INVESTIGATION OF *CASSIA SINGUEANA* LEAF EXTRACT FOR ANTIULCER EFFECTS USING ETHANOL-INDUCED GASTRIC ULCER MODEL IN RATS

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ABSTRACT: The antiulcer effects of the methanolic extract of *Cassia singueana* leaves were investigated using ethanol-induced gastric ulcer model in rats. The extract was prepared by cold marceration 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. *Cassia singueana* extract (CSE) exhibited a more gastro-protective effect against ethanol-induced stomach ulcers at 250 and 750 mg/kg than omeprazole (20 mg/kg) and solvent treated (control) rats. CSE had 59% while omeprazole and distilled water produced 55% and 0% respectively. Histopathological lesions were observed to deviate from massive severe lesions with marked disorientation of the gastric epithelium in the control to fairly protected mucosa with omeprazole and a better protected mucosa with intact epithelium in CSE (750 mg/kg) treated rats. *C. singueana* extract was found to be significantly protective against ethanol-induced gastric ulcers in the experimental rats.

Keywords: *Cassia sigueana*, Ethanol, Antiulcer, Preventive index, Gastric ulcer, Histopathology

INTRODUCTION

Peptic ulcer is a chronic, non-malignant inflammatory disease characterised by ulceration in the upper gastro-intestinal tract (stomach and duodenum) where parietal cells are found. The aetiology of gastric ulceration is multifactorial and not clearly defined, but some predisposing factors have been implicated. This include duration of starvation, 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, 1986).

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). Oxidative stress-induced tissue damage with reactive oxygen species (ROS) is implicated as a cause and consequence of a variety of disorders, including coronary heart disease, neurodegenerative disorders, autoimmune pathologies, cancer, apoptosis etc. (Kayode *et al*, 2009). Exposure of gastric mucosa to damaging factors such as ethanol, thermal stress or various irritants that are commonly named 'breakers' of gastric mucosal barrier produces pathological changes (Brzozowski *et al*, 1997).

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). Many have various adverse effects (Blum *et al*., 1986) and no drug proves solely effective in treating peptic ulcer. *Cassia singueana* leaf is noted for being effective in the treatment of different ulcer cases by the Fulani and Hausa herbal medicine practitioners of Northern Nigeria. A scientific investigation of this plant based on its folkloric use has not been done before. The aim of this study was to investigate the antiulcer effects of *Cassia singueana* leaves using ethanol gastric ulcer induction model in rats.

MATERIALS AND METHODS

Solutions, reagents, drugs and chemicals

Freshly prepared solutions and analytical grade chemicals were used in all the experiments. Methanol and ethanol were obtained from Riedel-deHaen, Germany; omeprazole (XL laboratories, Rajasthan, India), 10% formol saline were used.

Animals

Inbred matured albino Wistar rats of both sexes weighing 150-200 g, bred in the laboratory animal unit of the Faculty of Veterinary medicine, University of Nigeria, Nsukka were used in the experiments. The rats were kept in the same room with a temperature varying between 28 and 30^0; 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 Gerza, Limawa Local Government Area of Sokoto State in November, 2008. 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 (6th 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 C. *singueana* extract on ethanol-induced gastric ulcers in rats
Ethanol-induced ulcers were evaluated in rats as described by Morimoto *et al* (1991). Forty adult Wistar rats of either sex were marked, weighed and randomly assorted into 5 groups (A-E) with each group containing 8 rats. The rats were fasted for 24 h, the separate groups were then given distilled water or omeprazole (20 mg/kg) or CSE (100, 250 and 750 mg/kg) *per os*. One hour after the administration, 1 ml of absolute ethanol was administered orally to all the rats. Two hours after drenching the rats with ethanol, animals were sacrificed and their stomachs were carefully removed. 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 11.7 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 Cassia *singueana* extract on ethanol-induced gastric ulcers in rats

C. singueana extract (CSE) produced a significant reduction in the mean ulcer index of 1.24 ± 0.20 and 1.23 ± 0.28 at 250 and 750 mg/kg respectively when compared with 2.96 ± 0.13 in solvent treated rats. The extract had an equally better gastro-protective effect with the same preventive index of 59% at 250 and 750 mg/kg over 55% with omeprazole (Table 1).

Table 1: Effect of *Cassia singueana* extract on ethanol-induced gastric ulcer index in rats

Treatment	No. of animal	Mean ulcer index ± SE	Preventive index (%)
Distilled water (Control)	8	2.96 ± 0.13	0
Omeprazole (20 mg/kg)	8	1.28 ± 0.26*	55
CSE (100 mg/kg)	8	2.70 ± 0.26	7
CSE (250 mg/kg)	8	1.24 ± 0.20*	59
CSE (750 mg/kg)	8	1.23 ± 0.28*	59

*Superscript indicates significant difference at $p < 0.05$ when compared with the control.

Histopathology

Group A (Negative Control). Rats were given only distilled water orally prior to stomach ulcer induction with ethanol (1 ml) given *per os* (p.o.). The histopathologic findings revealed numerous severe erosions with marked disorientation of the surface epithelium (Plate 1). In some of the areas, the damage extended into the muscularis mucosa.

Group B (Positive Control). Animals in this group were given omeprazole (20 mg/kg), orally before ulcer induction. The mucosa was fairly protected even though; few areas of disorganisation of the villi and crypts were visible (Plate 2).

Group C. The histopathologic effect of a low dose (100 mg/kg) of *C. singueana* extract on ethanol-induced gastric ulceration in rats showed that the epithelium of the gastric mucosa had considerable levels of disorganisation.

Group D. Animals in this group were given oral treatment of *C. singueana* extract (250 mg/kg) prior to administration of ethanol (1 ml) *per os*. The gastric epithelium was fairly protected.

Group E. The rats in this group were given oral dose of CSE (750 mg/kg) before ulcer induction with 1 ml of ethanol. The mucosae were observed to be intact with minimal ulcer lesions (Plate 3).
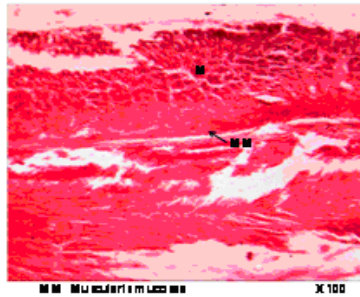

Plate 1: Micrograph of Control rat stomach showing severe ulcer lesions and desquamation of the surface epithelium in ethanol-induced gastric ulcers.
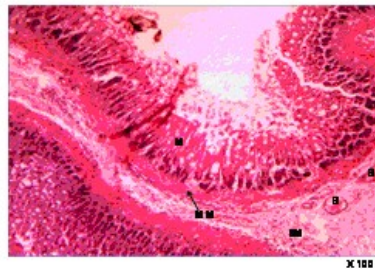

Plate 2: Micrograph of rat stomach fairly protected with omeprazole (20 mg/kg) in ethanol-induced ulceration
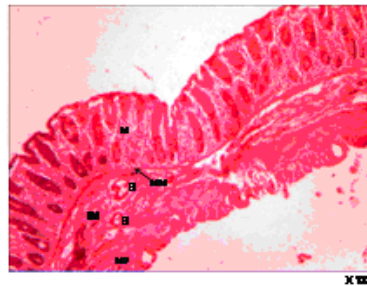

Plate 3: Micrograph of rat stomach showing a protected epithelium due to CSE (750 mg/kg) in ethanol-induced gastric ulceration

DISCUSSION

C. singueana extract (CSE) was safe, up to a dose of 4000 mg/kg did not cause mortality in the rats. Preliminary *in vivo* studies with extract doses above 750 mg/kg did not show appreciable difference between treated and normal rats. In the experimental rats, the extract exhibited maximal protective effect at 750 mg/kg against ethanol-induced gastric ulcers over omeprazole (20 mg/kg) and negative control (N.C). This is evidenced in the ulcer index (750 mg/kg CSE=1.23 $\pm$ 0.28; omeprazole = 1.28 $\pm$ 0.26 and N.C=2.96 $\pm$ 0.13), percentage ulcer preventive index (750 mg/kg CSE=59%; omeprazole =55% and N.C=0%) and histopathological lesions. In the negative control, there were severe and generalized erosions of the gastric epithelium with massive disorientation of the villi and crypts. The damage affected the submucosa and muscularis propria.

Omeprazole, a proton pump inhibitor (PPI) offered a fairly protected gastric mucosa while 750 mg/kg of the extract produced a more protective effect on the rat gastric mucosa (Plate 3). The results of the *in vivo* studies indicated that the effect of the extract at 250 and 750 mg/kg was almost similar to that of omeprazole, this suggests a possible inter-relationship in their mechanism of action. 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. By increasing intragastric pH through inhibition of acid secretion, PPIs inhibit activation of pepsin. They are effective in treating peptic ulcer disease and gastroesophageal reflux with both short and long-term use (Schneeweiss *et al*., 2006).

Ethanol disrupts the gastric mucosal barrier and cause profound micro-vascular changes with strong vaso-constriction accompanied by arteriolar dilatation responsible for engorgement of mucosal capillaries (Cho and Ogle, 1992). The pathogenesis of mucosal damage in the stomach includes the generation of ROS that seem to play a vital role in the formation of lipid peroxides, accompanied by impairement of anti-oxidative enzyme activity of cells (Konturek *et al*, 2000). CSE suppressed ulcerogenic tendencies of ethanol in the experimental rats at doses 250 and 750 mg/kg, an effect suggestive of antioxidant potential. Antioxidants consist of vitamins, polyphenols, flavonoids (Réka and Varga, 2002), minerals and endogenous enzymes such as superoxide dismutase, catalase and glutathione peroxidase that have the capability to neutralize unstable molecules called free radicals. Vitamin A (retinol), vitamin C (ascorbic acid), vitamin E (tocopherol) and selenium are valuable antioxidants. Antioxidants disrupt the chain reaction in which free radicals turn other molecules into free radicals like themselves, a process of chain-breaking or stabilization.

CONCLUSION AND RECOMMENDATION

The results of the investigation of *C. singueana* leaves for antiulcer effects using ethanol-induced gastric ulcer model in rats laid credence to traditional use of the plant leaves in ulcer treatment. The crude methanol extract of *C. singueana* leaves at 750 mg/kg demonstrated increased percentage preventive index compared to omeprazole or solvent treated rats. Further studies on *C. singueana* extract are however, recommended to evaluate its antioxidant power.

REFERENCES

Blum, J. and Fridovich, I. (1985). Inactivation of glutathione peroxidase by superoxide radical. *Arch. Biophys*. 240: 500.

Brzozowski, T., Konturek, P. C. H., Sliwowski, Z., Drozdowicz, D., Hahn, E. G. and Konturek, S. J. (1997). Importance of nitric oxide and capsaicin-sentitive afferent nerves in healing of stress lesions induced by epidermal growth factor. *J. Clin. Gastroenterol*. 25 (1): 28-38.

Cho, C. H. and Ogle, C. W. (1992). The Pharmacological differences and similarities between stress and ethanol-induced mucosal damage. *Life Sc*. 51: 1833-1842.

Coles, E. H. (1986). Determination of packed cell volume In: Coles E. H. Ed, Veterinary clinical Pathology. W. B. Saunders Co; Philadelphia. pp. 17-19.

Gerald, M. C. (1981). Pharmacology: An introduction to drugs, 2nd ed. Prentice-Hall Inc; New Jersey: 487-499.

Green, R. J. and Harris, N. D. (1993). Pathology and therapeutics for Pharmacists. Chapman & Hall, London. pp. 261-271.

Guidobono, F; Pagani F; Ticozzi, C; Sibilica, V; Pecile, A. and Netti, C. (1997). Protection by Amylin of gastric erosions induced by indomethacine or ethanol in rats. *Br. J. Pharmacol*. 120: 581-586.

Kayode, A.A.A; Kayode, O.T. and Odetola (2009). Anti-Ulcerogenic activity of two extracts of *Parquetina nigrescens* and their effects on mucousal antioxidants defence system on ethanol-induced ulcer in rats. *Res. J. Medicin. Plant* 3(3): 102-108.

Konturek, P. C. H., Duda, A. and Brzozowski, T. (2000). Activation of genes for superoxide dismutase, interleukin-1ß), tumour necrosis factor-α) and intercellular adhesion molecule-1 during healing of ischaemia-reperfusion gastric injury. *Scand. J. Gastroenterol.* 35: 452-463.

Laurence, D. R; Bennett, P. N. and Brown, M. J. (1997). Clinical Pharmacology (8th ed.). Churchill Livingstone, London. pp. 567-578.

Main, I. H. M. and Whittle, B. R. (1975). The effect of E and A Prostaglandin on gastric mucosal blood flow and acid secretion in Rats. *Br. J. Pharmacol*. 53: 217-224.

Morimoto, Y., Shimohara, K., Oshima, S. and Sukamoto, T. (1991). *The Japanese. J. pharmacol*. 57: 495.

Munson, P. L; Mueller, R. A. and Breese, G. R. (1995). Principles of pharmacology: Basic concepts and clinical applications. Chapman & Hall, USA. pp. 1063-1081.

Schneeweiss, S; Maclure, M; Dormuth, C. R; Glynn, R. J; Canning C. and Avorn, J. (2006). Clinical pharmacology & therapeutics. 79 (4): 379-386.

Takeuchi, K. and Okabe, S. (1982). Acid back-diffusion and mucosal H^+ handling in the rat stomach under normal and stress-induced conditions. *The Japanese J. Pharmacol.* 33, 1, 85-93.

Tanaka, H; Shuto, K. and Nakamizo, N. (1983). Exacerbation of acetic acid induced by Non-steroidal anti-inflammatory drugs in rats. *The Japanese J. Pharmacol*. 33, 2, 447-454.

Webster, C. R. L. (2001). Quicklook series in Veterinary medicine. Clinical Pharmacology. Teton Newmedia, Wyoming, U.S.A. pp. 102-103.

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