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Research Article

The Antiulcer Activities of the Methanol Extract of *Cassia singueana* Leaves Using Indomethacin-Induced Gastric Ulcer Model in Rats

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ABSTRACT

The antiulcer activities of the methanolic extract of *Cassia singueana* leaves were evaluated using indomethacin-induced gastric ulcer model in rats. The extract was prepared by cold marceration in 80% methanol at 37^{0}C 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 at 250 and 750 mg/kg produced significant ($p<0.05$) protective effect in rats with a preventive index of 71% against 52% with cimetidine (100 mg/kg) and 0% with distilled water. The histopathologic lesions deviated from numerous severe focal lesions and marked disorientation of the gastric epithelium in solvent treated control to fairly protected gastric mucosa with CSE (250 and 750 mg/kg) and cimetidine (100 mg/k) treated rats. *Cassia* leaf extract was found to be significantly protective against indomethacin-induced gastric ulcers in the experimental rats.

Keywords: *Cassia singueana*, Indomethacin, Anti-ulcer, Preventive index, Stomach ulcer, Histopathology

1. INTRODUCTION

The epithelium of gastrointestinal 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 duration of starvation, nature of food ingested, bile reflux [1], lessened mucosal resistance [2], alteration of gastric mucosal blood flow [3], disruption of gastric mucosal barrier by stress [4], decrease in alkaline mucosal bicarbonate and mucus secretion [5], over dosage and or prolonged administration of non-steroidal anti-inflammatory drugs [6], persistent infection with *Helicobacter pylori* [7], *Zollinger-Ellison* syndrome [8], 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 [9].

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 in the cyclooxygenase pathway. NSAIDs, besides producing the well recognized prostaglandin cytoprotective deficiency, also enhance gastric motility which may contribute to the pathogenesis of gastric injuries [10].

Histopathological changes in the gastric mucosa of animals infected with *H. pylori* involved dilatation of gastric glands, degenerative changes in superficial epithelia and necrosis of individual gastric glands. [11]. Histamine is regarded as the critical regulator of gastric acid secretion [12]. Most anti-ulcer drugs require prolong period of intake, yet ulcer relapse is a common occurrence [7]. The anti-ulcer drugs available in the market have numerous adverse effects [13] and no known drug proves solely effective in treating peptic ulcer. *Cassia* is indigenous to tropical and warm temperate regions. Its habitat is savannah and it is widespread in tropical Africa [14]. The local names of the plant in different languages are *rumfu* in Hausa, *rumfuhi* in Fulani, *tugelele* in Kanuri and *Shadarat al bashini* in Shuwa Arabic [15]. Concoction made from *C. singueana* leaves is popular in the treatment of different cases of stomach ulcer 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 evaluate the anti-ulcer effects of *Cassia singueana* leaves using indomethacin-induced gastric ulcer model in rats.

2. MATERIALS AND METHODS

2.1 Solutions, reagents, Drugs and chemicals

Freshly prepared solutions and analytical grade chemicals were used in all the experiments. Methanol was obtained from Riedel-deHaen, Germany., cimetidine, indomethacin (Sigma Aldrich, USA), and 10% formol saline were used.

2.2 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 experiments. The rats were kept in the same room with a temperature varying between 28 and 30^{0}C; 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.

2.3 Preparation of the plant extract

Fresh leaves of the plant were collected from Gerza, Limawa Local Government Area of Sokoto State in November, 2009. 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.

2.4 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.

2.5 Effect of Cassia singueana extract (CSE) on indomethacin-induced stomach ulcer lesions

Forty adult Wistar rats were randomly classed into 5 groups (A-E) of 8 rats per group. The rats were deprived of feed 24 h prior to the experiment. Group A served as the control and was given only distilled water *per os* by gastric intubation. Group B was given oral treatment of cimetidine (100 mg/kg) while groups C, D and E were equally treated with varying doses (100; 250 and 750 mg/kg) CSE respectively through the same route. After 30 min, indomethacin (40 mg/kg) was given orally to all the rats. The rats in all the groups were humanely killed by cervical dislocation at 6 h and their stomachs were 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 [16]. 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.

2.6 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.

3. RESULTS

3.1 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).

3.2 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.

3.3 Effect of C. singueana extract on indomethacin-induced stomach ulcer lesions in rats

The extract at 250 and 750 mg/kg produced significant ($p<0.05$) gastro-protective effect. At both doses, the extract had a mean preventive index of 71% against 52% with cimetidine (100 mg/kg) and 0% with distilled water (Table 1). CSE also reduced the ulcer index when compared to cimetidine or solvent control (CSE 250 mg/kg = 0.81 ± 0.08; CSE 750 mg/kg = 0.80 ± 0.09; cimetidine = 1.33 ± 0.12; control = 2.78 ± 0.10).

Table 1: Effect of Cassia singueana extract on indomethacin-induced stomach ulcer index in rats

Treatment	No. of animals	Mean ulcer index ± SE	Preventive index (%)
Distilled water (Control)	8	2.78 ± 0.10	0
Cimetidine (100 mg/kg)	8	1.33 ± 0.12^a	52
CSE (100 mg/kg)	8	1.83 ± 0.32^b	34
CSE (250 mg/kg)	8	0.81 ± 0.08^C	71
CSE (750 mg/kg)	8	0.80 ± 0.09^C	71

^{abc} *Superscripts denote mean differences at $p<0.05$.*

3.4 Histopathology

Group A (Negative control). Rats were given only distilled water orally prior to stomach ulcer induction with indomethacin (40 mg/kg) given *per os* (p.o.). The histopathologic findings revealed numerous severe focal erosions with marked disorientation of the surface epithelium (Plate 1). In some of the areas, the gastric epithelium was totally eroded leaving the basement of the muscularis mucosa exposed.

Group B (Positive control). Animals in this group were given cimetidine, 100 mg/kg orally before ulcer induction. The mucosa was fairly protected even though, early stages of desquamation of the surface epithelium were observed. Other layers of the gastric wall (mucosa muscularis, submucosa, inner circular and outer longitudinal layer of muscularis propria) appeared normal (Plate 2).

Group C. The histopathologic effects of 100 mg/kg of *C. singueana* extract on indomethacin-induced gastric ulceration revealed that the epithelium of the gastric mucosa had considerable level of erosions. The submucosal blood vessels were markedly congested.

Group D. Animals in this group were given oral treatment of *C. singueana* extract (250 mg/kg) prior to administration of indomethacin (40 mg/kg) *per os*. The gastric epithelium was fairly protected.

Group E. The rats in this group were given oral dose of *C. singueana* extract (750 mg/kg) before administration of indomethacin (40 mg/kg, p.o.) to induce ulcer. The mucosae were observed to be intact with minimal ulcer lesions (Plate 3).

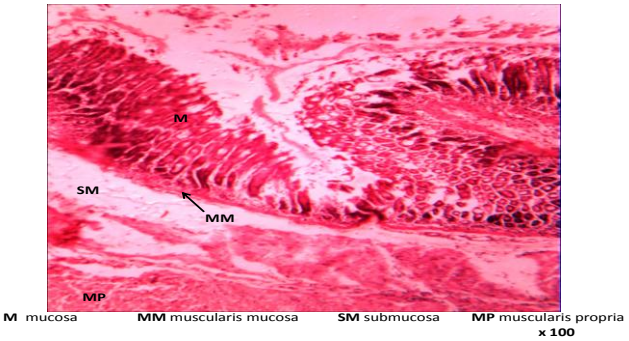

Plate 1: Control rat stomach showing indomethacin-induced generalized severe focal erosive lesions.

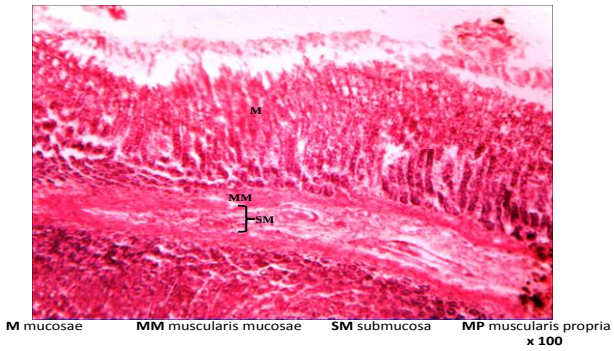

Plate 2: A fairly protected rat gastric mucosa with cimetidine in indomethacin ulcer

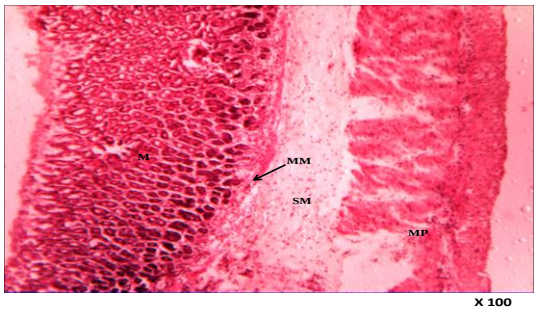

Plate 3: Rat stomach protected with 750 mg/kg of CSE in indomethacin-induced ulceration.

4. DISCUSSION

Cassia singueana extract (CSE) was safe,up to a dose of 4000 mg/kg did not cause mortality in the experimental rats. 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. The extract produced significant protection at 250 and 750 mg/kg against indomethacin-induced gastric ulcers in rats over cimetidine (100 mg/kg) and negative control (N.C). This is evidenced in the percentage preventive index (250 and 750 mg/kg CSE = 71%; Cimetidine = 52% and N.C = 0%) and histopathological lesions. In the negative control, there were generalized severe focal erosions with marked disorientation of the surface epithelium but in cimetidine and CSE (250 and 750 mg/kg) treated rats, the surface epithelia were fairly protected (Plate 1, 2, and 3). Because CSE treatment significantly inhibited the formation of stomach ulcers induced by indomethacin administration in rats, it suggests that CSE can suppress gastric damage induced by one of the aggressive factors. Preliminary *in* vivo studies showed that the extract had little or no gastro-protective effect above 750 mg/kg. The mechanism by which the extract inhibited ulcerogenesis by indomethacin is not well understood because indomethacin inhibits cyclooxygenase and produces the well recognized prostaglandin cytoprotective deficiency [10], which contributes to the pathogenesis of gastric injuries. Thus, it remains unclear if the extract mediated its action at the enzyme level or in stabilizing the integrity of gastro-intestinal tract. However, further studies are on-going using histamine and ethanol–induced gastric ulcer models and isolated tissue experiments to get a better understanding of the mode of action of the extract.

5. CONCLUSION

The methanol extract of *Cassia singueana* leaves demonstrated protective activity against indomethacin-induced gastric ulcer lesions in rats. This is evidenced in the increased preventive index with doses (250 and 750 mg/kg) of the extract and histopathological studies.

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