ANTIULCER ACTIVITY OF AQUEOUS EXTRACT OF FRESH LEAF OF *BRASSICA OLERACEAE* LINN. VAR. *ACEPHALA* (D.C) ALEF) (BRASSICACEAE)

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ABSTRACT

Brassica oleraceae (BOL) is the common cabbage and a familiar garden plant, widely used as spice all over the world. Cabbage is an excellent source of Vitamin C and has been widely employed locally to treat acute inflammation and peptic ulcers. The present study aimed at evaluating the antiulcer activity of *Brassica oleraceae*, while identifying the phytoconstituents responsible for the observed effects and exploring some of the possible mechanisms of its antiulcer activity, using standard laboratory procedures. Doses of 100, 300 and 750 mg / kg of *Brassica oleraceae* were separately administered to groups of overnight fasted rats, with appropriate standard drugs using Ethanol / HCl, Indomethacin and Cysteamine models to explore antiulcer property of BOL on the stomach and duodenum respectively. The extract in a dose dependent fashion, offered better protection against the ulcerogens in the gastric ulcer models, when compared with the positive control groups. However, the smallest dose of 100 mg / kg recorded the highest percentage protection in the cysteamine group. Up to 1200 mg / kg i.p and 10,000 mg / kg oral doses of *Brassica oleraceae* did not produce any mortality in mice. Phytochemical constituents identified included alkaloids, tannins, cardiac glycosides, flavonoids, phlobatannins, anthraquinones and saponins. The pH was 8.5. The present study has validated the local use of *Brassica oleraceae* in the treatment of peptic ulcer and the speculated mechanisms of action could be through acid neutralization, cytoprotection and antioxidation by flavonoids.

Keywords: Gastric ulcer, Duodenal ulcer, Ulcerogen, *Brassica oleraceae* Extract, Rat, Phytoconstituents.

INTRODUCTION

Ulcers are crater-like sores on the skin and mucous membrane. Peptic ulcer disease (PUD), the most prevalent gastrointestinal (GI) disorder¹ ensues when the stomach and duodenum are affected. For over a century, PUD has been one of the leading causes of GI surgery, with high morbidity and mortality rates. It affects several people worldwide and some researchers consider it a new plague of the twenty first century^{2, 3}. Pathophysiology of PUD involves an imbalance between the protective factors (mucin, prostaglandin, growth factors, nitric oxide and bicarbonate) and aggressive factors (acid, pepsin and *Helicobacter pylori*) in the stomach⁴. Provocative factors could also aggravate the pathological state of the individual⁵ for example, use of drugs such as the non steroidal anti inflammatory agents, alcohol, cigarette smoking and stress⁶. There have been two main approaches for treating PUD; one deals with reducing the production of gastric acid and mopping up of any excess acid secreted (proton pump inhibitors, histamine H₂ receptor antagonists, and antacids), while the second involves reinforcing the gastric mucosal protection (prostaglandin analogues, and cytoprotective agents)^{1,3}. Conventional treatments have a number of limitations; for example, long-term and / or high - dose administration often record several GI adverse effects, including small bowel bacterial overgrowth, spontaneous bacterial peritonitis, vitamin B₁₂ deficiency, community - acquired pneumonia and the risks of *Clostridium difficile* colitis^{7,8}. Herbal medicine is fast emerging as an alternative to available synthetic drugs used for treating diverse ailments and diseases, including peptic ulcer, possibly due to low cost, ready availability, and their speculated effectiveness⁹. A great variety of medicinal plants used, either the whole plant or a specific part of it (root, stem, leaf, seed, fruit and flower) is formulated into suitable preparations, which could be compressed as tablets or made into pills, infusions, extracts and tinctures among other uses. These drugs may be slow in action, but most of them compare effectively with standard

medications and are reliable with relative safety. The plant *Brassica oleraceae* (BOL) is the cultivated cabbage derived from a leafy plant called the wild mustard plant, sea cabbage and wild cabbage. It is native to the Mediterranean region along the seacoast. It is often considered one of the most ancient plants among all the common vegetables used by man, since history revealed that the consumption of cabbage by humans in the eastern world may have been as early as four millennia ago. Furthermore, it has been employed as an herbal remedy for the prevention and treatment of various diseases and disorders including arthritis, scurvy, leg ulcers, asthma, colitis, cough, peptic ulcer, acne, and constipation in different parts of the world¹⁰. Breast milk production was usually enhanced by giving cabbage to expectant mothers¹¹. The present study sought to investigate the anti ulcer activity of *Brassica oleraceae* in laboratory-induced gastric and duodenal ulcers, using standard experimental models, as well as elucidating the probable mechanisms of its antiulcer action.

MATERIALS AND METHODS

Preparation of Plant Extract

The plant *Brassica oleraceae* was obtained from the Vegetable Garden City in Lagos Metropolis, Lagos-Nigeria, and was identified by Mr. T.K. Odewo of the Department of Botany and Microbiology, University of Lagos, with voucher number LUH 2746. Fresh clean leaves of the plant were weighed (3 kg), chopped into bits and blended to obtain a mash of cabbage juice. Distilled water (0.9 L) was added to facilitate blending. The resultant juice was sieved through a sterile cotton wool, placed in a funnel to separate the residues and sediments from the juice and thereafter a clean handkerchief was employed to further sieve the juice to obtain the eventual fine pure cabbage juice used in the study. The juice was afterwards dried in the oven at 40°C, weighed, and stocked airtight in the freezer, as *Brassica oleraceae*

extract, until ready for use. Percentage yield and pH of the extract were recorded.

Animals

Healthy Wistar mice and Sprague Dawley rats of both sexes purchased from Laboratory Animal Centre, College of Medicine, University of Lagos, Lagos, Nigeria and weighing 15-20 g and 100-150 g respectively were used for the study. All animals were maintained under standard laboratory conditions (temperature $25 \pm 2^\circ\text{C}$) and humidity ($55 \pm 5\%$), with 12 h day: 12 h night cycle. The animals were fed with the normal laboratory diet (Ladokun Feeds PLC, Ibadan--Nigeria) and drinking water *ad libitum*. All the experimental procedures were in strict accordance with Institutional Animal Ethical Committee Guidelines for the care and use of laboratory animals.

Phytochemical Screening

The aqueous extract was tested for the presence or absence of secondary metabolites using standard phytochemical procedures^{12,13}.

Acute Toxicity Test

Toxicity of the plant was determined using a standardized method¹⁴. Doses of 300, 600 and 1200 mg / kg were given intraperitoneally (i.p) and up to 10,000 mg / kg administered orally to groups of fasted rats, which were thereafter monitored for behavioural changes, toxic symptoms and mortality.

Antiulcer Activity Study

Ethanol / HCl-induced gastric ulcer (Protective)

Six groups of rats (n = 5) were randomly selected and treated orally as follows: Group 1: Distilled water 10 ml / kg, Group 2: Cimetidine 100 mg / kg, Group 3: Omeprazole 20 mg / kg, Group 4: *Brassica oleraceae* 100 mg / kg, Group 5: *Brassica oleraceae* 300 mg / kg, Group 6: *Brassica oleraceae* 750 mg / kg

/ kg. One hour after gavage, one millilitre of 70 % ethanol in 250 mM HCl was delivered via oral cannula to the stomach of all the animals and the animals were humanely sacrificed 1 h later. The stomachs were dissected along the greater curvature and rinsed in normal saline in order to observe and score the lesions produced¹⁵.

Ethanol / HCl-Induced Gastric Ulcers (Curative)

Thirty rats were divided into two groups of 15 each, for distilled water (control) and *Brassica oleraceae* 750 mg / kg, respectively given 1 h after the ulcerogen ethanol / HCl¹⁶. Five animals were randomly selected from each group 4 h, 24 h and 72 h after drug therapy, and sacrificed with the stomach examined to determine the curative properties of the herbal drug, viz-a-viz, its time of onset and duration of action. Ulcer was scored as earlier described^{1, 15}.

Indomethacin-induced gastric ulcers

Groups of animals were given *Brassica oleraceae* 100, 300 and 750 mg / kg, distilled water 10 ml / kg (negative control) and misoprostol 200 µg / kg the positive control. Indomethacin 50 mg / kg was administered 1 h after the various test agents¹⁷ and all animals were sacrificed 6 h afterwards for ulcer scoring.

Cysteamine-induced duodenal ulcer

Randomly selected groups of animals were treated with 400 mg / kg cysteamine and 1 h after; rats were dosed as before with *Brassica oleraceae* and with 20 mg / kg omeprazole as the standard drug. Twenty four hours later, the animals were sacrificed and their duodena opened along the mesenteric side to observe and score the lesions¹⁸.

Statistical Analysis of Results

Analysis was done using the unpaired student's t-test and significance of difference was accepted as $p < 0.05$.

Table 1: Effect of *Brassica oleraceae* on Ethanol / HCl-induced gastric ulcer (protective)

Drug / Dose (mg / kg)	Ulcer index	% Protection
Distilled water 10 ml / kg	35	0
Cimetidine 100	*21	40
Omeprazole 20	*20	42.9
<i>Brassica oleraceae</i> 100	*20	42.9
<i>Brassica oleraceae</i> 300	*18	48.6
<i>Brassica oleraceae</i> 750	*16	54.3
P < 0.05 when compared with control		

Table 2: Effect of *Brassica oleraceae* on Indomethacin-induced gastric ulcer

Drug / Dose (mg / kg)	Ulcer index	% Protection
DW	16	0
Misoprostol 0.2	*2.5	84
<i>Brassica oleraceae</i> 100	*10.5	34.4
<i>Brassica oleraceae</i> 300	*6.5	59.3
<i>Brassica oleraceae</i> 750	a,* 1.5	90.6
a,* p<0.05 when compared with misoprostol and control respectively		

Table 3: Effect of *Brassica oleraceae* on cysteamine-induced duodenal ulcer

Drug / Dose (mg / kg)	Ulcer index	% Protection
DW	75	0
Omeprazole 20	*20.5	45
<i>Brassica oleraceae</i> 100	*5.5	85
<i>Brassica oleraceae</i> 300	*12.5	67
<i>Brassica oleraceae</i> 750	* 19.5	48

Table 4: Curative effect of *Brassica oleraceae* on Ethanol / HCl-induced gastric ulcer

Test Agents	Post drug therapy / ulcer index		
	4 h	24 h	72 h
DW	12	6	1.5
<i>Brassica oleraceae</i> 750 mg / kg	*1.5	0	0
% Protection	87.5	100	100
* p < 0.05 when compared with control

Figure 1: Ethanol / HCl induced gastric ulcer (Protective) – a and b = 300 and 750 mg / kg *Brassica oleraceae* respectively, c is Distilled water (negative control)

Figure 2: Indomethacin-induced gastric ulcer (Protective) d is distilled water (negative control), e and f = 750 and 300 mg / kg *Brassica oleraceae* respectively, g is misoprostol (positive control).

Figure 3: Ethanol / HCL-induced gastric ulcer (Curative) h and i = 750 mg / kg *Brassica oleraceae* and distilled water (negative control) 4 h post therapy

RESULTS

The extract, completely dissolved in distilled water and recorded a pH of 8.5

Acute Toxicity

No lethality was produced by i.p administration of *Brassica oleraceae*, except mild sedation and itching, both of which were transient.

Phytochemical Screening

Alkaloids, tannins, cardiac glycosides, flavonoids, phlobatanins, anthraquinones and saponins were identified.

Antiulcer Activity of *Brassica oleraceae* Ethanol / HCL-induced gastric ulcer (Protective)

The herbal preparation yielded a dose-dependent and significant effect when compared with the negative control group. In addition, its effect, especially in doses of 300 and 750 mg / kg, was superior to the standard drugs cimetidine and omeprazole (Table 1).

Effect of *Brassica oleraceae* on Indomethacin-induced gastric ulcer

The plant drug recorded a significant antiulcer activity when compared with both the negative and positive control groups (Table 2, Figure 2). The highest dose of *Brassica oleraceae* produced a higher efficacy than misoprostol.

Cysteamine-induced duodenal ulcer

Efficacy reduced with increasing doses, and 100 mg / kg, the least dose of *Brassica oleraceae* employed in this study gave 85.3 % efficacy. Omeprazole, the standard drug used recorded only 45 % effect (Table 3).

Curative Property of *Brassica oleraceae* in Ethanol / HCL-induced gastric ulcer

The herbal preparation possessed a relatively fast onset of action, since 87.5 % protection was recorded within 4 h of drug administration. The herbal drug antiulcer effect was also sustained as all the sores were totally cleared by 24 h. (Table 4, Figure 3).

DISCUSSION

BOL (Cabbage) is a local vegetable used as spice by majority of people, who may not necessarily have any knowledge of its medicinal benefits. Administration of the aqueous extract up to 10,000 mg / kg did not produce any mortality in the animals, which showed its relative safety. The present investigation explored both the protective and curative potentials of *Brassica oleraceae* in laboratory-induced ulcers, while employing ethanol / HCl and indomethacin as ulcerogens in gastric ulcers, and cysteamine HCl in duodenal ulcers. *Brassica oleraceae*, in a dose dependent fashion protected against ulcer in both the gastric ulcer models, however, a higher efficacy was recorded in the indomethacin model, where the highest dose of the herbal drug, 750 mg / kg produced 90.6 % antiulcer effect, which was not only significant when compared with both the negative and

positive control groups, but also better than misoprostol, the standard drug used. Ethanol and HCl, the most common necrotizing agents produce severe gastric erosions when given intragastrically. Oxygen-free radicals which lead to an increased lipid peroxidation and damage to cell and cell membranes, are implicated in the pathogenesis of ethanol / HCl-induced gastric mucosal injury¹⁹. Ethanol / HCl, in addition to its direct damage of gastric mucosal cells through free radicals formation, also causes solubilisation of mucus constituents and depresses tissue levels of proteins, leading to flow stasis in gastric blood¹⁹. Flavonoids, one of the phytoconstituents in *Brassica oleraceae*, have been confirmed to possess anti ulcerogenic property through cytoprotection, free radical scavenging and antioxidation, also through increased mucus production, antisecretory actions, and inhibition of the *Helicobacter pylori* growth^{20,21}. Furthermore, Vitamin C, an antioxidant has also been reported present in *Brassica oleraceae*¹² and the herbal drug has been observed to produce antimicrobial activity²². Tannins another group of constituents in *Brassica oleraceae*, prevent ulcer formation as a result of their protein precipitating and vasoconstricting effects,²³ their astringent action helps to precipitate microproteins on the ulcer site, thereby, forming an impervious layer over the lining, which hinders induced gastric ulcer in rats²³. Indomethacin produces gastric lesions by inhibiting the synthesis of cytoprotective prostaglandins through modulation of cyclooxygenase isoforms, COX₁ and COX₂; infiltrating inflammatory cells, and the involvement of inducible nitric oxide synthase (iNOS) and its products. Therefore, involvement of oxygen derived free radicals such as the superoxide anion, hydrogen peroxide, and hydroxyl radical are well established in the pathogenesis of nonsteroidal anti inflammatory drug-induced ulcer. The resulting products, through oxidative stress produce gastric mucosal lesions, leading to disruption of gastric mucosal barrier. Agents which have cytoprotective and/or antacid secretory effect, prevent gastric lesions induced by indomethacin¹². Fresh cabbage juice has also been reported to contain zinc, phosphorus, glutamine and S-methyl methionine¹² all of which enhance antiulcer activity via different mechanisms. In cysteamine-induced duodenal ulcer, the herbal drug's efficacy was similarly sustained. Mechanism of ulcer formation in this model has been shown to involve an increase of oxygen-derived free radicals and a decrease of Cu, Zn, and superoxide dismutase (SOD) activity in the duodenal mucosa^{18,24}. Other factors involved include increased duodenal motility³¹ decreased duodenal bicarbonate secretion in response to acid^{25,26} delayed gastric emptying and enhanced or unchanged gastric secretion. The protective effect of *Brassica oleraceae* against cysteamine-induced duodenal ulcers could be due to the strengthening of the duodenal mucosa or by other mechanisms like increased gastric and duodenal alkaline secretion or by increased luminal prostaglandin levels^{18,27}. *Brassica oleraceae* recorded a pH of 8.5 and its antiinflammatory property has been well documented²⁸.

CONCLUSION

The present study has shown the antiulcer property of *Brassica oleraceae* and the findings may support the local use of *Brassica oleraceae* in treating peptic ulcer disease. It also demonstrated the superiority and safety of *Brassica oleraceae* over the commonly used conventional antiulcer drugs and has been able to suggest some of the possible pathways for *Brassica oleraceae* antiulcer activity.

Nevertheless, further studies on kinetics and safety (subacute and chronic toxicity studies involving the evaluation of the haematological and biochemical parameters) are necessary.

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