar albino rats weighing 100-200 g of either sex were divided into 3 groups, each group consists of 6 animals. Animals were provided with standard rodent pellet diet and the food was withdrawn 18-24h before the experiment though water was allowed ad libitum. All the experiments were performed in the morning according to current guidelines for the case of laboratory animals and care was taken to avoid coprography. Animals were anaesthetized using ketamine (65 mg/Kg), the abdomen were opened and pylorus ligation was done without causing any damage to its blood supply. The stomach was replaced carefully and the abdomen wall was closed in two layers with interrupted sutures. The animals were deprived of water during the post-operative period. After 4h, stomachs were dissected out and cut open along the greater curvature and ulcers were scored by a person unaware of the experimental protocol in the glandular portion of the stomach (9). The gastric content was titrated against 0.01N NaOH (Table-1).

Ethanol Induced Ulcers in mice

The gastric ulcers were induced in rats by administrating 100% ethanol (1ml/200g) and the animals were sacrificed by cervical dislocation and stomach was incised along the greater curvature and examined for ulcers (10). The ulcer index was scored, based upon the product of length and width of the ulcers in the glandular portion of the stomach (square millimeters per rat) (Table-2).

Cold-restraint stress induced ulcers in rats (CRS)

Rats were deprived of food, but not water, for about 18 h before the experiment. On day six, the experimental rats were immobilized by strapping the fore and hind limbs on a wooden plank and kept for 2h, at temperature of 4-6°C(11). Two hours later, the animals were sacrificed by cervical dislocation and ulcers were examined on the dissected stomach as described above (Table-3).

Statistical Analysis

The result are expressed as mean \pm standard deviation and standard error of means .ANOVA was applied and showed significant difference between groups with $P<0.001$ & $P<0.05$. The statistical calculations were done using Graphpad Prism 5 software.

Results and Conclusions

From the screening results it was observed that, in aspirin pylorus ligation induced gastric ulcer method, the ethanol extract of entada phaseoloides showed moderate reduction in gastric volume, free acidity and ulcer score as compared to control (Table-1). From the table-2 & table-3 it shows that HCl-induced lesion in mice, cold restraint ulcer in mice have shown moderate activity when compared with the standard. Though we have not studied the active principles responsible for antiulcer activity, it is likely that the seeds of entada phaseoloides contain chemical constituents entadamide A, B & C, phaseoloides may likely be responsible for antiulcer activity. Gastric ulcers result from imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous mechanism (12). The excessive gastric formation by prostaglandin includes both increases the mucosal resistance as well as decrease in aggressive factors, mainly acid and pepsin (13). Inhibition of prostaglandin synthesis by aspirin in comparison with earlier stages of damage to cell membrane of mucosal, parietal & endothelial cells (14). Preliminary phytochemical screening revealed the presence of steroids, saponins, alkaloids.

Table-1 Effect of Entada phaseoloides ethanol extract on gastric secretion, acidity, P^H and ulcer score in aspirin plus pylorous ligated rats

Treatment (mg/kg.b.wt)	Volume of gastric secretion (ml/100g)	Free acidity (mEq/l/100g)	Total acidity (mEq/l/100g)	P ^H	Ulcer Score	Ulcer Inhibition (%)
Vehicle control(normal saline 2 ml)	7.5±0.11	22.6±0.05	60±0.20	2.100±0.142	2.5±0.04	-----
Ranitidine 50	6.1±0.01	14.30±0.03	40±0.12	3.523±0.146	2.0±0.03	88.75
Entada phaseoloides 200	5.0±0.01	9.1±0.02	35±0.20	3.311±0.120	1.0±0.02	50.00

Values are expressed as mean ± SEM P<0.001 when compared with control

Table-2 Effect of Entada phaseoloides ethanol extract against HCl-ethanol induced gastric lesion in mice

Treatment	Dose (mg/kg.b.wt)	Gastric lesion (Mean± SEM)	Ulcer Inhibition (%)
Control	2 ml	19.258±2.346	-----
Sucralfate	100	1.0±0.0.442	92.42
Entada phaseoloides	200	0.684±0.446	52.42

Values are expressed as mean± SEM P<0.05 when compared with control

Table-3 Effect of Entada phaseoloides ethanol extract against Cold restraint stress induced ulcer in rats

Treatment	Dose (mg/kg.b.wt)	Mean ulcer score	Ulcer Inhibition (%)
Control	2ml	22.5±3.0	-----
Omeprazole	20	0.0±0.0	100
Entada phaseoloides	200	1.26±1.0	50

Values are expressed as mean± SEM P<0.001 when compared with control

The ethanol extract of entada phaseoloides was evaluated for antiulcer activity against aspirin plus pylorous ligation, HCl-ethanol induced, Cold restraint induced animal models. The various parameters like gastric secretion, total acidity, ulcer index, gastric lesions were evaluated. In aspirin plus pylorous ligation ulcers are due to antisecretory mechanism, autodigestion of gastric mucosa and breakdown of gastric mucosal barrier

(15). Ethanol induced ulcer are more predominant in the glandular part of the stomach stimulates leukotrienes, 5-lipoxygenase pathway, mast cell secretory product and break down of reactive oxygen species resulting in the damage of gastric mucosa(16,17). Stress plays an important role in inducing ulceration. Increase in gastric motility, vagal over activity, mast cell degranulation, decreased prostaglandin synthesis are involved in the genesis of stress induced ulcers. Entada phaseoloides has shown medium activity in different models when compared with standard drugs. Further studies are required to isolate the active principle responsible for activity.

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