INTERNATIONAL JOURNAL OF PLANT, ANIMAL AND ENVIRONMENTAL SCIENCES

Volume: 1: Issue-1: March-May -2011 *www.ijpaes.com*

THE PROTECTIVE EFFECTS OF THE METHANOL EXTRACT OF *Cassia singueana* LEAVES AGAINST HISTAMINE-INDUCED STOMACH ULCERS IN ALBINO RATS

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ABSTRACT: The anti-ulcer potentials of the methanolic extract of *Cassia singueana* leaves were evaluated using pyloric ligation/histamine-induced stomach ulcer model in rats. The extract was prepared by cold maceration in 80% methanol at 37^0C with intermittent shaking for 48 h. A yield of 12.6% w/w dry extract was obtained. The extract was safe, up to a dose of 4000 mg/kg given *per os* did not cause mortality in the rats. The extract exhibited profound protective effect at 250 and 750 mg/kg against histamine-induced gastric ulcers in rats over cimetidine (100 mg/kg) and negative control. C. singueana extract (250 mg/kg) gave a significantly higher preventive index when compared with cimetidine and solvent treated control (CSE=73%; Cimetidine=53% and Negative control=0%). Histopathologic lesions deviated from a highly protected gastric mucosa with 250 mg/kg extract treated rats to fairly protected stomach epithelium with cimetidine and a generalized, severe ulcerative lesions in the gastric mucosa of negative control rats that received only distilled water prior to ulcer induction. *C. singueana* extract was therefore significantly protective against the ulcerogenic activities of histamine in the experimental rats.
Keywords: *Cassia sigueana*; Histamine; Pyloric ligation; Gastro-protective activity; stomach ulcer; Histopathology.

INTRODUCTION

The epithelium of gastro-intestinal tract is continually exposed to damaging effects of noxious substances on daily basis. The aetiology of gastric ulceration is multifactorial and not clearly defined, but some predisposing factors have been implicated. This include the nature of food ingested, bile reflux (Gerald, 1981), lessened mucosal resistance (Cho and Ogle, 1992), alteration of gastric mucosal blood flow (Guidobono *et al*., 1997), disruption of gastric mucosal barrier by stress (Takeuchi and Okabe, 1982), decrease in alkaline mucosal bicarbonate and mucus secretion (Webster, 2001), over dosage and or prolonged administration of non-steroidal anti-inflammatory drugs (Tanaka *et al*., 1983), persistent infection with *Helicobacter pylori* (Munson *et al*., 1995), *Zollinger-Ellison* syndrome (Greene and Harris, 1993), and genetic factors as suggested by a higher incidence of duodenal ulcers in patients with positive family history of this disorder or blood type O (Coles, 1968).

Pathophysiology of ulcer is due to an imbalance between aggressive factors (acid, pepsin, H. *pylori* and non-steroidal anti-inflammatory agents) and local mucosal defensive factors (mucus bicarbonate, blood flow and prostaglandins). Non-steroidal anti-inflammatory drugs (NSAIDs) inhibit synthesis of prostaglandins from Arachidonic acid in the cyclooxygenase pathway (Brestel and Dyke, 1994). NSAIDs, besides producing the well recognized prostaglandin cytoprotective deficiency, also enhance gastric motility which may contribute to the pathogenesis of gastric injuries (Takeuchi *et al*., 1994).

Histopathological changes in the gastric mucosa of animals infected with *H. pylori* involve dilatation of gastric glands, degenerative changes in superficial epithelia and necrosis of individual gastric glands. There are inflammatory changes with increased number of neutrophils and lymphocytes in gastric epithelia, swelling of the mucosa and lymphocytic inflammation of plasma cells and eosinophils (Robbic *et al*, 2007). Histamine is regarded as the critical regulator of gastric acid secretion (Laurence *et al*., 1997). Most antiulcer drugs require prolong period of intake, yet ulcer relapse is a common occurrence (Munson *et al*., 1995). The antiulcer drugs available in the market have numerous adverse effects (Blum *et al*., 1986) and no known drug proves solely effective in treating peptic ulcer. *Cassia singueana* leaf is popular for its efficacy in the treatment of long standing cases of peptic ulcer by the *Fulani* and *Hausa* traditionalists of Northern Nigeria. A scientific investigation of this plant for protective activity in histamine challenged rats had not been done before. The aim of this study was therefore to evaluate the antiulcer effects of the methanolic leaf extract of *Cassia singueana* plant using pyloric ligation/histamine-induced gastric ulcer in rats.

MATERIALS AND METHODS

Solutions, reagents, drugs and chemicals

Freshly prepared solutions and analytical grade chemicals were used in the experiment. Cimetidine, histamine and ketamine hydrochloride purchased from Sigma Aldrich, USA and 10% formol saline were used for the study.

Animals

Inbred albino Wistar rats of both sexes weighing 130-200 g, bred in the laboratory animal unit of the Faculty of Veterinary medicine, University of Nigeria, Nsukka were used in the experiment. The rats were kept in the same room with a temperature varying between 28 and 30^0C; lighting period was between 15 and 17 hours daily. The rats were kept in stainless steel wire mesh cages which separated them from their faeces to prevent coprophagy. They were supplied clean drinking water and fed standard feed (Grower mash pellets, Vital feed®, Nigeria). Ethical rules guiding the use of animals for experimentation were strictly adhered to.

Preparation of the plant extract

Fresh leaves of the plant were collected from Kumbotso local Government Area within Kano metropolis, Northern Nigeria September, 2010. The plant was duely identified as *Cassia singueana* by Mr. Ozioko, a taxonomist, with Botany department, University of Nigeria, Nsukka (UNN). The plant leaves were dried under mild sunlight, pulverized into coarse powder with mortar and pestle before grinding into fine particles. Cold extraction was performed using 80% methanol for 48 h with intermittent shaking at 2 h interval. The extract was concentrated by vacuum rotary evaporation and stored in a refrigerator at 4^0C. The concentration and percentage yield of the extract were determined.

Acute toxicity test

Thirty (30) matured albino Wistar rats of both sexes were marked with 10% picric acid, weighed and randomly separated into 6 groups (A – F) with each group having 5 rats. Groups A–E were dosed orally with varying doses (250; 500; 1000; 2000 and 4000 mg/kg) of the leaf extract *of C. singueana* plant respectively while group F (6^{th} group) was given an equivalent volume of distilled water. The rats were allowed access to feed and water *ad libitum* for 48 h and observed for signs of toxicity and death.

Effect of *Cassia singueana* extract (CSE) on pyloric ligation/histamine-induced gastric ulcers

Forty (40) adult wistar rats of mixed sexes were marked, weighed and then placed in 5 different groups (A–E) consisting of 8 rats in a group. The rats were starved for 24 h; water was made available but withdrawn 1 h before the experiment.

Treatments were given orally by stomach intubation. Thirty (30) min before surgery, group A was given only distilled water and it served as the negative control (N.C) group; group B: the positive control received 100 mg/kg of cimetidine (a potent H_2-receptor blocker), while groups C – E were given 100, 250 and 750 mg/kg CSE respectively.

Surgery

Following a light ketamine (3 mg/kg) anaesthesia, a midline incision was made on the abdominal wall of each rat. The pylorus of the stomach exteriorized, located and ligated loosely with a silk thread. Incision was then sutured. After 30 min post operation, 100 mg/kg of histamine was administered intraperitoneally to all the rats. At the 1st and 3rd h following histamine administration, each group was given a repeat of the treatment it had before histamine administration. After 6 h of histamine administration, each rat was terminally anaesthesized with ether in a glass hood; the stomach was carefully removed after ligating the cardiac end. Each stomach was cut open through the greater curvature with a scapel blade and after rinsing with distilled water, it was pinned to a white background on a wooden board for examination and assessment of ulcers.

The stomachs were examined for ulcer with the aid of a magnifying lens (x10). The ulcer index was assessed as follows: less than 1 mm =1, between 1 and 2 mm =2, greater than or equal to 3 mm= 3. The sum of the scores were divided by 10 (the magnification of the lens) to obtain the ulcer index for each rat (Main and Whittle, 1975). The mean ulcer index for each group was subjected to Mann-Whitney test and the effectiveness of the extract and drug was calculated using the formula: Preventive index (%) = Ulcer index of control - Ulcer index of treated/ Ulcer index of control x 100.

Histopathology

Tissue samples from the stomach of rats in each group (A – E) of the experiment were fixed in 10% formol-saline for a minimum of 24 h and then dehydrated by washing in ascending grades of ethanol before clearing with xylene and embedding in paraffin wax. The samples were sectioned with a microtome, stained with Hematoxyline and Eosin (H and E) and mounted on Canada balsam. All sections were examined under light microscope (x10, x20 and x40) magnification. Photographs of the lesions were taken with an Olympus photo microscope for observation and documentation of histopathologic lesions.

RESULTS

Extraction of the Plant material

Cassia singueana extract (CSE) was dark brown in colour with a pleasant smell and a pasty consistency. The total solids recovered from extracts were 11.8 percent (w/w).

Acute toxicity

No death was recorded in the rats treated orally with varying doses (250; 500; 1000; 2000 and 4000 mg/kg) of the leaf extract *of C. singueana*. The extract was well tolerated by the rats without any overt signs of toxicity.

Effect of *C. singueana* extract on pyloric ligation/histamine-induced gastric ulcers

Negative control (N.C) rats that were given only distilled water orally had mean ulcer index of 1.53 ± 0.15 and a percentage preventive index of 0. *C*assia *singueana* extract at doses 250 and 750 mg/kg was more effective than cimetidine (100 m/kg) in protecting rats from the ulcerogenic activity of histamine (100 mg/kg). Cimetidine produced a mean ulcer index of 0.78 ± 0.09 while the extract had reduced ulcer index of 0.42 ± 0.14 and 0.50 ± 0.06 at 250 and 750 mg/kg respectively (Table 1). Similarly, cimetidine had a percentage preventive index of 53 but 250 and 750 mg/kg of the extract exerted preventive indices of 73 and 67 percent respectively.

Table 1: Effect of *C. singueana* Extract (CSE) on pyloric ligation/histamine-induced gastric ulcers in rats

Treatment	No. of animal	Mean ulcer index ± SE	Preventive index (%)
Distilled water (N.C.)	6	1.53 ± 0.15	0
Cimetidine (100 mg/kg)	6	0.78 ± 0.09[a]	53
CSE (100 mg/kg)	6	1.23 ± 0.06	0
CSE (250 mg/kg)	6	0.42 ± 0.14[b]	73
CSE (750 mg/kg)	6	0.50 ± 0.06[b]	67

[ab] Superscripts indicate significant differences at $p<0.05$.

Histopathology

Group A (Negative control). Rats in this group were given only distilled water orally before intraperitoneal (i.p.) injection of histamine (100 mg/kg). The microscopic picture of the epithelium of the mucosa appeared severely eroded with massive disorientation of the villi and crypts (Plate 1). The lamina propria of the gastric mucosa was highly disorganized and the destruction seemed to have penetrated the muscularis mucosa in many areas. The damage was also observed to be generalized.

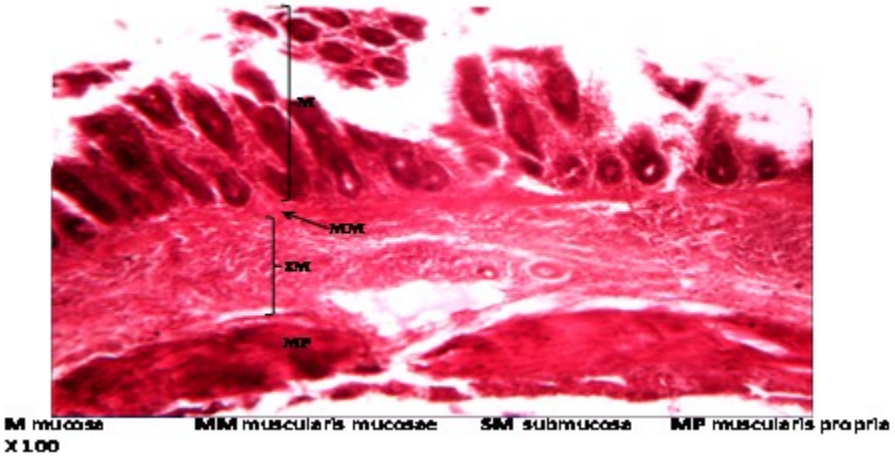
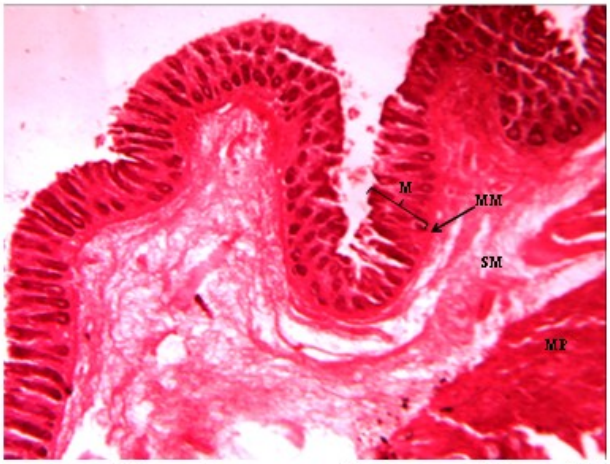


Plate 1: Micrograph of control rat stomach showing severely eroded gastric mucosa in pyloric ligation/histamine ulcer induction.

Group B (Positive Control). This group was given cimetidine (100 mg/kg) orally prior to ulcer induction with histamine (100 mg/kg, i.p.). The epithelium of the gastric mucosa seemed to be fairly protected as evidenced by the presence of some degree of intact mucousal linning. There was however, slight sloughing-off of the surface epithelium in some areas. The general orientation of the crypts appeared intact. Other layers of the gastric wall (mucosa muscularis, submucosa, inner circular and outer longitudinal layer of muscularis propria) were observed to be normal (Plate 2).



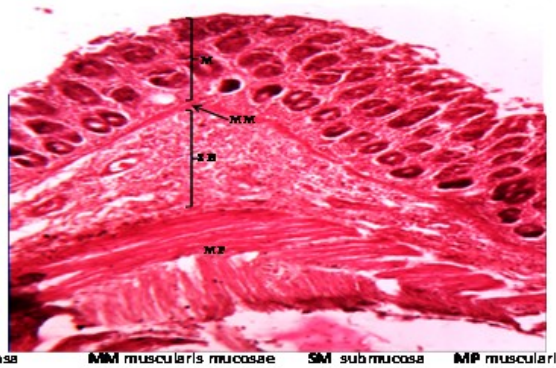
M mucosae **MM** muscularis mucosae **SM** submucosa **MP** muscularis propria
X 40

Plate 2: Rat stomach showing fairly protected gastric mucosa with cimetidine in pyloric ligation/histamine-induced gastric ulcers.

Group C. The effect of a low oral dose (100 mg/kg) of *C. singueana* extract on histamine ulcer induction showed that there were regions of severe and fair erosions of the gastric mucosa. Other segments of the stomach wall appeared normal.

Group D. This group received a medium dose (250 mg/kg) of *C. singueana* extract prior to ulcer induction with histamine (100 mg/kg, i.p.). The mucosa appeared well protected as evidenced by the appearance of intact surface epithelium (Plate 3)

Group E. High oral dose of *C. singueana* extract (750 mg/kg) gave a protective effect against stomach ulcers induced with 100 mg/kg of histamine administration. The epithelium of the mucosa appeared protected even though early stages of disorientation could be observed. There was focal desquamation of the villi in some areas.



M mucosa **MM** muscularis mucosae **SM** submucosa **MP** muscularis propria
X 100

Plate 3**:** Rat stomach showing well protected gastric mucosa with 250 mg/kg of CSE in pyloric ligation/histamine induced gastric ulcer model.

DISCUSSION

*C*assia *singueana* extract was safe, up to a dose of 4000 mg/kg did not cause mortality in the rats. The extract exhibited maximal protective effect at 250 mg/kg against histamine-induced gastric ulcer lesions in rats over cimetidine (100 mg/kg). This is evidenced in the ulcer index (250 mg/kg CSE=0.42 ± 0.14; Cimitidine=0.78 ± 0.09 and N.C=1.53 ± 0.15), percentage preventive index (250 mg/kg CSE=73%; Cimetidine=53% and N.C=0%) and histopathological lesions. In the negative control, there was severe and generalized erosion of the epithelium with massive disorientation of the villi and crypts. The damage even penetrated deep into the submucosa and muscularis propria. Cimetidine provided a fairly protective effect on the gastric mucosa while 250 mg/kg of the extract offered a highly protective effect with the result that the rat gastric mucosae remained without ulcerative lesions after exposure to the damaging effects of histamine.

The production of gastric HCl is mediated through histamine which in-turn is produced by enterochromaffin-like cell or mast cell (Munson *et al*., 1995). Treatment with 250 and 750 mg/kg of the extract significantly inhibited the formation of stomach ulcers caused by histamine administration in rats; it therefore suggests that the crude extract of *C. singueana* leaves can suppress gastric damage induced by one of the aggressive factors. The activity of the extract could have been mediated through blockage of histamine H_2-receptors or by inhibition of hydrogen, potassium adenosine triphosphatase (H^+/K^+-ATpase) or proton pump activity. The effects leading to ulcerogenesis are mediated at the parietal cells by the transmitter substances histamines, gastrin and acetylcholine which through a common path involving cyclic Adenosine monophosphate ($_c$Amp) and calcium ions, interact with the gastric proton pump (H^+/K^+-ATpase) that is ultimately responsible for secreting acid into the lumen of the stomach (Munson *et al*, 1995). Histamine appears to be necessary for the action of gastrin and acetylcholine and it is the critical regulator of gastric acid secretion (Laurence *et al*., 1997).

CONCLUSION AND RECOMMENDATION

The study demonstrated that 250 and 750 mg/kg of *C*assia *singueana* extract exhibited profound protective effects against histamine-induced stomach ulcers in rats. The extract could have blocked histamine receptors or inhibited proton pump activities.

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