



Research Article

GASTROPROTECTIVE POTENTIAL OF SAATHILADHI CHOORANAM: AN EXPERIMENTAL STUDY IN ASPIRIN AND PYLORUS LIGATION-INDUCED ULCER MODELS

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ABSTRACT

Gastric ulcer is a common gastrointestinal disorder caused by an imbalance between aggressive factors such as gastric acid, pepsin, and nonsteroidal anti-inflammatory drugs, and protective factors including mucus secretion and mucosal integrity. *Saathiladhi Chooranam* (SC) is a Siddha herbal formulation traditionally prescribed for stomach ulcers and other gastrointestinal ailments. The present study was designed to evaluate the anti-ulcer activity of SC in aspirin-induced and pylorus ligation-induced gastric ulcer models in Wistar albino rats. In the aspirin-induced ulcer model, animals were divided into four groups: control, standard, and test groups, standard drug (omeprazole 10mg/kg), and test drug SC at low and high doses. Ulcer index, percentage protection, and histopathological changes were evaluated. In the pylorus ligation model, gastric parameters such as pH, volume of gastric juice, free acidity, and total acidity were assessed. Statistical data were analysed by One-way ANOVA followed by Student's t-test. SC showed a significant and dose-dependent reduction in ulcer index in both models. The high dose of SC demonstrated greater protection comparable to the standard drug. In pylorus ligation, SC significantly reduced gastric secretion, free acidity, and total acidity, and increased gastric pH. Histopathological studies confirmed reduced mucosal damage and inflammatory cell infiltration in the test groups. The findings suggest that SC possesses significant anti-ulcer activity, possibly due to its anti-secretory and cytoprotective properties. Further studies are required to elucidate its exact mechanism of action and to confirm its safety and efficacy. Acute toxicity studies revealed no mortality or observable signs of toxicity up to 2000mg/kg, indicating the safety of the test compound.

INTRODUCTION

Gastric ulcer is one of the most prevalent gastrointestinal disorders worldwide, characterized by mucosal erosion due to an imbalance between aggressive factors such as gastric acid, pepsin, and reactive oxygen species, and protective factors including mucus secretion, prostaglandins, and mucosal blood flow [4,7]. Among the various etiological factors, the prolonged use of nonsteroidal anti-inflammatory drugs (NSAIDs), particularly aspirin, is a

major contributor to gastric mucosal injury, leading to ulcer formation through inhibition of prostaglandin synthesis and increased gastric acid secretion. [2,3]

Despite the availability of several pharmacological agents such as proton pump inhibitors and H₂ receptor antagonists, their long-term use is often associated with adverse effects and relapse of symptoms. This has led to growing interest in the exploration of alternative and complementary therapies, especially those derived from traditional systems of medicine. Medicinal plants and polyherbal formulations have been widely investigated for their potential gastroprotective effects due to their antioxidant, anti-inflammatory, and cytoprotective properties [5,6].

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Siddha medicine, one of the oldest traditional systems practiced in India, offers numerous formulations for the management of gastrointestinal disorders.

Saathiladhi Chooranam (SC)^[1] is a classical Siddha polyherbal formulation traditionally used in the treatment of gastric ulcers and related conditions. However, scientific validation of its therapeutic efficacy and mechanism of action remains limited.

Therefore, the present study was designed to evaluate the gastroprotective potential of *Saathiladhi Chooranam* using experimental models of gastric ulcer, namely aspirin-induced and pylorus ligation-induced ulcer models in Wistar albino rats. The study aims to assess its anti-ulcer activity by analyzing ulcer index, gastric secretion parameters, and histopathological changes, thereby providing experimental evidence to support its traditional use.

MATERIALS AND METHODS

Treatment of Animals

Following physical and behavioural veterinary examination, healthy male and female Wistar albino rats weighing 150–200 g and aged 4–8 weeks were selected from the Institutional Animal House of Arulmigu Kalasalingam College of Pharmacy, Krishnankoil. All the experiments involving animals complied with the ethical standards of animal handling and were approved by Institutional Animal Ethics Committee (Approval No: AKCP/IAEC/19/25-26). At the start of the experiment, the body weight of the animals was within $\pm 20\%$ of the average body weight for each sex.

All selected animals were acclimatized under standard laboratory conditions for at least five days before starting the treatment. The rats were housed in groups of six in polypropylene cages fitted with stainless steel grill tops.

The animals were maintained under a 12-hour light and 12-hour dark cycle. The room temperature was maintained at $22 \pm 3^\circ\text{C}$, and relative humidity was kept between 30–70%. Standard pellet diet and water were provided ad libitum throughout the study, except during overnight fasting prior to blood collection. After

blood collection, feed was provided immediately. Reverse osmosis (R.O.) drinking water was supplied in polypropylene bottles with stainless steel sipper tubes.

Acute Toxicity Study

The acute oral toxicity study was conducted at the Department of Pharmacology, Madras Veterinary College. Healthy experimental animals were used following OECD guidelines (425) ^[8] and approved by Institutional Animal Ethics Committee (44/SA/IAEC/2024). The test compound was administered in graded doses, and the animals were observed continuously for the first 4 hours and periodically for 24 hours, with special attention during the first 48 hours, and thereafter daily for a total of 14 days.

No mortality was observed in animals treated with the test drug at doses up to 2000mg/kg body weight. The animals did not exhibit any significant behavioural changes such as tremors, convulsions, salivation, diarrhoea, lethargy, or coma during the observation period.

All treated animals appeared normal with respect to:

- Skin and fur condition.
- Eyes and mucous membranes.
- Respiratory and circulatory signs.
- Autonomic and central nervous system behaviour.
- Somato motor activity

No significant changes in body weight were observed when compared to the control group throughout the study duration.

Based on these observations, the LD₅₀ of the test drug is considered to be greater than 2000mg/kg body weight, indicating that the drug is relatively safe as per OECD classification.

The dose selected for the anti-ulcer activity study was selected based on OECD toxicity study results of the maximum safe dose (200 and 400mg/kg).

Aspirin-induced gastric ulcer

Animals were divided into four groups, each consisting of six rats (n = 6).

S.No	Group	Treatment	No. of Animal
1	Group I	Control	6
2	Group II	Positive control 10mg/kg	6
3	Group III	Test drug low dose of SC	6
4	Group IV	Test drug high dose of SC	6

Group I served as the ulcer control and received aspirin (400mg/kg).

Group II received the standard drug omeprazole (10mg/kg).

Groups III and IV received the test drug SC at dose level 200 and 400mg/kg respectively.

Aspirin (400mg/kg) was administered^[2] orally 30 minutes prior to the administration of the standard

drug, test drug, or vehicle. After 6 hours, the animals were sacrificed. The stomachs were removed and injected with 2% formalin. Each stomach was opened along the greater curvature and immersed in 2% formalin solution.

The length of gastric lesions was measured using a dissecting microscope. The lesion index was calculated by summing the lengths (mm) of all lesions in each rat. Gastric lesions were scored as follows:

- 0 = Normal stomach
- 0.5 = Pink to red coloration
- 1 = Spot ulcer
- 1.5 = Hemorrhagic streaks
- 2 = Number of ulcers
- 4 = Ulcers with bleeding

The ulcer score was determined using a 10× magnifying lens. Severity grading was as follows:

- Pinpoint ulcer (1 mm) = 1
- 1–2 mm = 2
- > 2 mm = 3
- > 3 mm = 4

The mean ulcer score was calculated as:

Ulcer score = Total ulcer index in group / Total number of animals

Ulcer Index

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

Where:

- UI = Ulcer Index
- UN = Average number of ulcers per animal

- US = Average severity score
- UP = Percentage of animals with ulcers

Percentage Protection

% Protection =

$$(\text{Control mean ulcer index} - \text{Test mean ulcer index}) / \text{Control mean ulcer index}$$

Pylorus Ligation-Induced Ulcer Models

Male albino rats weighing 150–200g were used. Animals were divided into four groups (n=6). Rats were fasted for 24 hours prior to the experiment.

- Group I received normal saline (2ml/kg) and served as negative control.
- Group II received omeprazole (10mg/kg) as standard drug.
- Groups III and IV received SC at dose level 200 and 400mg/kg by oral route respectively, 30 minutes before pyloric ligation.^[3]

After 4 hours, the animals were sacrificed and the stomachs were opened to collect gastric contents. The total volume of gastric juice was measured and centrifuged at 1000 rpm for 10 minutes.

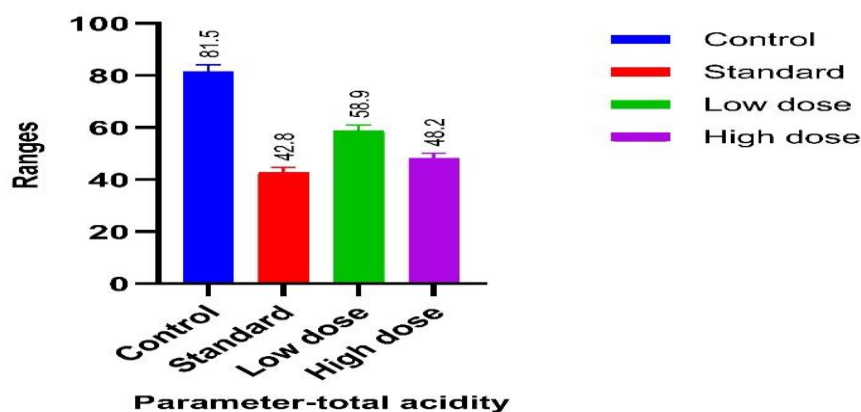
One ml of supernatant was diluted to 10ml with distilled water and titrated with 0.01 N NaOH using Topfer's reagent as indicator until the solution turned orange. This was recorded as free acidity. Titration was continued until a pink colour appeared and recorded as total acidity.

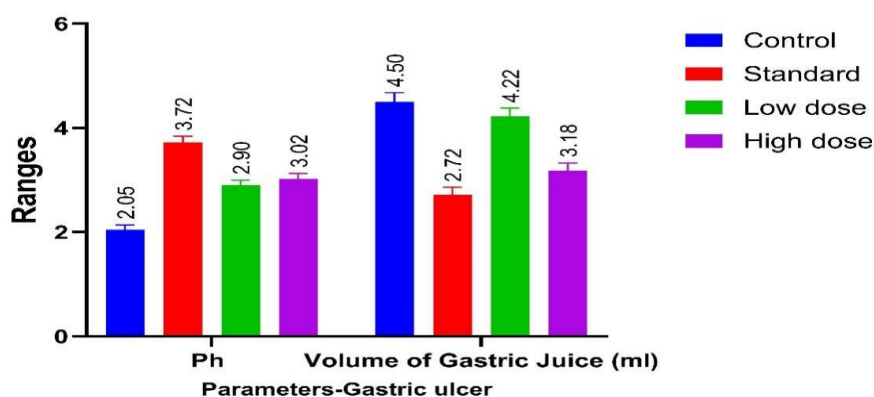
Acidity was calculated as:

$$\text{Acidity} = \text{Volume of NaOH} \times \text{Normality} \times 100 \text{ mEq} / 0.1$$

Table 1: Effect of SC Against Aspirin and Pylorus Ligation-Induced Gastric Ulcer in Rats

Treatment	Ulcer index	% Inhibition	pH	Free acidity	Total acidity	Volume of gastric juice (ml)
Control	28.9±1.2	-	2.05±0.09	30.8 ± 1.4	81.5 ± 2.6	4.50 ± 0.18
Standard	11.2±0.8	61%	3.72 ± 0.12	17.0 ± 0.9	42.8 ± 1.8	2.72 ± 0.14
Test drug low dose	18.4±1.0	41%	2.90 ± 0.10	24.8 ± 1.2	58.9 ± 2.1	4.22 ± 0.16
Test drug high dose	10.8±0.7	55%	3.02 ± 0.11	18.6 ± 1.0	48.2 ± 1.9	3.18 ± 0.15





EFFECT OF SC AGAINST ASPIRIN AND PYLORUS LIGATION INDUCED GASTRIC ULCER IN RATS

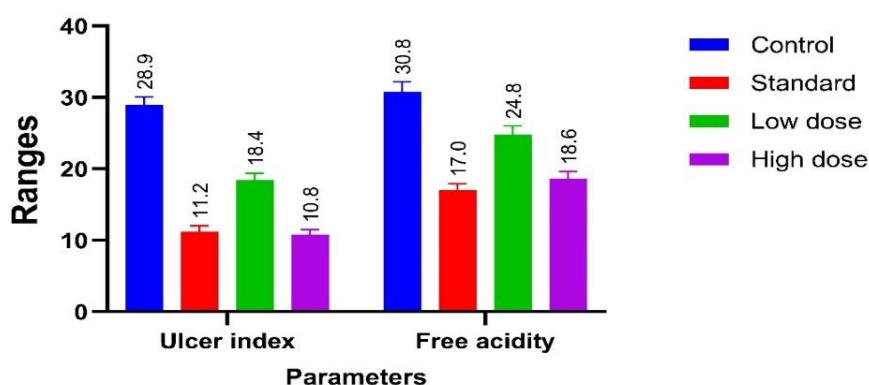
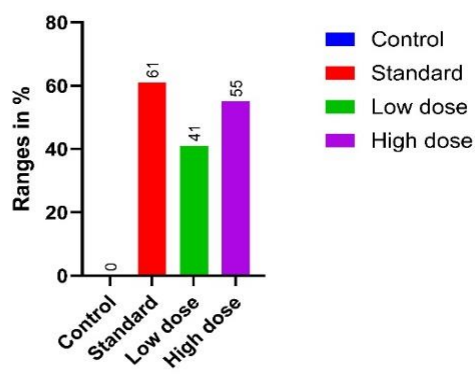


Figure 1a: Negative control (Aspirin 400mg/kg)



Figure 1b: Positive control omeprazole 10mg/kg



Figure 1c: SC low dose



Figure 1D: SC High dose may involve H₂ receptor antagonism or proton pump inhibition

Histological evaluation

Control animals receiving distilled water and aspirin showed mucosal erosion with mild focal damage. The standard drug group showed minimal focal mononuclear infiltration in the submucosa. Both low and high doses of SC showed minimal inflammatory infiltration with protection of the gastric mucosa.

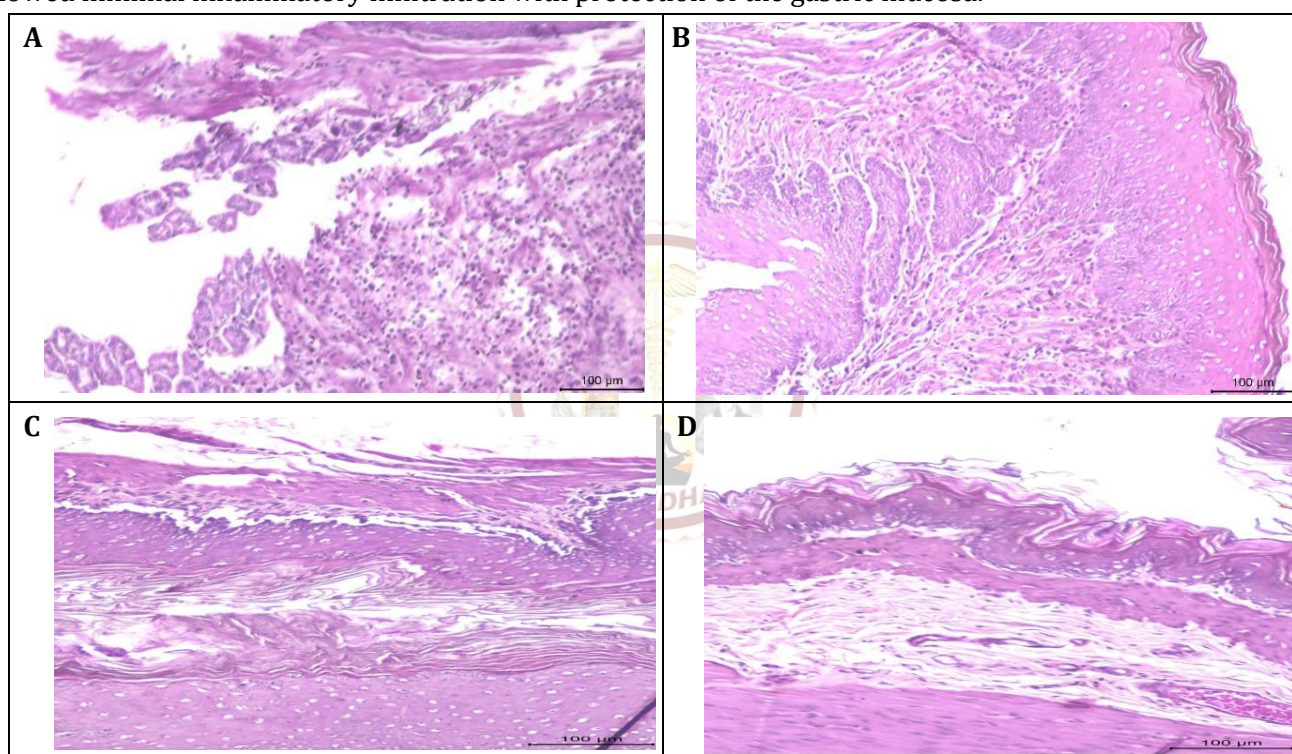


Fig: 2 Histological evaluations of anti-ulcer activity of SC. 'A' received orally on distilled water group served as control, and were administered Aspirin 400mg/kg showing Erosion, mucosal, focal, mild. H&E. 'B' served as a positive control and were treated with standard drug Omeprazole (10mg/kg and were administered Aspirin 400mg/kg showing Infiltrates, mononuclear, sub-mucosal, focal, minimal. H&E. 'C' received SC low dose -p.o. and were administered Aspirin 400mg/kg showing Infiltrates, mononuclear, sub-mucosal, focal, minimal. H&E. 'D' received SC high dose -p.o. and were administered Aspirin 400mg/kg showing Infiltrates, mononuclear, sub-mucosal, focal, minimal. H&E.

DISCUSSION

The study demonstrated dose-dependent protection against aspirin-induced gastric ulcers in rats. [2,4] SC produced a significant reduction in ulcer index, especially at the high dose. The anti-ulcer activity of SC may be due to its anti-secretory effect^[4], as evidenced by reduction in gastric volume, free acidity, and total acidity in the pylorus ligation model.

SC showed statistically significant results ($P < 0.05$ and $P < 0.01$) at low and high doses, respectively, compared to the control group. The increase in gastric pH further supports its protective action [6].

The mechanism of action of SC may involve possible anti-secretory mechanisms such as H₂ receptor modulation or proton pump inhibition [9,10], which requires further investigation.

Histopathological evaluation revealed inflammatory cell infiltration^[7], mucosal damage, erosion, and hemorrhage in the control group. The standard drug group showed minimal infiltration. The test drug groups showed reduced inflammatory infiltration, indicating protective effects.

CONCLUSION

SC exhibited significant protective and curative effects against gastric ulcers in both aspirin-induced and pylorus ligation models. Both low and high doses showed anti-ulcer activity; however, the high dose demonstrated greater protection and results comparable to the standard drug omeprazole.

Therefore, SC may serve as a potential anti-ulcer agent, warranting further investigation. Further studies are required to confirm its mechanism of action and long-term safety.

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