



Research Article

ANTI-ULCER ACTIVITY OF ETHANOLIC *SARCOSTEMMA ACIDUM* STEM EXTRACT

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ABSTRACT

The use of natural herbs for the prevention or treatment of ailments gives sound recommendations fostering the development of quality medicines with excellent phytonutrients. So, attempt was made to test the antiulcer activity of selected plant. Objective of the study was to evaluate the gastric anti-ulcer activity of ethanolic extract of *Sarcostemma acidus* (ESA) stem in experimental animals. Antiulcer activity of ESA was investigated in indomethacin induced ulcer models in Wistar rats. In models, the parameters determined were ulcer index and % protection, pH and gastric volume. The extract (150 mg/kg) showed significant ($P < 0.001$) reduction in ulcer index and % protection as compared to control. It can be concluded that ESA possess anti ulcerogenic property due to the presence of flavonoids and antioxidants which can scavenge free radicals responsible of ulcer induction.

Keywords: Antiulcer, *Sarcostemma acidus*, ethanol, wistar rats, ulcer index

INTRODUCTION

Sarcostemma acidum (Roxb) is a xerophytic plant

Family: Asclepiadaceae.

Synonyms: khir khimp, khursani tanto, Artthor, soma and somavalli, moon plant and moon creeper, somalata.

Description: Branched, leafless straggling shrub, climbing on euphorbiacacuducifolia haines on hills¹.

Uses

1. Dried stems - emetic, leprosy.
2. Roots - snake bite and rabies². The root is taken as infusion in dog bites cases³.
3. Plant is acrid, cooling, narcotic, emetic, antiviral and rejuvenating, useful in vitiated conditions of pitta, dipsia, hydrophobia, psychopathy and general debility⁴.
4. In Vedic literature, it has been called soma, a sacred plant. The juice of this plant is considered as the divine drink offered to gods, contemplated with medicinal efficacy, used as natural restorative for health that makes the consumer awakened and alert⁵.

Chemical constituents: Phytochemicals like malic acid, succinic acid, reducing sugars and βamyrins, lupeol, lupeolacetate, β-sitosterol, four lignans, sacidumignans A–D(1–4), and two degraded lignan derivatives, sacidumols A (5) and B (6), (+)-pinoselinol, 9αhydroxypinoselinol, perforatic acid, and peucenine-7-O-methyl ether, isolated from the ethanolic extract of the whole plant of *Sarcostemma acidum*. Among these Sacidumignan A showed moderate antimicrobial activities against two Gram positive bacteria *in vitro*⁶. The species of *Sarcostemma* are used by cultivators to extirpate white ants from sugarcane fields. A bundle of twigs is put into trough from which the field is watered together with a bag of salt and water thus impregnated destroys the white ants without affecting the crop⁷.

Physiology of Ulcer: Gastric ulcers are mucosal lesions that result from an imbalance between aggressive factors such as acid and pepsin, and defensive mechanisms like gastric mucous, high mucosal blood flow and high mucosal turnover rate that work towards maintenance of mucosal integrity⁸. Another factor that has been implicated in the pathogenesis of gastric ulcers is oxidative stress in the gastric mucosa. Studies have shown a positive correlation between increased free radicals and the extent of gastric ulceration in experimental animals⁹⁻¹¹. Agents that are currently available for the treatment of gastric ulcers act by either reducing gastric acid secretion (H₂ blockers, proton pump inhibitors, anti muscarinic agents), acting as physical barriers (sucralfate, colloidal bismuth sub citrate), or increasing the mucous and bicarbonate secretion (prostaglandin analogues, carbenoxolone). Even though these agents are effective in healing of gastric ulcers, continued use is required to prevent recurrence. Continued use of these agents can in turn lead to a plethora of side effects ranging from dryness of mouth to achlorhydria, atrophic gastritis, osteodystrophy and encephalopathy¹². A new approach would be the use of cytoprotective agents that can also modulate the antioxidant defenses in the body and thus prevent mucosal damage and gastric ulceration. As plants are a rich source of active principles and antioxidants, there has been a growing interest to identify and scientifically validate agents that have traditionally been used in folk medicine in the treatment of gastric ulcers and related diseases.

MATERIALS AND METHODS

Chemicals and drugs

All chemicals required for laboratory experimentation were purchased from Fischer scientific products, India. Indomethacin and omeprazole used were of Ranbaxy laboratories.

Plant collection and authentication

Fresh stems were collected from *Sarcostemma acidum* plant growing at Chalapathi Institute of Pharmaceutical Sciences garden. The plant was identified and authenticated by (Acharya Nagarjuna University, Guntur, Andhra Pradesh, India). Stems were dried under shade and powdered and stored in an airtight container. For extraction 50g of dried stem powder was loosely packed in the thimble of soxhlet apparatus and extracted with ethanol for 24hrs at 50° C. The extract was dried in a hot air oven at 35° C and weighed

Experimental Animals

Wistar rats (12,195-200g) were obtained from the animal house, Chalapathi Institute of Pharmaceutical Sciences and kept in standard environmental conditions. They were fed with rodent diet and water *ad libitum*. Experiments were carried out in accordance with CPCSEA guidelines and the study was approved by Institutional Animal Ethical Committee (22/IAEC/CLPT/2016-17).

Preparation of extracts

Stems of *Sarcostemma acidum* plant were washed under running tap water and dried in shade for two weeks. Dried stems were powdered, sieved and stored in an air tight container at room temperature. Dried powder (400 g) was extracted with ethanol by using soxhlet apparatus. The extracts were concentrated to dryness using rotary evaporator. The extracts were preserved in refrigerator at 40 C for further use.

Phytochemical screening

The ethanolic extract of the *S.acidum* stem was evaluated for the presence of flavonoids, tannins, alkaloids, saponins, glycosides and sterols/triterpenes¹³.

Animal grouping, ulcer induction and treatments

Group of 12 wistar rats weighing 195-200g were used. They were fasted with free access to water 24 h prior to ulcer induction. All the animals were randomized into three groups of four rats each as below:

Group 1 (ulcerated control) – indomethacin (30mg/kg body weight)

Groups 2 - 150 mg/kg b.w. of ethanolic extract.

Group 3 - omeprazole (20 mg/kg b.w.) after pretreatment with indomethacin.

After six hours, the rats were sacrificed in CO₂ anesthesia and their stomachs were removed, stored in formal saline (2% v/v) over night. Next day the stomachs were opened along the greatest curvature, washed in warm water and examined for lesions and total score (in mm for each animal) and the mean count for each group was calculated¹⁴.

Estimation of total acid output and Ph

Total acid output of the gastric juice was estimated by titration of 0.1 ml of gastric juice with 0.01 N sodium hydroxide using phenolphthalein as indicator. Total acid output was expressed as mEq/L per 100 gm of body weight. pH of the gastric juice is determined using a pH meter¹⁵.

Quantification of ulceration

The following groups of animals were used.

Group I, normal control;

Group II, ulcer: indomethacin (20 mg/kg body wt, p.o);

Group III, drug control: Garcinia cambogia fruit extract (1 g/kg body wt/day) alone for 15 days;

Group IV, pretreated ulcer: Garcinia cambogia fruit extract (1 g/kg body wt/day) for 5 days □ indomethacin (20 mg/kg body wt, p.o);

Group V, pre-treated ulcer: Garcinia cambogia fruit extract (1g/kg body wt/day) for 10 days □ indomethacin (20 mg/kg body wt, p.o);

Group VI, pretreated ulcer: Garcinia cambogia fruit extract (1g/kg body wt/day) for 15 days □ indomethacin (20 mg/kg body wt, p.o). used.

The stomachs were harvested, opened along the greater curvature and the mucosa was exposed for macroscopic evaluation. The ulcer index (UI, mm²) was calculated as the arithmetic mean for each treatment¹⁶.

Severity of scores

Normal coloration - 0

Red coloration - 0.5

Spot ulcer - 1.0

Hemorrhagic ulcer - 1.5

Deep ulcer - 2.0

Perforations - 3.0

Ulcer index (UI)

$$UI = (UN + US + UP) \cdot 10^{-1}$$

UN – average number of ulcers per animal.

US- average of severity score

UP- percentage of animal with ulcer.

$$\% \text{protection} = \frac{\text{control mean ulcer index} - \text{test mean ulcer index}}{\text{control mean ulcer index}} \times 100$$

Statistical analysis

Inhibition against ulceration was expressed in percentage. Other results were expressed as mean of three determinations ± standard error of mean. One-way analysis of variance (ANOVA) complemented with Student's t-test using PRISM software package for windows (Version 16) for differences between means was used to detect any significant difference (p < 0.001) between the treatment groups in this study.

RESULTS

The effects of ESA on the ulcer index and % inhibition against ulcer in the experimental animals are shown in Fig. 1, 2 and table1. Oral administration of 30 mg/kg b.w. of indomethacin caused a significant (p < 0.001) increase in the degree of ulceration (ulcer index) in the rats. A significant improvement in the level of inhibition against ulceration was however observed in the extracts of treated animals. Indomethacin administration caused significant (p < 0.001) decrease in pH value with a corresponding significant (p < 0.001) increase in gastric volume of gastric content. Pre-treatment with the extracts produced significant increase in pH value coupled with significant decrease in gastric volume when compared with ulcerated control rats.

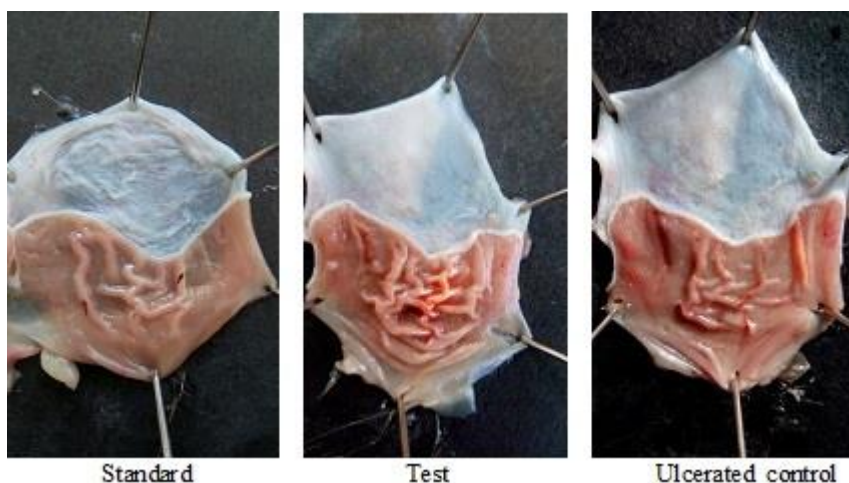


Figure 1: Images of animal tissue

Table 1: Effects of ESA extract on various parameters of indomethacin induced gastric ulcer

| Groups | Treatments | Dose(mg/kg) | Ulcer index | % protection | pH of gastric juice | Gastric volume |
|--------|-------------------|-------------|-----------------|--------------|---------------------|----------------|
| 1 | Ulcerated Control | 20 | 7.52±0.129*** | - | 3.21±0.0425 | 5.525±0.125 |
| 2 | Standard | 30 | 3.53±0.056*** | 53 | 5.35±0.0955 | 3.28±0.082 |
| 3 | ESA | 150 | 3.722±0.0045*** | 50 | 5.05±0.0645 | 4.3±0.0705 |

All values are mentioned in Mean ±SEM, *** indicates (p<0.001)

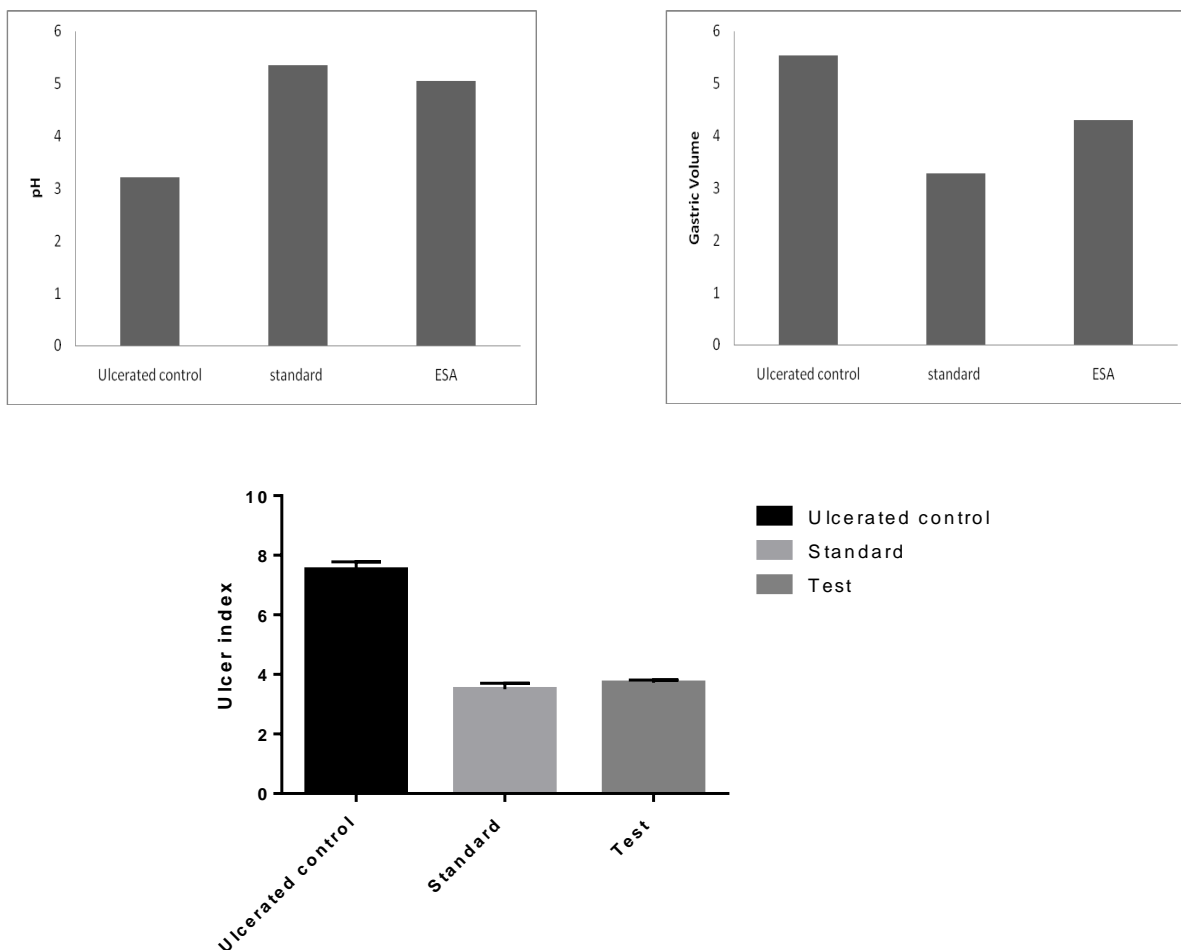


Figure 2: Effect of ESA on pH, gastric volume and ulcer index of indomethacin ulcerated rats

DISCUSSION

The pathogenesis of peptic ulcer is generally due to imbalance between aggressive factors and the maintenance of mucosal integrity by endogenous defense mechanism. Impediment of prostaglandin synthesis and free radicals formation by indomethacin has propounded the critical biochemical events in the pathogenesis of gastric ulceration. Use of costly synthetic drugs with major side effects, made the use of natural products to be non-toxic, efficacious and affordable in the treatment of gastric ulcer. Studies have revealed the presence of bioactive principles in ESA that promote good health. In the present study, the significant increase in ulcer index and gastric volume following oral administration of indomethacin in the ulcerated rats may be ascribed to either free radicals formation or inhibition of prostaglandin synthesis.

CONCLUSION

In a nut shell, the debilitation of gastric injury of indomethacin by administration of ethanolic extracts of *Sarcostemma acidus* at 150mg/kg body weight indicates gastro protective and anti ulcer potentiality in animals. Still research has to be done to identify the exact antiulcerogenic principle(s) in these extracts and also harness their possible synergistic efficacy against gastric ulcer.

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