

(RESEARCH ARTICLE)



## Evaluation of *Siphomeris lingu* Bojer (Rubiaceae) anti gastric ulcer activity in rat

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### Abstract

This work was carried out to evaluate the anti-gastric ulcer activity of the hydroalcoholic extract of *Siphomeris lingu*. Gastric ulcer was induced by two methods: 100 mg/kg/day of indomethacin administered orally for 5 days, and pylorus ligation. The study was done on rat, the *S. lingu* extract was administered orally at the doses 150, 300 and 600 mg/kg. The results of this study indicate that *S. lingu* reduces indomethacin induced mucosa lesion surface area. It is equal to  $24.12 \pm 0.21$  mm<sup>2</sup> in control group, versus  $15.32 \pm 0.12$ ,  $9.13 \pm 0.25$  and  $5.22 \pm 0.26$  mm<sup>2</sup> in animals treated with the extract at doses 150, 300 and 600 mg/kg ( $p < 0.05$ ), and  $6.30 \pm 0.12$  mm<sup>2</sup> in animals treated with misoprostol at the dose of 1.4 µg/kg. It also reduces gastric content acidity. The pH is equal to  $2.64 \pm 0.31$  in the control group, versus  $4.81 \pm 0.25$ ,  $5.2 \pm 0.31$  and  $5.9 \pm 0.12$  in the groups treated with the extract at doses 150, 300 and 600 mg/kg ( $p < 0.05$ ), and  $6.34 \pm 0.26$  for the positive control group treated with cimetidine at the dose of 100 mg/kg. These results reveal the anti-gastric ulcer action of *S. lingu* extract, justifying its use in traditional medicine. Alkaloids and flavonoids in it might be responsible of this activity.

**Keywords:** Gastric ulcer; *Siphomeris lingu*; Rat; Pylorus ligation

### 1. Introduction

Gastroduodenal ulcer is characterized by a loss of a part of the gastroduodenal lining which can get to the muscular structure. This is due to the imbalance between the protective and aggressive endogenous agents. The protection is assured by mucous, bicarbonate and a good blood flow on the mucosa. While aggressive endogenous factors such as HCl, pepsin and bile acid corrodes the mucosa. Prostaglandins in mucosa increase the local blood flow and stimulate mucous and bicarbonate secretion from the collet cells. Non-steroidal anti-inflammatory agents like indomethacin inhibit the enzyme responsible for prostaglandins synthesis (COX) [1]. While acetylcholine from its M3 receptor, histamine on its H1 receptors and gastrin are responsible for HCl secretion from parietal cells. Alcohol precipitates the mucous and stimulates histamine secretion. Predominance of aggressive factors or reduction of protective factors leads to ulcer formation. It is worsened by exogenous factors like *Helicobacter pylori*, non-steroidal anti-inflammatory agents, tobacco, alcohol or stress [2,3]. Treatment of gastro duodenal ulcer aims to reduce the aggressive factors or to reinforce the mucosa protection [4]. There are anti-secretory drugs which inhibit HCl secretion, or prostaglandin analogues which protect the mucosa [5].

In Madagascar, especially in urban areas, this pathology comes at 12<sup>th</sup> in ranking [6] and most of those who suffer from stomach ache prefer to take medicinal plants taken as beverage. According to the data that we have collected during an ethnobotanical survey in the Menabe region (southern part of Madagascar), the decoction prepared with *S. lingu* leaves relieves epigastric pain. This information led us to postulate that this plant might have an antigastroduodenal ulcer activity. It might reduce gastric acidity or increase mucoprotection.

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## 2. Material and methods

### 2.1. Extraction and phytochemical screening

The leaves of *S. lingun* were collected from the southern part of Madagascar in May 2021. They were dried under shade and ground with an electric grinder. The powder was macerated in a mixture of ethanol and water (60:40) for 72 hours. The macerate was filtered and evaporated to dryness under vacuum with a rotavapor (Büchi®). Phytochemical screening was conducted on the dry extract following the method of Fong [7]. The dry extract was used for the biological tests.

### 2.2. Experimental animals

Male and female albino rats Wistar strain of 3 months, weighing from 180 to 200 grams were used for the biological tests. They were reared on a standard laboratory diet and given water ad libitum. They were kept in a room where temperature was ( $24 \pm 2^\circ\text{C}$ ), with humidity (65–70%), and day/night cycle (12 h/12 h).

The experiments were conducted following the guidelines of the ethic committee of Sciences Faculty, University of Antananarivo, Madagascar (Ref: 06/2021). Rats were deprived of food but had free access to water 18 h prior to tests.

### 2.3. Evaluation of *S. lingun* effect on indomethacin induced ulcer

Indomethacin-induced gastric ulceration was used to evaluate the extract's mucoprotective activity [8]. Rats were fasted 18 hours prior to this experiment. They were separated into 5 groups of 6 rats per group: the first group served as neutral control group and received distilled water by oral route; 3 groups were given the extract orally at the dose of 150, 300 and 600 mg/kg respectively, while the last group served as positive group and received 1.4 µg/kg of misoprostol. All the products were administered by oral route in a volume of 10 ml/kg.

Indomethacin was administered at the dose of 30 mg/kg, 30 minutes after administration of the extract, for 5 days. On the sixth day, the animals were anesthetized using 100 mg/kg of Phenobarbital i.m. and exsanguinated by cutting the carotids. A laparotomy was practiced, and the animals' stomach was isolated, opened along the big curvature and rinsed with saline solution (NaCl 9%). The lesions on the gastric mucosa were measured by direct planimetry, using a transparent millimetric paper.

### 2.4. Evaluation of the effect of the extract on gastric acid secretion

Pylorus ligation was carried out to evaluate *S. lingun* extract anti secretory activity [9]. Four groups of 6 rats per group were used during this experiment. The animals were fasted 18 hours prior to the test. The first group received distilled water, the second group served as positive group and received cimetidine at the dose of 100 mg/kg, while the remaining 3 groups of animals received the extract at dose 150, 300 and 600 mg/kg respectively. The different products were administered in a volume of 10 ml/kg by oral route. After 1 hour, the animals were anesthetized using 40 mg/kg of phenobarbital i.m. The upper part of the abdomen was incised for pylorus ligation, and afterwards sutured. During the six hours following ligation, the animals were put in separate cages and deprived of food, after which they were anesthetized with 100 mg/kg of Phenobarbital i.m. and exsanguinated. A laparotomy was performed, the oesophagus was clamped, and the stomach isolated. The gastric content was poured into test tubes and centrifuged at 3000 rpm for 10 minutes. The pH of the supernatant was measured with a pH meter (PIERRON).

### 2.5. Data expression and analysis

The results were expressed as mean with standard error of the mean. Statistical significance was determined by ANOVA (analysis of variance) and 't' test of Student, p values less than 0.05 were considered as statistically significant.

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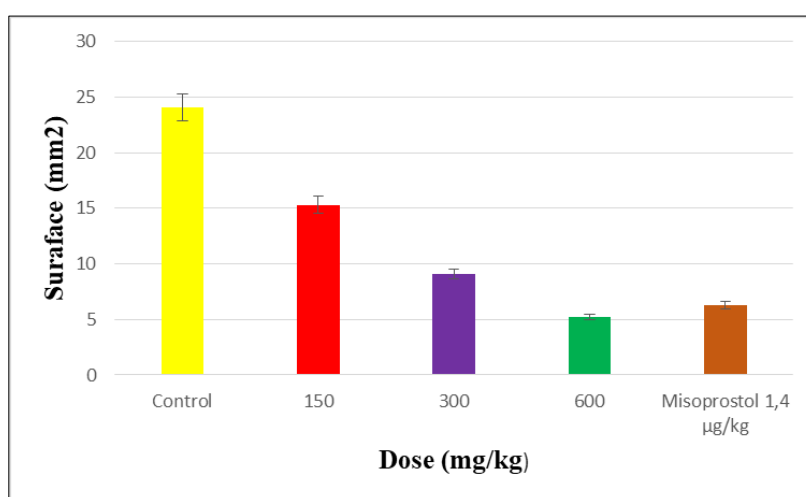
### 3. Results

#### 3.1. Principal secondary metabolites in the extract

Phytochemical screening revealed the presence of alkaloids, flavonoids, steroids, terpenes, reducing sugars, tannins, anthocyanins, leucoanthocyanins, polysaccharides, phenolic compounds and saponins.

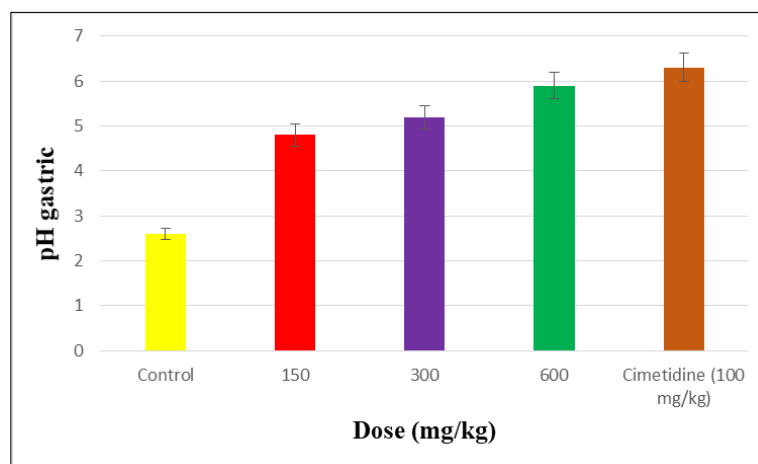
#### 3.2. Effect of *S. lingun* on indomethacin induced ulcer

Indomethacin (40 mg/kg) administered orally for 5 days induced erosion of gastric mucosa. The lesion surface area in neutral control group is inferior to those measured in the groups treated with the extract, and its variation is dose dependant. The lesion surface area of the control group is equal to  $24.12 \pm 0.21 \text{ mm}^2$ , versus  $15.32 \pm 0.12$ ,  $9.13 \pm 0.25$  and  $5.22 \pm 0.26 \text{ mm}^2$  in animals treated with the extract at doses 150, 300 and 600 mg/kg ( $p < 0.05$ ), and  $6.30 \pm 0.12 \text{ mm}^2$  in animals treated with misoprostol at the dose of  $1.4 \mu\text{g/kg}$  (Figure 1). The surface area of the lesion observed on gastric mucosa of the rat treated with the extract at the dose 600 mg/kg is comparable to the positive control group. These results indicate the mucoprotective activity of *S. lingun* extract.



**Figure 1** Variation of the lesion surface area on gastric mucosa of control group and the group treated with *S. lingun* hydroalcoholic extract, administered orally, at doses 150, 300 and 600 mg/kg, and treated with misoprostol, administered orally at the dose of  $1.4 \mu\text{g/kg}$  ( $\bar{x} \pm \bar{\sigma}$ ;  $n = 6$ ;  $p < 0.05$ )

#### 3.3. Effect of *S. lingun* on pylorus ligation induced acid secretion



**Figure 2** Variation of gastric content acidity of control group and the group treated with *S. lingun* hydroalcoholic extract, administered orally, at doses 150, 300 and 600 mg/kg and treated with cimetidine, administered orally at the dose of  $100 \text{ mg/kg}$  ( $\bar{x} \pm \bar{\sigma}$ ;  $n = 6$ ;  $p < 0.05$ )

Pylorus ligation provokes HCL secretion and increases the gastric content acidity. In this experiment, the pH of the gastric content of the control animals is inferior to the ones treated with the extract, and the increase is dose dependant. It is equal to  $2.64 \pm 0.31$  in the control group, versus  $4.81 \pm 0.25$ ,  $5.2 \pm 0.31$  and  $5.9 \pm 0.12$  in the groups treated with the extract at doses 150, 300 and 600 mg/kg ( $p < 0.05$ ), and  $6.34 \pm 0.26$  for the positive control group treated with cimetidine at the dose of 100 mg/kg. The pH value for the group treated with the extract at the dose of 600 mg/kg is comparable to the positive control group treated with cimetidine at the dose of 100 mg/kg (Figure 2). These results demonstrate the reduction of the gastric content acidity, indicating the anti-secretory activity of the extract.

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#### 4. Discussion

*S. lingun* leaves are used as traditional medicine in Madagascar for wound healing and gastrointestinal disorders. To verify this activity, hydro alcoholic extract was prepared and tested in rat. Different mechanisms can induce experimental gastric ulcer, the commonly used are pyloric ligation and non-steroidal anti-inflammatory drugs. Pyloric ligation mostly stimulates the sympathetic system and results in accumulation of gastric acid and activation of pepsin, leading to the auto digestion of gastric mucosa. These will result in ulcer formation [9]. Endogenous prostaglandins are important in maintaining gastro duodenal integrity. Indomethacin is a commonly NSAIDs used in animal experiment to induce gastric ulcer. Its ulcerogenic effect is due to cyclooxygenase inhibition, which reduces prostaglandins synthesis, leading to the decrease of bicarbonate and mucus secretion. It leads to erosion on gastric and duodenal mucosa as it abrogates the protective mechanism [8, 10].

The hydro alcoholic extract of *S. lingun* leaves was evaluated for its anti-ulcer activity in pylorus ligation and indomethacin induced ulcer models in Wistar rats. The results of our investigation exhibit significant ulcer inhibition in both induced ulcer models. The extract produced a significant anti-ulcer activity almost similar to the standard drugs used as references.

Reducing gastric acidity is one of the important means for muco-protection. In this model, *S. lingun* leaves extract show significant rise in pH of gastric contents compared to the negative control, and its effect is dose dependant and comparable to standard drug cimetidine. This exhibits the extract's anti-secretory activity. Since pylorus ligation is mostly a parasympathetic reflex, we suggest the acidity decrease might be due to inhibition of HCl secretion via anti-cholinergic action [11]. Alkaloidal constituents present in the extract might be also responsible for this activity [12].

On the other hand, increasing the mucoprotection is also important in the treatment of gastric ulcer. Or according to the results that we obtained, the extract reduces the lesion surface area induced by indomethacin, in dose dependent manner. This finding indicates that the extract possesses a gastro protective effect. It might be due to mucus and bicarbonate secretion increase. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed. These active compounds are able to stimulate prostaglandin secretion via COX1 activation, hence increasing mucus and bicarbonate secretion [13, 14]. Alkaloids might also be involved in this activity since they can activate cyclooxygenase enzyme, which subsequently stimulates prostaglandins synthesis and increases bicarbonate and mucus levels in the gastric lumen [15].

Even though this study was conducted in animal models, which may not transitional to human subjects, the results suggest the probable mechanism(s) of how the extract exhibited the tested biological activity. Flavonoids and alkaloids might be responsible for the gastro protective effects of this plant extract via stimulation of prostaglandins synthesis and acid secretion.

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#### 5. Conclusion

Authors are expected to conclude their presentation comprehensively in the conclusion. Authors have to freedom to include future research details as part of the conclusion or as a separate section before the conclusion, depending on the appropriateness. Conclusion should not repeat the main text; instead it should try to help the reader to have a strong view on the article's claims. Following a critical approach on own research methods and experiments can show maturity and impartial evaluation, which enhance the quality of your article.

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## Compliance with ethical standards

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### *Disclosure of conflict of interest*

The authors declare no conflict of interests.

### *Statement of ethical approval*

The experimental protocols were approved by the Sciences Faculty Animal Care Ethics Committee (Ref: FacSc/CE013/22).

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