

## Gastric secretagogue action of *Cryptolepis sanguinolenta* in the perfused stomach of anesthetized rats

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### ABSTRACT

**Background:** *Cryptolepis sanguinolenta* has been reported to have antimalarial and antidiabetic effects. **Aim:** The gastric secretagogue action of ethanolic extract of *Cryptolepis sanguinolenta* stem was studied in rat model to evaluate its possible role in gastric acid secretion. Food intake and weight gain were also observed. **Materials and Methods:** The animals used were divided into four groups. The control received 2 ml of distilled water, while treated groups received 50, 150, and 250 mg/kgbw of *Cryptolepis sanguinolenta* extract per oral respectively daily for 21 days. **Results:** *Cryptolepis sanguinolenta* caused dose-dependent increase in gastric acid secretion. This secretagogue effect was augmented by histamine. *Cryptolepis sanguinolenta* also significantly increased food intake, body weight gain, and the sizes and number of gastric parietal cells. **Conclusion:** The results suggest that *Cryptolepis sanguinolenta* is ulcerogenic, and the gastric secretagogue action is histamine-mediated. This effect could be attributed to the presence of cryptolepine, the major bioactive agent, and other alkaloids present in the botanical.

**Key words:** *Cryptolepis sanguinolenta*, body weight, food intake, gastric acid, ulcer

### INTRODUCTION

Alteration in gastric acid secretion has been implicated in some diseases<sup>[1]</sup> with excessive secretion leading to gastritis and peptic ulcer disease.<sup>[2]</sup> Peptic ulcer is a common disease throughout the world and in the past one or two decades, there has been a phenomenal increase in the contribution to the knowledge on the treatment of the disease.<sup>[3]</sup> Peptic ulcer is a significant health challenge with multifactorial aetiology. The pathogenesis of gastric ulcer occurs with acidic digestion of the mucosal defence.

Several studies have been carried out on the effect of plant extract on gastric acid secretion. Such studies include the effect of garlic (*Allium sativum*) and onion (*Allium cepa*), plants greatly used in traditional and complementary medicine. Its anti-hypertensive, lipid-lowering, oxidative activities, anti-bacterial, anti-viral, anti-fungal activities have been documented.<sup>[4]</sup> Garlic extract have a stimulatory effect on gastric acid and pepsin secretion. The likely mechanism is a rise in parietal or chief cell activities in response to acetylcholine release or its stimulatory effect on histamine releases. However, on pentagastrin stimulation, acid secretion decline and pepsin level remain

same in study group and this effect may be due to rapid emptying of gastric acid stock or due to inhibition of gastric activity because of attachment of some components in garlic extracts to gastrin receptors on parietal cells.<sup>[5]</sup>

*Cryptolepis sanguinolenta* (Periplocaceae) is an established antimalarial in West African ethnomedicine.<sup>[6]</sup> Its major alkaloid, cryptolepine (CLP), is reported to possess a multiplicity of biological effects such as antimicrobial, antimuscarinic, anti-inflammatory, and hypoglycaemic activities.<sup>[7-9]</sup> CLP also exhibits cytotoxic and topoisomerase II inhibitory actions, intercalates and inhibits DNA synthesis<sup>[10]</sup> and induces apoptosis in HL-60 leukaemia cells.<sup>[11]</sup>

The use of *C. sanguinolenta* has become popular since it has been discovered to have several valuable medicinal potentials. This study thus investigated its effect on gastric acid secretion and possible mechanisms involved.

## METHODS AND MATERIALS

### Plants materials

*C. sanguinolenta* stems were obtained from Ojurin Akobo- Olorunda road, Oyo, Oyo state, Nigeria. The plant was authenticated by Ugbogu. A, Chukwuma E.C and Shasanya O.S of the Forest Herbarium, Ibadan, Nigeria. A specimen voucher (FHI.108847) was deposited at the Herbarium. The stems were dried and pulverized. The powder formed after pulverization was weighed and stored below 4°C until required.

### Preparation of ethanolic extract of *Cryptolepis sanguinolenta*

1,226g of the pulverized stem was dissolved in 4.8 litres of 65% ethanol.<sup>[12]</sup> The mixture was dissolved to stand for 48 hours. The extract was filtered and evaporated at 40°C. From the viscous solution gotten, a 0.1M solution of extract was prepared by dissolving 5ml of viscous solution of extract in 45ml of distilled water.

### Animals

24 Adult Sprague-Dawley rats (weighing 150–200g) were used for the experiment (n=6). Rats were housed in a well-ventilated room maintained at 25°C ± 2, on a 12:12hour light/dark cycle. Rats fed on standard rat chow and water ad lib'itum. The rats were acclimatized for two weeks.

### Ethics

The study was approved by the ethics committee. All animals had humane care according to the institution's guideline and criteria for humane care as stated in the National Institute of Health Guidelines for the Care and Use of Laboratory Animals.<sup>[13]</sup>

### Animal treatment

Group I: Treated with 2ml/kg body weight of distilled water per oral.

Group II: Treated with *Cryptolepis sanguinolenta* per oral at 50mg/kg body weight.

Group III: Treated with *Cryptolepis sanguinolenta* per oral at 150mg/kg body weight.

Group IV: Treated with *Cryptolepis sanguinolenta* per oral at 250mg/kg body weight.

Doses were as used in our previous studies (as commonly used in folklore medicine).<sup>[14]</sup> Treatments lasted for 21 days.

### Procedures

Gastric acid was collected by continuous perfusion technique titration method and acidity determined by titration. Acid secretion was measured using the Langerdoff apparatus.<sup>[15]</sup> Animal was anesthetized with an intradermal injection of Urethane (25g of ethylcarbamate dissolved in 100ml). The animal was then placed on the dissecting board and the limbs are tied to the board. The trachea was located and isolated around the neck it is cannulated and slightly opened to aid breathing. The stomach is then located and a cannula was placed in it from the duodenal end of the stomach as previously described.<sup>[16]</sup> Normal saline (9g of sodium chloride in 1litre of distilled water) was then passed to the stomach from the mouth through the aid of a cannula. The gastric content of the stomach was collected after every 10 minutes. This was for the basal recording of gastric acid. After about three different recordings, histamine was injected into the animal's system via the hepatic portal vein. The gastric content was also collected after every 10 minutes. The immediate response of the histamine injection was increased heart-rate which was then followed by an increase in gastric acid secretion. The acidity of each 10minutes effluent collected was assayed by titration. Sodium hydroxide (1ml of NaOH was dissolved in 399mls of distilled water) and titrated against 10mls of gastric collection after every 10 minutes. The change in volume on the burette was then recorded for each titration. The indicator used for the titration was phenolphthalein.

After the treatment period, rats were sacrificed by cervical dislocation and dissected. The stomach were harvested and preserved in 10% formalin. Tissue was processed by autotechnicon; 5µm thick sections were prepared and mounted on glass slides and stained with hematoxylin and eosin (H&E). Stained sections were evaluated morphologically and the microphotograph was taken.

**Statistical analysis**

All values presented in the tables are expressed as means ± Standard error of Means (SEM). Comparisons between each treatment group and the control group were made using student’s t- test. ANOVA was used to compare values across all groups. All analyses were done using the Statistical Package for Social Sciences (SPSS Inc., Chicago, IL, USA). The difference between the groups is taken to be significant at  $p < 0.05$

**RESULTS**

Figure 1 shows that *Cryptolepis sanguinolenta* significantly increased food consumption when compared to the control. Similarly, there was significant body weight gain in *Cryptolepis sanguinolenta*-treated rats (table 1). The increase in food consumption was in a dose-dependent pattern, though the increase in body weight gain was not

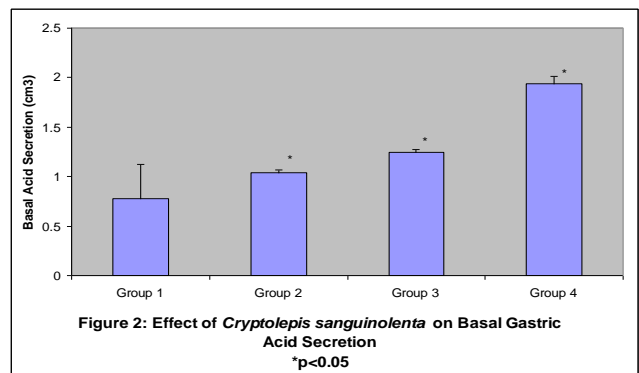
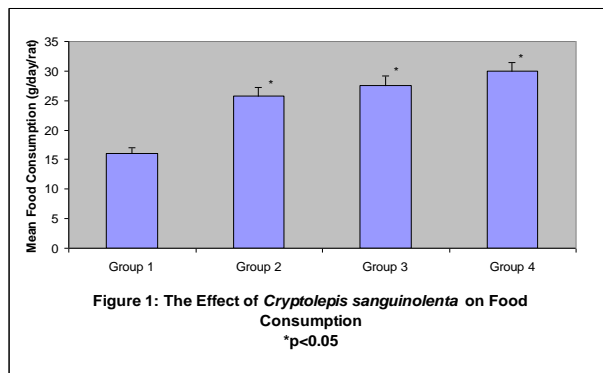
dose-related. The food consumption of treated groups 2, 3, and 4 when compared to the control was 60, 71, and 86% higher respectively; suggesting a dose-dependent increase in food consumption. However, the weight gains of the control and treated groups 2, 3, and 4 were 2, 6, 5, and 11% respectively; implying dose-non dependence.

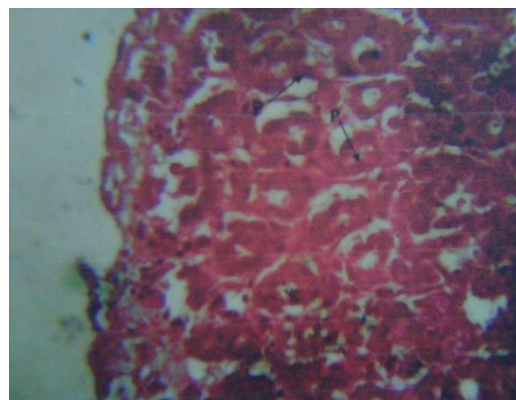
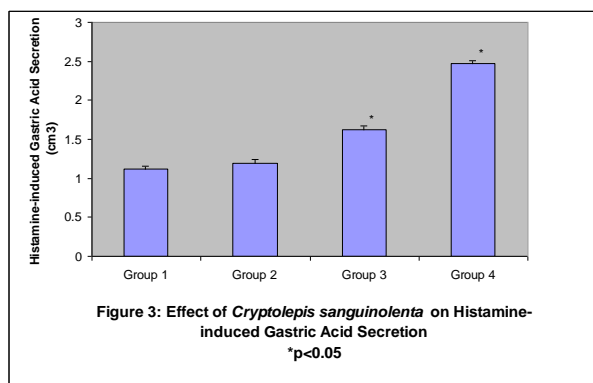
*Cryptolepis sanguinolenta* also significantly increased basal gastric acid secretion. The increase in basal and histamine-induced gastric secretion, and the number of gastric parietal cells observed in the treated rats were also dose-dependent. The increase in basal gastric secretion seen in the treated groups 2, 3, and 4 when compared to the control were 34, 62, and 151% respectively. Similarly, histamine-induced gastric secretion was 6.5, 44, and 120% higher in the treated groups 2, 3, and 4 respectively. 21, 115, and 277% increase in the number of gastric parietal cells were observed in the treated groups 2, 3, and 4 respectively. However, significant increases in histamine-induced gastric acid secretion and number of gastric parietal cells per field were seen in rats treated with 150 and 250mg/kg only (figures 2, 3, and 4). Correspondingly, significant increases in the sizes of gastric parietal cells were seen in treated rats (figure 5).

**Table 1: Effect of ethanolic extract of *Cryptolepis sanguinolenta* stem on body weight gain in Sprague-Dawley rats.**

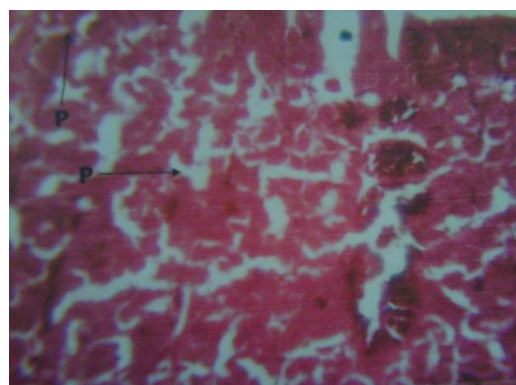
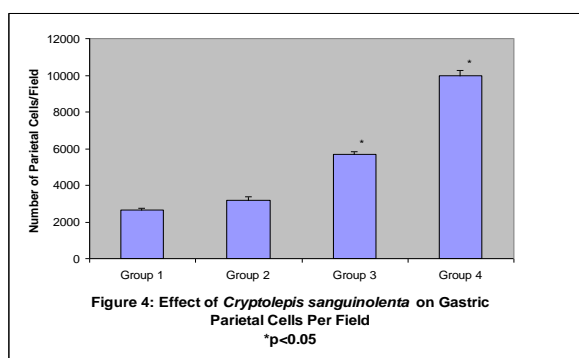
VALUES (g)	GROUP 1	GROUP 2	GROUP 3	GROUP 4
Initial	195.667±1.647	162.833±1.222	174.833±1.721	182.833±2.301
Final	199.167±2.023	173.000±1.461	183.333±1.606	202.333±2.445
Change	3.5±0.376	10.167±0.239*	8.500±0.115*	19.500±0.144 *

\* $p < 0.05$

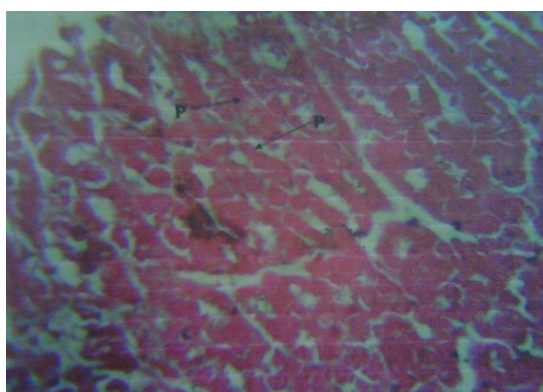




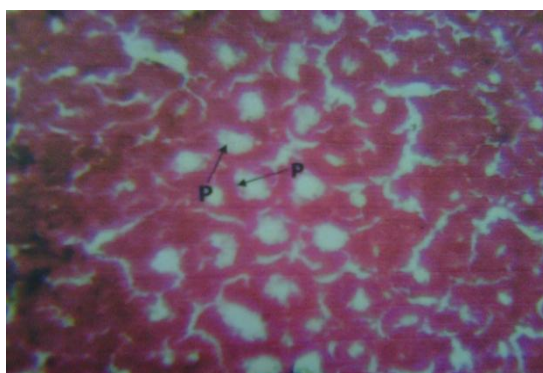
**Figure 5c**



**Figure 5d**



**Figure 5a**



**Figure 5b**

**Figure 5: Photomicrograph of the histological sections of the gastric parietal cells in the control and *Cryptolepis sanguinolenta*-treated rats. These show increase in number and sizes of the parietal cells**

## DISCUSSION

The major bioactive agent in *Cryptolepis sanguinolenta* has been reported to be cryptolepine, an indoloquinoline alkaloid. In addition, several minor alkaloids and their salts have also been isolated from the botanical which include the hydrochloride and 11-hydroxy derivatives of cryptolepine, cryptoheptine, iso- and neo-cryptolepine, quindoline, the dimers of biscryptolepine, cryptoquindoline and cryptospirepine, and the monomers of cryptosanguinolentine, cryptoakienine and cryptomisrine.<sup>[17-20]</sup>

This seems to be the first study to report the effect of *Cryptolepis sanguinolenta* on food consumption and body weight gain. The results from this study show that *Cryptolepis sanguinolenta* causes significant rise in food intake and body weight gain.

This is consistent with previous study<sup>[21]</sup> that relates body weight gain with increased food consumption. Increase in weight gain associated with food consumption seen in *Cryptolepis sanguinolenta*-treated rats suggests that the botanical may stimulate the appetite center, thus causing increase food intake with consequent increase in the weight of the treated rats.<sup>[21]</sup> It could also infer that *Cryptolepis sanguinolenta* has anabolic effect, thus preventing tissue loss.

This study also shows that *Cryptolepis sanguinolenta* stimulates gastric acid secretion. The results from the study revealed that *Cryptolepis sanguinolenta* significantly increased basal and histamine-stimulated gastric acid secretions. This is in agreement with previous study<sup>[22,23]</sup> that associated alkaloids with increased in gastric acid secretion. The increase in gastric acid secretion could be via nitric oxide increment, which enhances histamine release from enterochromafin-like cells.<sup>[24-26]</sup> The increase in basal and histamine-stimulated gastric acid secretion seen in the present study was dose-dependent.

Parietal cells are the gastric epithelial cells responsible for the secretion of gastric acid and intrinsic factors in response to histamine, acetylcholine and gastrin.<sup>[27]</sup> This study shows that administration of *Cryptolepis sanguinolenta* caused significant increase in the sizes and number of gastric parietal cells. This could explain the increase in gastric acid secretion. The *Cryptolepis sanguinolenta*-induced gastric acid secretion observed in the study is in response to its hyperplastic and hypertrophic effects on the gastric parietal cells.

Cryptolepine, the major alkaloid, and other alkaloids present in the botanical, are responsible for the gastric secretagogue activity of *Cryptolepis sanguinolenta*. Alkaloids have been reported to stimulate gastric acid secretion.<sup>[28]</sup> Alkaloids cause fat burning and are capable of digesting gastric epithelium.<sup>[23]</sup> There is also increasing evidence that alkaloids increase gastric mucosal cyclic adenosine monophosphate (cAMP),<sup>[28]</sup> which enhances hydrogen and chloride ions transport to the gastric lumen. It could be suggested that the alkaloids in *Cryptolepis sanguinolenta* inhibited phosphodiesterase, an enzyme responsible for the metabolism of cAMP to 5'AMP or they cause the synthesis of adenylate cyclase enzyme which catalyzes the formation of cAMP from ATP.

## CONCLUSION

This study shows that *Cryptolepis sanguinolenta* has ulcerogenic potentials. This effect was augmented by histamine treatment. The increase in gastric acid secretion seen in *Cryptolepis sanguinolenta*-treatment is due to its hypertrophic and hyperplastic effects on gastric parietal cells.

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**Conflict of Interest:** None declared