

ANTI-ULCER EFFECT OF *ARGYREIA SPECIOSA* ETHANOLIC ROOT EXTRACT IN RATS

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Summary

The objective of this study was to investigate the antiulcer activity of *Ethanollic root extract of Argyreia speciosa* in rats. Antiulcer effects of the ethanolic root extract at 25, 50 and 100 mg/kg were evaluated in rats using ethanol, indomethacin and aspirin induced ulcer methods. Phytochemical analysis and lethality tests (LD50) were carried out using standard methods. Results showed that the ethanolic root extract exhibited significant ($p < 0.05$) and dose dependent anti-ulcer activity in all ulcer models. Percentage ulcer inhibitions of extract at 100 mg/kg for ethanol, aspirin and indomethacin induced ulcers were 73.5, 60.5 and 87.5%, respectively. Ulcer protections in all the models by the extract were dose-dependent. The ulcer inhibitory effects of the extract were comparable with those of standard drugs especially in the drug-induced ulcers. Oral LD50 value greater than 5000 mg/kg was obtained indicating the safety of the plant for consumption. Phytochemical analysis showed the presence of glycosides, tannins, alkaloids, saponins and flavonoids. Therefore results of our study suggest the ethanolic root extract of *Argyreia speciosa* possesses antiulcer activity in rats.

Key words: *Argyreia speciosa*, anti-ulcer activity, sucralfate, omeprazole.

Introduction

Ulcer is erosion in the lining of the stomach or duodenum which is caused by the disruptions of the gastric mucosal defense and repair systems^[1]. Ulcers are produced when any factor causes an imbalance between the protective factors (mucus and bicarbonate) and aggressive factors (acid and pepsin) in the stomach. Such factors could range from natural causes (gastric cancer), infections (*H. pylori*), lifestyle (drugs like non steroidal antiinflammatory agents, alcohol, stress and cigarette smoking)^[2]. Even though a range of drugs are available for the treatment of ulcer, many of these do not fulfill all the requirements and have side effects^[3,4].

Current treatment of ulcers in developing countries has been largely suppression of pain, with little or no strategy aimed at a cure. Recently, there has been much interest in herbal medicines derived from the traditional knowledge of plant pharmacological properties as it is fast emerging as an alternative treatment to available synthetic drugs for treatment of ulcer possibly due to lower costs, availability, fewer adverse effects and effectiveness. Many tropical herbs have been scientifically reported to possess potent antiulcer activity^[5,6].

Argyreia speciosa (Convolvulaceae), commonly known as elephant creeper is a woody climber distributed throughout India and has been used as a 'rasayana' drug in the traditional Ayurvedic system of medicine. The leaves are used by natives as a local stimulant and rubefacient in skin diseases, the seeds are a rich source of ergoline alkaloids while the roots are reported to be a tonic, aphrodisiac, bitter, diuretic and used in rheumatism, gonorrhea, chronic ulcer and diseases of nervous system^[7,8]. Phytochemical screenings of the plant have shown the presence of alkaloids, flavonoids, triterpenes, phenylpropanoids, lipids, tannin and resin^[9-10]. Pharmacological studies on *Argyreia speciosa* have been reported it to possess anti-inflammatory^[11], anti-arthritic^[11], immunomodulatory^[12], wound healing^[13], hepatoprotective activity^[14] and nootropic effect^[15]. This study was designed to evaluate the antiulcer activity of ethanolic root extract of *Argyreia speciosa*(ERAS).

Materials and Methods

Plant material, chemicals, and drugs

ERAS was procured from Green Chem. Pvt. Ltd, Bangalore, India (ARG/8001). All chemicals used in the present study were of analytical grade and purchased from SD fine chemicals Ltd (Mumbai, India). Omeprazole, sucralfate (reference drug) was procured from Dr.Reddy's laboratories (Hyderabad, India).

Animals: Adult Swiss albino mice (20- 25g) and Wistar Rats (150 -200g) of either sex were used for the study. The mice and rats were fed with standard pellet and water *ad libitum*. The animals were maintained under standard 12-hr light / dark cycle throughout the study. The study protocol was approved by Institutional Animal Ethical Committee (IAEC) (No.CPCSEA/IAEC/PC-01/346).

Phytochemical screening^[1]: ERAS was tested for the presence or absence of secondary metabolites using standard phytochemical procedures and tests.

Acute toxicity study^[2]: Five groups (n = 5) of male albino mice were used in the acute toxicity study of ERAS. Animals from all groups were fasted overnight and administered (p.o) with single dose (250, 500, 2000 and 5000 mg/kg) of ERAS. A group of animals which received equal volume of PBS served as control. Changes in the behavior of animals were observed for 24 h after extract administration. For any signs of toxicity and mortality, animals were observed for 14 days.

Anti-ulcer activity: Three models (Ethanol, Aspirin, and Indomethacin) with effective induction of ulcer experimentally in rats were employed to evaluate the antiulcer activity of ERAS. All the rats used were fasted for eighteen hours but were given water *ad libitum* till the start of the experiment.

Ethanol-induced ulcer^[16]: Thirty fasted animals were used in five groups of six animals each. Groups A and B received 2 ml/kg distilled water (negative control) and 100 mg/kg p.o. sucralfate, while rats in groups C, D and E were given 25, 50 and 100 mg/kg p.o. of ERAS respectively. After one hour all animals received 1 ml/kg of 80% ethanol orally. The rats were sacrificed with chloroform anesthesia after one hour. The stomachs were isolated, washed gently under clean flowing water and cut open along the greater curvature. The stomachs were then fixed in 10% formalin and craters observed and ulcer scores were recorded.

Aspirin-induced ulcer^[17]: Thirty fasted rats were also used this model as five groups of six rats each. Groups A and B of this model received distilled water (2 ml/kg) and omeprazole 20 mg/kg p.o respectively, while groups C, D and E received 25 mg/kg, 50 mg/kg and 100 mg/kg p.o of ERAS. After one hour, 200 mg/kg p.o of aspirin was given to each rat, and was sacrificed 4h later as described above. Stomachs were isolated, fixed and ulcers counted.

Indomethacin-induced ulcer^[18]: Animals (five groups of six rats each) in groups A, B, C, D and E received distilled water 2 ml/kg p.o., omeprazole 20 mg/kg p.o, 25, 50 and 100 mg/kg p.o ERAS respectively. After 30 min, indomethacin 40 mg/kg p.o was administered to each rat. After 8 h of drug treatment, stomachs were isolated, cut and ulcers counted.

Statistical analysis: Ulcer indices were shown as the mean±standard error of mean and level of ulcer protection presented as percentage inhibition. The significance of the differences in mean ulcer indices between extract and negative control was calculated at 95% confidence interval using Student's t-test.

Results

Phytochemical screening showed that the extract contains alkaloids, glycosides, saponins, tannins, flavonoids and resins. Acute toxicity results showed that the LD₅₀ was greater than 5000 mg/kg. The minimum doses of ERAS 25, 50 and 100mg/kg body weight were selected for the study.

Ethanol-induced ulcer: In Table 1, ulcer inhibition was evident in all treatment of ERAS extract compared to the negative control. However, statistically significant ulcer inhibition (59.5 and 73.5%, $p < 0.05$) could be seen only at doses of 50 and 100 mg/kg of the ERAS. The protection from ulcer was dose dependent even as ulcer was produced in all rats in this model.

Aspirin-induced ulcer: ERAS extract at all the doses provided protection from ulcer and the protection was dose dependent. ERAS at doses of 50 mg/kg and 100 mg/kg provided statistically significant protection (74.6% and 87.5%, $p < 0.05$) when compared with the negative control (Table 2).

Indomethacin-induced ulcer: ERAS extract protected the rats from experimentally-induced ulcers at all dose levels but the lesions produced in this model were noticeably more severe than the aspirin model (Table 3). However, the percentage ulcer inhibition was the least when compared to values obtained in the other two models. The dose of 100 mg/kg of extract proved to be the only dose with statistically significant protection (60.5%, $p < 0.05$).

Treatments	Dose mg/kg p.o	Quantal ulcer incidence	Ulcer index	Ulcer inhibition(%)
Distilled water	2ml/kg	6/6	1.90 ± 0.15	
sucralfate	100	6/6	0.52 ± 0.14	84.3
ERAS	25	6/6	1.30 ± 0.24	31.50
ERAS	50	5/6	0.77 ± 0.09*	59.50
ERAS	100	4/6	0.50 ± 0.09*	73.50

Table 1: Effects of aqueous leaf extract of EREAS on Ethanol Induced Ulcers in Rats (n = 6)

Treatments	Dose mg/kg p.o	Quantal ulcer incidence	Ulcer index	Ulcer inhibition(%)
Distilled water	2ml/kg	6/6	0.60 ± 0.10	
Omeprazole	20	5/6	0.11 ± 0.04	84.45
ERAS	25	6/6	0.25 ± 0.06	62.70
ERAS	50	6/6	0.17 ± 0.02*	74.60
ERAS	100	2/6	0.08 ± 0.04*	87.50

Table 2: Effects of aqueous leaf extract of EREAS on Aspirin Induced Ulcers in Rats (n = 6)

Treatments	Dose mg/kg p.o	Quantal ulcer incidence	Ulcer index	Ulcer inhibition(%)
Distilled water	2ml/kg	6/6	3.60 ± 0.45	
Omeprazole	20	6/6	0.55 ± 0.14	84.3
ERAS	25	6/6	3.20 ± 0.38	35.00
ERAS	50	6/6	2.67 ± 0.29*	26.50
ERAS	100	6/6	1.35 ± 0.15*	60.50

Table 3: Effects of aqueous leaf extract of EREAS on Indomethacin Induced Ulcers in Rats (n = 6)

Values are expressed in mean ± SEM: n = number of animals in each group.

*: p<0.05 vs negative control (Students t-test)

Discussion

The anti-ulcer activity of EREAS against ethanol-, aspirin- and indomethacin-induced ulcers was established in this study. Results of acute toxicity showed that the plant is safe as exemplified by its use as food in domestic and wild animals. Ethanol induced gastric ulcer was employed to study the cytoprotective effect of the extracts. Ethanol induced gastric lesion formation may be due to stasis in gastric blood flow which contributes to the development of the haemorrhage and necrotic aspects of tissue injury. Alcohol rapidly penetrates the gastric mucosa apparently causing cell and plasma membrane damage leading to increased intra cellular membrane permeability to sodium and water. The massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury. This leads to cell death and exfoliation in the surface epithelium^[19] The extract protected the stomach against ethanol's necrotic damage and its effect was more pronounced than sucralfate, a cytoprotective agent. may be due to both reductions in gastric acid secretion and gastric cytoprotection earlier study has suggested the plant's ability to protect against HCl/Ethanol challenge by prostaglandin-like cytoprotection. Treatment of rats with However, an antisecretory effect might be indicated as the extract protected the stomach mucosa from NSAIDS (aspirin and indomethacin) induce gastric damage through mechanisms which include suppression of prostaglandin generation, overproduction of leukotrienes, acting as a topical irritant and by reducing the local blood-flow^[2].

Rats pretreated with ERAS produced significant protection. This damage is elicited by the inhibition of prostaglandin synthesis, which is essential for mucosal integrity and regeneration^[20]. This results to a sustained reduction in mucosal blood flow and a subsequent generation of ulcer. Sucralfate and omeprazole were employed as positive control in this study for the cytoprotective effect^[21] against experimentally induced ethanol ulcers and omeprazole exhibits an anti-secretory and protective effect against ulcers and agents providing ulcer healing against NSAID induced ulcers may provide similar effect.

The preliminary phytochemical screening of *Argyrea speciosa* showed the presence of alkaloids, flavonoids, and triterpenes. Previous studies have shown that flavonoids may be related to the anti-ulcer activity^[21] and play a major role in the mechanism of gastroprotection^[22-23]. In addition to flavonoids, other constituents in ERAS such as triterpenes are known for their antioxidant activities, which may contribute to some of the anti-ulcer mechanisms^[24].

Conclusion

It may be concluded that ethanolic root extract of *Argyrea speciosa* exerts a significant protection against ethanol, aspirin and indomethacin induced gastric ulcers in rats compared to the standard reference drugs.

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