

EVALUATION OF *Cassia singueana* EXTRACT ON STOMACH HCL PRODUCTION AND GASTRIC EMPTYING IN RATS

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ABSTRACT : Investigation of the effects of the extract from *C. singueana* leaves on stomach hydrochloric acid (Hcl) production following histamine administration and on gastric emptying was carried out in rats. *Cassia singueana* extract (CSE) at 250 and 750 mg/kg significantly ($p < 0.050$) decreased both gastric free-Hcl and total acids as well as the quantity of meal emptied from the stomach when compared with solvent control. The extract at 250 mg/kg exerted a significant decrease in gastric emptying more than cimetidine (100 mg/kg). CSE contains alkaloids, tannins, sterols and terpenes but no flavonoids, saponins, carbohydrates, reducing sugars, starch nor polyuronides.

Keywords: *Cassia sigueana*; Histamine; Pyloric ligation; $H^+, K^+ - ATPase$; stomach ulcer; Histopathology.

INTRODUCTION

Peptic ulcer affects up to 10-15% of general human population (Gerald, 1981). Ritter *et al* (1995) estimated that, up to 1 million of the UK population suffers from peptic ulceration in a 12-month period. Peptic ulcer is a chronic, non-malignant inflammatory disease characterized by ulceration in the upper gastro-intestinal tract (stomach and duodenum) where parietal cells are found and where they secrete HCl and pepsin. The mucosa is damaged by the pepsin and hydrochloric acid of the gastric juice with consequent inflammation of the underlying and surrounding tissue (Greene and Harris, 1993). Erosion may then occur into the lamina propria and sub-mucosa to cause bleeding. It would be difficult to imagine a more corrosive environment for living tissue than what exists in the lumen of the human stomach and duodenum. The mucosa is subjected to constant forceful churning, a warm temperature, and to a juice with a pH of 0.7 to 4.0 (DiPalma, 1971).

In addition, the gastric acid contains the potent proteolytic enzyme pepsin. Protection from self digestion of the stomach depends upon delicate mechanisms which are easily disturbed. However, antacids to neutralize excess HCl in the stomach are combined with other anti-ulcer agents in the management of peptic ulcer. The persistent problem of acid rebound and reduction in bioavailability of other drugs are serious constraints in frequent medication with antacids. Agents that reduced gastric emptying are known to delay passage of drugs in the stomach. The delay in emptying would allow the extract more contact time with the stomach cells, undoubtedly assisting in the ulcer healing process (Asuzu and Anaga, 1995).

There is a global resurgence of interest in traditional medicines in the last ten years probably because many of the known synthetic drugs for treatment of diverse ailments are failing or that the causes of these various diseases are developing resistance to the known drugs (Olapade, 1998). Concoctions from *Cassia singueana* leaves are widely employed locally to treat diverse cases of ulcer by herbalists in Northern Nigeria. The study aims to evaluate the effects of *Cassia singueana* extract (CSE) on stomach HCl production and gastric emptying in rats.

MATERIALS AND METHODS

Freshly prepared solutions and analytical grade chemicals were used in the experiments. Cimetidine, histamine and ketamine hydrochloride were got from Sigma Aldrich, USA.

Animals

Matured inbred Wistar albino rats of both sexes weighing 140-200 g were purchased from the National Veterinary Research Institute (NVRI), Vom in Plateau State, Nigeria. The animals were allowed two weeks to acclimatize.

Plant Material

Fresh leaves of *Cassia singueana* plant were dried under mild sunlight, pulverized into powder. The extract was prepared by cold maceration using 80% methanol and concentrated by vacuum rotary evaporation. The concentration of the extract was determined.

Effect of *Cassia singueana* extract (CSE) on stomach HCl production

Thirty (30) adult wistar rats of either sex were marked, weighed and then randomly classed into 5 groups (A–E) consisting of 6 rats per group. The rats were fasted for 48 h with free access to water which was withdrawn 1 h before the experiment. Pyloric ligation was performed under light ether anaesthesia to each animal (Shay *et al*, 1945). Animals in group A were given distilled water; group B received cimetidine (100 mg/kg) while groups C, D and E were given CSE 100, 250 and 750 mg/kg respectively. Treatments were given orally by stomach intubation. The rats were allowed 30 min, before 100 mg/kg of histamine was administered intraperitoneally to all the rats. Treatments were repeated for all rats at 1 h and 3 h after histamine treatment. At 5 h post histamine treatment, animals were sacrificed. The stomachs were carefully removed and the gastric contents were collected.

The stomach content of each rat was washed with 10 ml distilled water into a test tube and centrifuged at 10,000 rpm for 5 minutes. The supernatant from each of the test tubes was decanted into a new set of test tubes. The total acid and gastric-free HCl were determined by titration with 0.1N NaOH using thymol blue as indicator (Ohiri, 1983). Colour change was from reddish orange to orange yellow at 1st endpoint and full blue pink colour at 2nd endpoint. The 1st endpoint indicated free hydrochloric acid while the 2nd endpoint marked total acid level (Asuzu and Anaga, 1995). Free hydrochloric acid (mmol/L) and Total acid (mmol/L) for each rat were determined after titration using the method adopted by Hepler (1965).

Total HCl (mmol/L) = $\frac{\text{Volume of 0.1N NaOH at 2}^{\text{nd}} \text{ endpoint} \times 0.1}{\text{Volume of gastric acid content used.}} \times 1000$

Volume of gastric acid content used. 1

pH ranges of thymol blue: pH 1.2-2.8 (Orange – Orange – Yellow); pH 8.0 – 9.6 (Yellow – Green – blue).

Test for mean difference in total and free acids was performed using one-way ANOVA followed by Duncan New Multiple Range post-hoc test, p-value < 0.05 was considered statistically significant.

Effect of *C. singueana* extract on Gastric emptying in rats

The method of Droppleman *et al* (1980) as modified by Asuzu and Njoku (1992) was adopted to prepare the test meal. Sixteen (16g) methylcellulose (methocil®; Fluka, Switzerland) was added slowly to 200 ml ice water being agitated in a blender at 20,000 rpm for 5 minutes.

Two Maggi® cubes (Food specialities, Nigeria) dissolved in 100 ml warm water was blended into the Methocil® solution. Then 16g of casein (Merk Darmstadt), 8g of granulated confectioner's sugar and 8g of cornstarch was added. The ingredients were added slowly and thoroughly mixed for 2 min each, to produce a semisolid homogenous paste. The test meal was refrigerated for 48 h to allow any trapped air in the mixture to escape before use.

Test rats were randomly arranged into 5 groups containing 6 animals each and treated in the same manner as those rats used in studies on effect of CSE on stomach HCl production. Following treatment, each rat was given 3.0 ml of the test meal (average weight 3.11 g) by a stomach tube. At 30 min after administering the test meal, the animals were killed by cervical dislocation. The cardiac and pyloric ends of the stomach were subsequently tied and the organ was removed from the carcass. Full stomachs were weighed and then opened by cutting through the greater curvature. The organs were rinsed and the excess moisture removed by dabbing gently with paper tissue.

The difference in weight between the full stomach and the empty stomach was considered to represent the amount of meal remaining in the stomach. These remaining meal values were subtracted from the weight of the test meal to obtain the quantity of meal emptied from the stomach during the test period. The results were analysed by one-way analysis of variance and the level of significance was tested at $p < 0.05$ using Newman Kuel's range test.

Phytochemical tests

Preliminary phytochemical tests were carried out on CSE using the method of Trease and Evans (1989). Equal volume of distilled water in a separate test tube served as the control for each of the tests.

Test for alkaloids

To a test tube containing 2 ml (100 mg/ml) of CSE each time, 3 drops of Dragendorff's, Mayer's or Wagner's reagent were added, shaken and the mixture observed for colour change or presence of precipitate.

Test for flavonoids

Ammonia test

To 2 ml of the extract in a test tube was added 3 drops of ammonia solution. The content was mixed and then observed for colour change or presence of precipitate.

Test for tannins

To a test tube containing 2 ml of the extract (100 mg/ml) was added 3 drops of ferric chloride (FeCl_3) solution. The mixture was observed for colour change or presence of precipitate.

Test for saponins

Emulsifying test

To a test tube containing 2 ml of the extract (100 mg/ml) was added 3 drops of olive oil and the content shaken vigorously, properly mixed by inverting the tubes several times for formation of frothing foam.

Test for sugar

Freshly prepared Fehling's solutions A and B were added to 1 ml of the extract (100 mg/ml) in a test tube and then boiled in water bath for 5 min and observed. Presence of brick-red precipitate indicates presence of a reducing sugar.

Test for carbohydrates (reduction test)

To 1 ml of the extract (100 mg/ml) was added 2 drops of 1 % iodine solution, and then observed for blue black colouration.

Molisch's test

This reaction is a general test for the presence of carbohydrate and other organic compounds that could form furfuraldehyde (furfural) or hydroxymethyl furfuraldehyde (hydroxyl-methylfurfural) in the presence of concentrated tetraoxosulphate (IV) acid (H_2SO_4). In the Molisch's test, the basic principle is one in which H_2SO_4 hydrolyses glycosidic bonds to give the monosaccharides, which are then dehydrated to furfural and its derivatives.

For the test, two drops of α -naphthol solution was added to 2 ml of the extract and mixed thoroughly. Then 1 ml quantity of concentrated H_2SO_4 was carefully poured down the side of the tube and observed.

Test for sterols and terpenes

C. singueana extract (5 ml) was evaporated to dryness in a beaker. The residue was dissolved in 1 ml of acetic anhydride and 1 ml of chloroform. The solution was transferred to a dry test tube and 2 ml of concentrated H_2SO_4 was added. Formation of a brownish or violet ring at the zone of contact with the supernatant indicates presence of sterols and terpenes.

Test for polyuronides

To a test tube containing 2 ml of absolute ethanol, 1 ml of *C. singueana* extract (100 mg/ml) was added drop-wise and then observed for formation of violet or blue precipitate.

RESULTS

Effect of *Cassia singueana* extract (CSE) on gastric acid production

CSE at 250 and 750 mg/kg significantly ($p < 0.05$) decreased the volume of gastric secretions when compared with distilled water (negative control): 250 mg/kg CSE = 2.08 ± 0.16 ; 750 mg/kg CSE = 2.22 ± 0.14 and negative control = 3.12 ± 0.05 . Similarly, the extract at doses 250 and 750 mg/kg significantly ($p < 0.050$) decreased both the gastric free-HCl and total acids in the stomach when compared with negative control (distilled water). However, the action of the extract was not significantly different from that of the positive control (cimetidine) in this regard (Table 1).

Gastric emptying

The extract at 250 and 750 mg/kg and cimetidine significantly decreased the quantity of meal emptied from the stomach when compared with control. (Table 2). However, 250 mg/kg of the extract exerted a significant decrease in gastric emptying more than cimetidine (100 mg/kg) or distilled water.

TABLE 1 Effects of the methanolic leaf extract of *Cassia singueana* on gastric acid secretions in rats

Treatment	Number of rats	Volume of secretions (ml) \pm SE	Free HCl (mM/l) \pm SE	Total acids (mM/l) \pm SE
Distilled Water (Control)	6	3.12 \pm 0.05	1.22 \pm 0.05	4.25 \pm 0.18
Cimetidine (100 mg/kg)	6	2.48 \pm 0.14 ^a	0.78 \pm 0.04 ^a	1.9 \pm 0.10 ^a
Extract (100 mg/kg)	6	3.04 \pm 0.08	1.12 \pm 0.07	3.70 \pm 0.12
Extract (250 mg/kg)	6	2.08 \pm 0.16 ^b	0.60 \pm 0.04 ^a	1.4 \pm 0.07 ^a
Extract (750 mg/kg)	6	2.22 \pm 0.14 ^{ab}	0.68 \pm 0.03 ^a	1.7 \pm 0.10 ^a

^{ab} Superscripts in the same column indicate significant differences ($p < 0.05$) when compared with control.

TABLE 2 Effects of *C. singueana* extract on Gastric emptying in rats

Treatment	Number of Animals	Weight of test meal emptied (g) \pm SE	% test meal emptied from stomach
Distilled water	6	1.20 \pm 0.2	34
Cimetidine (100 mg/kg)	6	0.72 \pm 0.1*	21
CSE (100 mg/kg)	6	1.13 \pm 0.06	32
CSE (250 mg/kg)	6	0.52 \pm 0.1**	15
CSE (750 mg/kg)	6	0.44 \pm 0.1**	13

*Superscript indicates significant difference ($p < 0.05$), ** $p < 0.01$ when compared with control.

Phytochemical tests

The crude extract of *C. singueana* contains alkaloids, tannins, sterols and terpenes but no flavonoids, saponins, starch, reducing sugars or polyuronides.

DISCUSSION

Preliminary *in vivo* studies with doses above 750 mg/kg of the extract did not produce appreciable difference in the effects between treated and normal rats. *C. singueana* extract (250 and 750 mg/kg) and cimetidine produced significant (0.05) reduction in gastric secretions following histamine administration.

This similarity in action between the extract and cimetidine suggests that both may act through the same mechanism. It is possible that CSE inhibited histamine H₂-receptor sites or H⁺,K⁺-ATPase (Hydrogen, potassium ion adenosine triphosphatase) activities. Potent histamine H₂-receptor antagonists e.g. cimetidine, famotidine, ranitidine and nizatidine, and H⁺,K⁺-ATPase or proton pump inhibitors (PPIs) e.g. omeprazole, pantoprazole, lansoprazole, rabeprazole and esomeprazole inhibit HCl secretions in the stomach. PPIs are capable of producing almost complete suppression of acid secretion. The mechanism of action of omeprazole is such that it binds very specifically to a single subunit of the H⁺,K⁺-ATPase at the secretory surface of parietal cell and inactivate it (Munson *et al.*, 1995). It reduces acid secretion regardless of the source of secretory stimulation.

CSE also reduced values of total acids and free HCl in the rat stomachs, an effect similar to the action of antacids in neutralizing excess HCl in the stomach. The extract at 250 mg/kg was more effective than cimetidine or distilled water in decreasing the quantity of meal emptied from rat stomachs. The delay in gastric emptying would allow the extract more contact time with the stomach cells, undoubtedly assisting in the ulcer healing process (Asuzu and Anaga, 1995).

CONCLUSION AND RECOMMENDATION

C. singueana extract demonstrated considerable effect at 250 and 750 mg/kg in reducing both gastric free-HCl and total acids following histamine administration in rats. The extract also caused a highly significant (0.01) decreased in the quantity of meal emptied from the rat stomachs. These activities are suggestive that the extract could be useful as an anti-ulcer agent but further studies with various gastric ulcer-inducing models should be conducted to verify this assertion.

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