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Gastro-duodenal protective effect of aqueous leaf extract of *Daucuscarota sativus* Linn. (Apiaceae) in rats and its possible mechanism of action

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ABSTRACT

Background: *Daucuscarota sativus* L. (Apiaceae) commonly known as 'carrot' is a multipurpose herb cultivated in different parts of the world for its edible roots, juice, oils and leaves. Carrot root as well as its leaves has been credited with many medicinal properties, including cleansing of the intestine and maintenance of acid-alkaline balance in the body. Carrot leaves also known as carrot tops or carrot greens have been used locally as a decoction for healing mouth sores, and in some cases, mixed with honey to clean festering wounds. The present study was therefore designed to evaluate the possible gastro-duodenal protective property of *Daucuscarota sativus* (DCS) aqueous leaf extract on laboratory-induced ulcers. Aqueous leaf extract prepared by maceration was orally administered one hour before ulcerogens in doses of 50, 100 and 200 mg/kg to groups of randomized adult rats of both sexes. Gastric ulcers were induced using pyloric ligation, methylene-blue, and acetic acid, while cysteamine, and indomethacin-histamine were separately employed for induction of duodenal ulcers in the animals. Positive controls were given standard drugs appropriate for each experimental model. Phytochemical screening of the freshly prepared extract was also carried out, as well as evaluating its antioxidant activity. In each of the models, the aqueous leaf extract of DCS showed a significant (p<0.05-0.001) dose-independent protection against peptic ulcer. The effects produced by the aqueous leaf extract of DCS were comparable to those of the standard drugs (omeprazole; 200 µg/kg, ranitidine; 50 mg/kg, and cimetidine; 50 mg/kg). Phytochemical analysis of the aqueous leaf extract of *Daucuscarota sativus* revealed the presence of flavonoids, tannins, alkaloids, and saponins, some of which have been reported to elicit cytoprotective effect. Antioxidant analysis showed significant scavenging effect of free radical using nitric oxide, lipid peroxidation and DPPH assay. The findings in this study suggest that the aqueous leaf extract of DCS possesses cytoprotective effect and also reduces secretion of secretagogues, thereby protecting against gastric and duodenal ulcers.

Keywords: *Daucus carota sativus*, Ulcerogen, Antioxidant, Anti-Ulcer, Lipid Peroxidation, Cytoprotective.

INTRODUCTION

Gastro-duodenal ulcer also known as peptic ulcer, is a disease characterized by the disruption of mucosal integrity of the esophagus, stomach and duodenum [1], and it affects 10-15% of the population at any one time [2].Ulcers are primarily caused by an imbalance between some aggressive (acid-pepsin secretion) and protective (mucous secretion, blood flow, cellular regeneration, prostaglandins, growth factors and integrity of mucosal barrier) endogenous factors in the gastrointestinal tract [3]. Several factors could also predispose to peptic ulcer, among which are: use of steroidal and non-steroidal anti-inflammatory drugs (NSAIDs), stress, alcohol consumption, *Helicobacter pylori* infections, smoking, lower socio-economic status and family history [4]. It is noteworthy that ulcer disease is not deadly but could lead to severe complications, such as gastrointestinal bleeding, perforations, penetration of ulcer into adjacent organs and gastric outlet obstruction [5]. Different medications among which are antacids, antibiotics, proton pump inhibitors, other antisecretory and cytoprotective agents are employed for pain relief, healing of ulcers and for delaying ulcer recurrence [2, 6]; clinical evaluation of most of these drugs has shown incidences of relapses, side effects and drug interactions. In view of this, there is a need to search for drugs with greater or equal therapeutic benefits but with reduced or minimal side effects among plants that have been used locally for treating peptic ulcer and one of such is DCS.

Daucuscarota sativus L. (Apiaceae) is a tall biennial plant, with alternate and compound leaves arranged in a spiral form and having leaf blades that are pinnate [7]. It is widely grown during the dry season in the arid zones of West and Central Africa and in the highlands of East and southern Africa.

The leaves of *Daucuscarota sativus* (DCS) are edible, but are only occasionally eaten by humans. When used for this purpose, they are harvested young in high-density plantings, before significant root development, and typically used as a garnish for roasted meats, pastas, and soups, or blended into other herb-based salad dressings [7]. In ancient and medieval times, carrot leaves were used to bind wounds, owing in large part to their mild antiseptic and antioxidant properties. They were also chewed to promote fresh breath, as well as to relieve the pain of the common toothache and gum irritation. The leaves were also brewed into a tea, which was believed to have detoxifying effects. Their high potassium content makes them useful in lowering blood pressure; support for metabolism and prevention of osteoporosis [8].Other pharmacological activities of DCS include hepatoprotective [9], hypotensive [10], and anticancer [11] effects. Furthermore, protective and curative effects of renal ischemia [12], anti-dementia potential [13], and lowering of intraocular pressure by topical application [14] have also been reported.

In view of its folkloric use for maintenance of acid-alkaline balance in the body as well as its antioxidant property, DCS was investigated for its antiulcer potentials in acute and chronic ulcer models.

MATERIALS AND METHODS

Plant collection and extraction

The fresh leaves of *Daucuscarota sativus* (Apiaceae) were purchased from the vegetable garden at Idi-Araba, Lagos-Nigeria, and was identified and authenticated by Mr. T. K. Odewo of the Department of Botany and Microbiology, University of Lagos, Nigeria, where a voucher specimen was deposited (LUH 6154) for reference purpose. The fresh leaves were dried at room temperature and pulverized in a mechanical grinder. The powdered material (500 g) was macerated with boiled distilled water for 24 h. The resulting mixture was filtered and evaporated to dryness on water bath at 40°C with a percentage yield (w/w) of 50.5%.

Animals

Adult albino mice (18-25 g) and Sprague Dawley rats (120-150 g) of both sexes purchased from Laboratory Animal Centre, College of Medicine, University of Lagos, Nigeria were used. The animals were maintained under standard environmental conditions in accordance with Experimentation Ethics Committee of the College of Medicine, University of Lagos, Nigeria. All the animals were acclimatized for one week, fed on standard rodent diet (Livestock Feeds Plc, Ibadan, Oyo State, Nigeria) and had free access to drinking water. However, the animals were fasted at least 24 hours prior to experiments.

Drugs and chemicals

Omeprazole (Bristol laboratories Ltd. UK), Ranitidine (SKG Pharma, London), Indomethacin, Cimetidine (Yangzhou Pharma, China), Histamine, Cysteamine HCl, Acetic Acid (Sigma-Aldich Chemical Company, United Kingdom) and Phenobarbitone (Ganes Chemical, USA).

Acute toxicity

The acute toxicity of the plant extract was evaluated using the method earlier described [15]. Eight groups of mice (n=5), fasted for 12 h were orally administered the plant extract in doses of 2.5, 3, and 5 g/kg orally and 0.25, 0.75, 0.15, 0.2, and 0.8 g/kg intra-peritoneally. The animals in each group were closely monitored during the initial 2 hours to observe the immediate signs of toxicity. Deaths that occurred within 24 h were recorded and the survivors were further observed for 14 days for any delayed toxicity or mortality.

Anti-ulcer activities

Pyloric ligation-induced gastric ulcer

Adult rats fasted for 24 h were divided into five groups of five animals each and the following treatments given: distilled water (10 ml/kg); DCS (50, 100 and 200 mg/kg); Omeprazole (200 µg/kg) and Ranitidine (50 mg/kg). Sixty minutes later, the animals were anaesthetized using 25% urethane at a dose of 1 mL/100g weight of rats. The abdomen was opened by a small midline incision below the xiphoid process. The pylorus connected to the duodenum was ligated without causing any damage to its blood vessels. The stomach was isolated carefully and the abdominal wall was sealed by interrupted sutures [16, 17]. The animals were deprived of water during the postoperative period. Four hours after ligation, the stomachs were dissected out and contents were collected into clean tubes; volume, pH and total acid content of gastric juice were determined. Each stomach wascut opened along the greater curvature and examined for ulcer lesions in the glandular portion of the stomach. Total acid secretion in the gastric juice supernatant was determined by titration to pH 7.0, using a 0.01 N NaOH solution, and phenolphthalein as indicator [18].

Acetic acid induced ulcer

Adult rats were divided into five groups of five animals each, andpre-treated for 5 days as follows: distilled water (10 mL/kg); DCS (50, 100 and 200 mg/kg) and ranitidine (50 mg/kg). On the 6th day, the rats were fasted for 24 hours with access to water and treated as above. After one hour they were anesthetized with phenobarbitone (20 mg/kg) at a dose of 1ml/100g; the stomachs were dissected and dilute acetic acid (4%) (1mL) was injected in each of the stomach and sutured back, and animals allowed to recover. After 5 days, the animals were sacrificed and their stomachs removed, cut opened along the greater curvature and ulcers were scored in the glandular portion of the stomach [19, 20].

Cysteamine-induced duodenal ulcer

Adult rats fasted for 24 hours were divided into five groups of five animals each and treated as before, using omeprazole (200 µg/kg) and ranitidine (50 mg/kg) as reference drugs. Sixty minutes after treatment, cysteamine was administered twice at an interval of 4 h, first 400 mg/kg (*p.o*) followed by the addition of the second dose to drinking water [21]. The animals were sacrificed 24 h later to excise the duodenum, which was cut opened along the antimesenteric side, for ulcer scoring.

Indomethacin - histamine induced duodenal ulcer

Adult rats fasted for 24 hours were divided into five groups of five animals each and treated as before, using omeprazole (200 µg/kg) and ranitidine (50 mg/kg) as reference drugs. Sixty minutes after treatment, indomethacin (5 mg/kg, *s.c*) was administered and 30 min thereafter, histamine dihydrochloride (40 mg/kg, *s.c*) was also administered three times at 2.5 h intervals [22]. After 3 h, the animals

were similarly prepared for ulcer scoring.

Methylene-blue induced ulcer

Adult rats fasted for 24 hours were divided into five groups of five animals each and treated as before, using omeprazole (200 µg/kg) and ranitidine (50 mg/kg) as reference drugs. Sixty minutes post treatment; methylene blue (125 mg/kg, *p.o*) was administered. After 4 h, the animals were similarly prepared for ulcer scoring (Shah *et al*., 2006) [23].

Rating of Ulcer Lesion [24]

0 = No lesion
0.5 = Haemorrhage
1 = 1-3 small lesions
2 = 1-3 large lesions
3 = 1-3 thickened lesions
4 = more than 3 small lesions
5 = more than 3 large lesions
6 = more than 3 thickened lesions

Total antioxidant capacity

DPPH radical scavenging activity assay

The free radical scavenging activity of the extract, based on the scavenging of the stable 1,1-diphenyl-2-picrylhydrazyl (DPPH) free radical was estimated according to the procedure described by Burits and Bucar (2000) [25]. An aliquot of 0.5 ml of extract in ethanol (95%) at different concentrations (25, 50, 75, 100µg/mL) was mixed with 2.0 ml of reagent solution (0.004 g of DPPH in 100 mL methanol). The control contained only DPPH solution in place of the sample, while methanol was used as the blank. The mixture was vigorously shaken and left to stand at room temperature. After 30 minutes the decrease in absorbance of test mixture (due to quenching of DPPH free radicals) was read at 517 nm. The scavenging effect was calculated using the expression:

$$\% \text{ inhibition} = \frac{[A0-A1] \times 100}{A0}$$

Where A0 is the absorption of the blank sample and A1 is the absorption of the extract.

Lipid peroxidation assay

Lipid peroxidation was induced by Fe^{2+} ascorbate system in liver homogenate and estimated as thiobarbituric acid reacting substances (TBARS) [26]. Freshly excised rat liver was sliced and processed to obtain 10% homogenate in cold 150 mM KCl−Tris−HCl buffer. The reaction mixture contained liver homogenate, Tris−HCl buffer (20 mM pH 7.0), $FeCl_2$ (2 mM), ascorbic acid (10 mM), and 0.5 ml plant extract (25−100 µg/ml) in a final volume of 1 ml. The reaction mixture was incubated at 37 °C for 1 hour. Lipid peroxidation was measured as malondialdehyde (MDA) equivalent using trichloroacetic acid (TCA), thiobarbituric acid (TBA) and HCl (TBA-TCA reagent: 0.375 % $^{w}/_{v}$ TBA; 15 % $^{w}/_{v}$ TCA and 0.25 N HCl). The incubated reaction mixture was mixed with 2 mL of TBA-TCA reagent and heated in a boiling water bath for fifteen minutes. After cooling, the flocculent precipitate was removed by centrifugation at 10,000 g for 5 minutes. Finally, malondialdehyde concentration in the supernatant fraction was determined spectrophotometrically at 535 nm. Using ascorbic acid as standard, the concentrations of extract that would cause 50% inhibition of the production of thiobarbituric acid reactive substances (IC_{50} values) were calculated.

Nitric oxide scavenging activity assay

A four milliliter sample of plant extract or standard solution of different concentrations (25, 50, 75, 100 µg/mL) were taken in different test tubes and 1 ml of sodium nitroprusside (5 mM in phosphate buffered saline) solution was added into the test tubes. They were incubated for 2 hours at 30 °C to complete the reaction. Two milliliter sample was withdrawn from the mixture and mixed with 1.2 mL of Griess reagent (1% sulphanilamide, 0.1% naphthylethylene diamine dihydrochloride in 2% H_3PO_4). The absorbance of the chromophore formed during diazotization of nitrite with sulphanilamide and its subsequent coupling with napthylethylene diamine was measured at 550 nm [27]. Ascorbic acid was used as standard andthe percentage (%) inhibition was calculated from the following equation: [(A0 – A1)/A0] x 100. Where, A0 is the absorbance of the control and A1 is the absorbance of the extract or standard.

Phytochemical screening

The presence of various secondary metabolites in the extract of *Daucuscarota sativus* was evaluated using simple chemical tests [28].

Trace elements determination

The extract was prepared using standard procedure meant for Atomic Absorption Spectroscopy (AAS) [29, 30].

Statistical analysis

Data were expressed as mean ± SEM and analyzed using one way ANOVA followed by Tukey's multiple comparison test or by two-way ANOVA followed by Bonferroni posttest using Graph Pad Prism 5 (Graph Pad Software Inc., CA, USA). Results were considered significant when $p<0.05$, $p<0.01$ and $p<0.001$.

RESULTS

Acute toxicity

None of the mice treated with 5 g/kg oral dose ofDCS died and neither were any visible side effects recorded within 14 days of observation. However, the animal treated via intra-peritoneal route, showed tachypnea, catalepsy, sedation and convulsion before death. The LD_{50} was determined as 126 mg/kg i.p.

Pyloric ligation induced gastric ulcer

Treatment with DCSshowed a significant ($p<0.01$-0.001) dose independent antiulcer effect compared to control. Peak effect was observed at 50 mg/kg (56.25 %) (Fig. 1). DCS treated group demonstrated a significant ($p<0.01$) reduction in both the total acidity as well as in the volume of gastric acid secreted. Howbeit, there was no significant ($p>0.05$) alteration in pH by both the herbal and the standard drugs (Table 1).

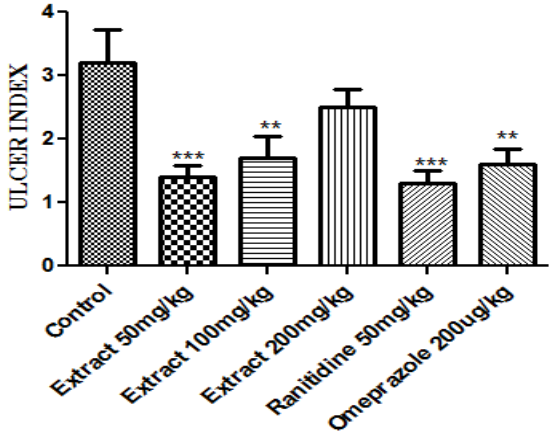


Figure 1: Bar chart showing pylorus ligation ulcer model results

Values are mean ± S.E.M (n=5). **p<0.01, ***p<0.001 when compared to control. (One way ANOVA followed by Dunnett's multiple comparison tests)

Table 1: Effect of DCS on gastric content volume, pH and titratable acidity *in the pylorus ligation model*

Treatment (mg/kg)	Dose Volume (mL)	p^H	Titratable acidity	(mEq/L)
Daucuscarota sativus	50	1.40±0.18^{c}	5.74±0.38^{b}	3.27±0.69^{b}
	100	1.66±0.05$^{c\beta}$	6.46±0.42^{c}	3.73±1.13^{b}
	200	2.00±0.16$^{c\gamma}$	5.58±0.73^{b}	7.29±0.58$^{\gamma}$
Ranitidine	50	1.02±0.06^{c}	6.68±0.30^{c}	2.08±0.45^{c}
Omeprazole	200 µg/kg	1.50±0.09^{c}	5.48±0.19^{b}	5.13±0.92
Distilled H_2O	10ml/kg	4.10±0.18	3.90±0.47	7.88±0.54

Values are represented as mean ± S.E.M (n=5).^{b}p<0.01, ^{c}p<0.001 when compared to control; βp<0.01, γp<0.01 when compared to standard (Ranitidine) (One way ANOVA followed by Dunnett's and Turkey's multiple comparison test).

Acetic acid-induced ulcer

The extract DCS produced a significant (p<0.05-0.01) dose independent reduction in ulcer index compared to control; maximum inhibitory effect was observed at 50 mg/kg (67.30 %). Ranitidine on the other hand offered a superior protection (80% inhibition), which was similarly significant (Figure 2).

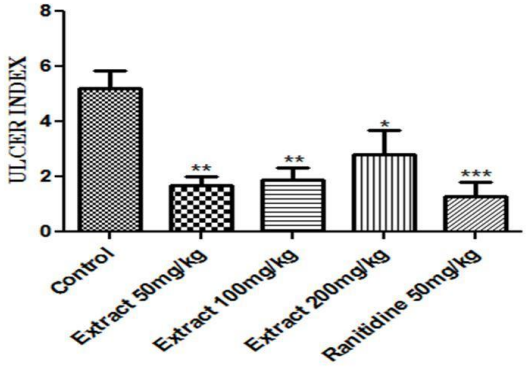


Figure 2: Bar chart showing Acetic acid induced gastric ulcer

Values are mean ± S.E.M (n=5) *p<0.05, **p<0.01, ***p<0.001 when compared to control. (One way ANOVA followed by Dunnet's multiple comparison tests).

Cysteamine-induced duodenal ulcer

The effect of orally administered DCS on duodenal ulcer induced by cysteamine is shown in figure 3. The effect produced by DCS was significant (p<0.05-0.01) but dose independent, when compared to control.

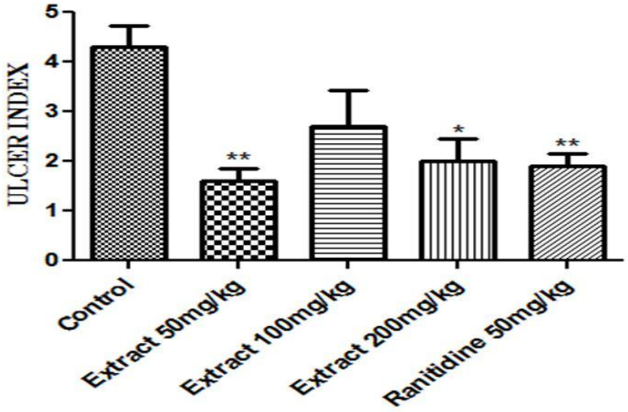


Figure 3: Bar chart showing cysteamine induced duodenal ulcers results

Values are mean ± S.E.M (n=5) *p<0.05, **p<0.01 when compared to control. (One way ANOVA followed by Dunnett's multiple comparison tests).

Indomethacin-histamine induced duodenal ulcer

Treatment with DCS (50, 100 mg/kg) and cimetidine (50 mg/kg) significantly (p<0.05-0.01) reduced ulcer index compared to control (Figure 4). The effect produced at 200 mg/kg was not statistically significant. Similar to cysteamine model, maximum protection was recorded at 50 mg/kg.

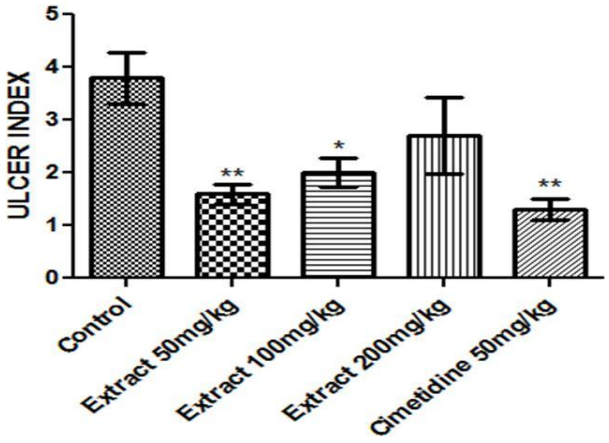


Figure 4: Bar chart showing Indomethacin-Histamine induced duodenal ulcer model results.

Values are mean ± S.E.M (n=5) p<0.05, p<0.01 when compared to control. (One way ANOVA followed by Dunnett's multiple comparison tests).

Methylene blue-induced gastric ulcer

Oral administration of aqueous leaf extract of DCS produced a significant (p<0.05-0.01) protection of methylene blue-induced gastric ulcer in a dose independent manner. The maximum effect produced at 50 mg/kg compared effectively with the standard (omeprazole 200 µg/mg) (Figure 5).

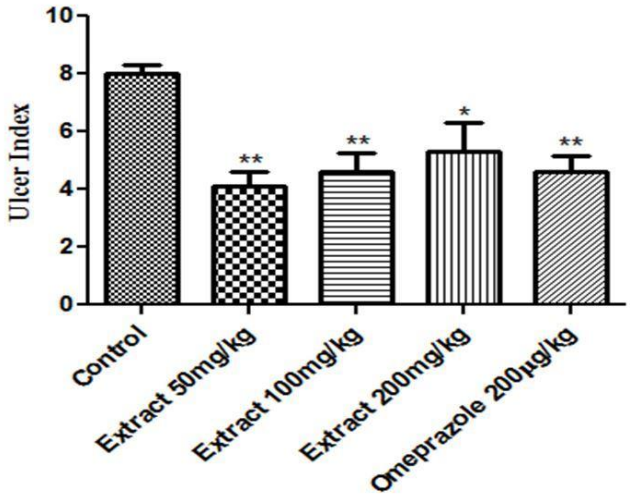


Figure 5: Bar chart showing Methylene-blue induced gastric ulcer model results.

Values are mean ± S.E.M (n=5) p<0.05, p<0.01 when compared to control. (One way ANOVA followed by Dunnett's multiple comparison tests).

Mineral content

Using Atomic Absorption Spectroscopy (AAS) various elements were found to be present in DCS in different amounts (Table 2).

Table 2: Mineral content

Mineral content	Quantity (mg/ 100g)
Calcium	150.56
Sodium	110.54
Potassium	110.49
Magnesium	76.63
Zinc	49.99
Iron	174.76
Copper	14.69
cobalt	0.51

Phytochemical analysis

Preliminary analysis of the chemical composition of DCS extracts revealed the presence of alkaloid, steroid, flavonoids, cardiac glycosides, tannin phlobatannin, terpenoids phenol, reducing sugar and saponins (Table 3).

Table 3: Quantitative phytoconstituents

Phytoconstituents	Quantity (mg/ 100g)
Tannin	27.14 ± 0.36
Saponin	41.28 ± 0.06
Cardiac glycosides	11.38 ± 0.09
Alkaloid	7.98 ± 0.06
Reducing sugar	5.79 ± 0.06

Values are expressed as mean ± S.E.M

Antioxidant capacity

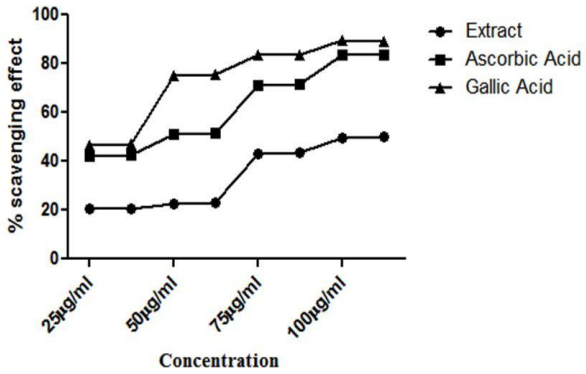


Figure 8: 1,1-diphenyl-2-picrylhydrazyl (DPPH) Radical Scavenging Activity

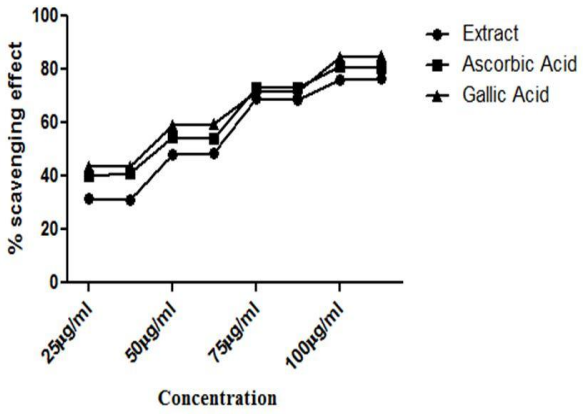


Figure 9: Lipid Peroxidation Assay

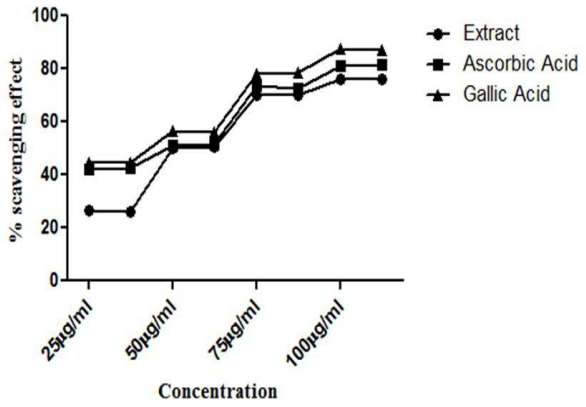
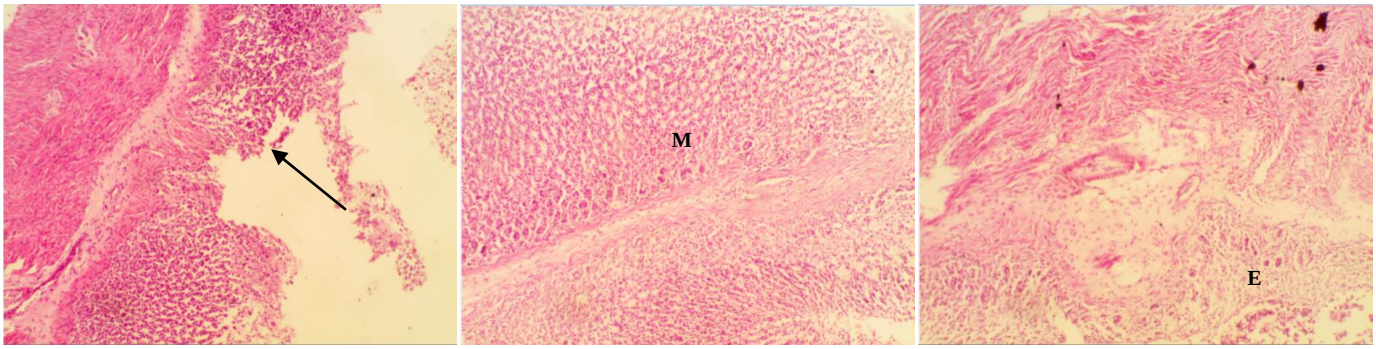


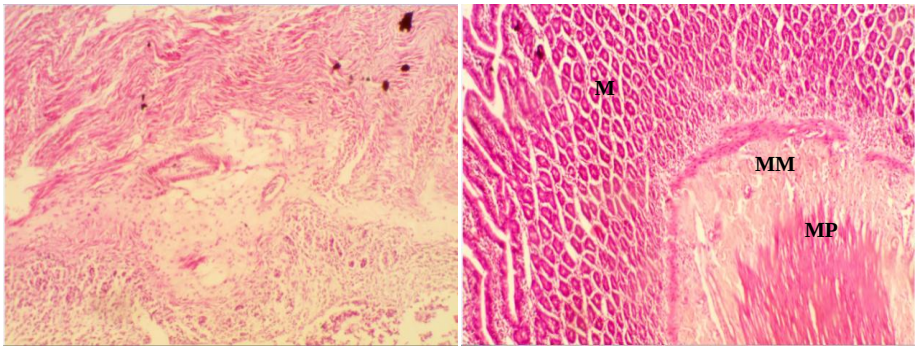
Figure 10: Nitric Oxide Scavenging Activity Assay



A) Distilled water 10 ml/kg - arrow shows ulceration

B) Extract 50 mg/kg - Well regenerated epithelium of the mucosa

C) Extract 100 mg/kg - few distortions in the mucosa with submucosa edema

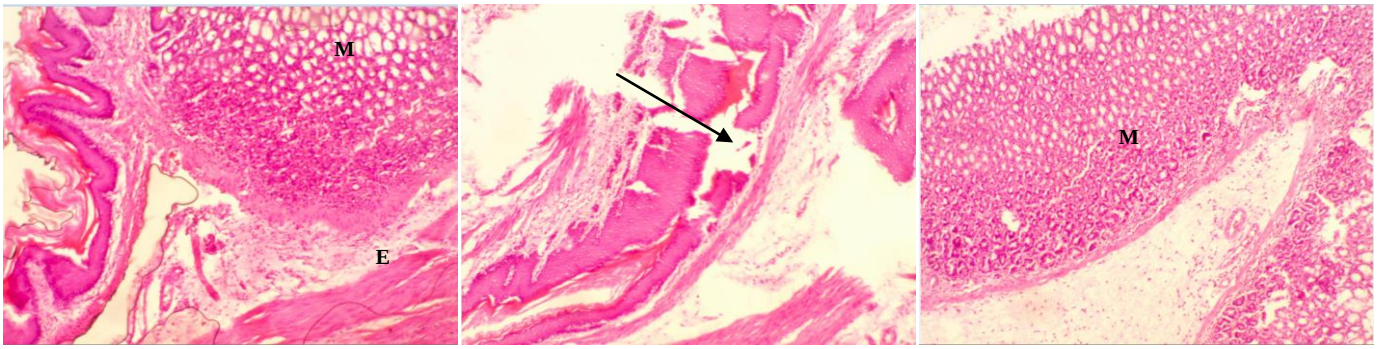


D) Extract 200 mg/kg - Well regenerated epithelium of the mucosa

E) Ranitidine 50 mg/kg - Well regenerated epithelium of the mucosa M with obvious distinct glands

M = Mucosa. MM = Muscularis mucosae. MP = Muscularis propriae, Arrow points to the area of ulceration. E = submucosa edema.

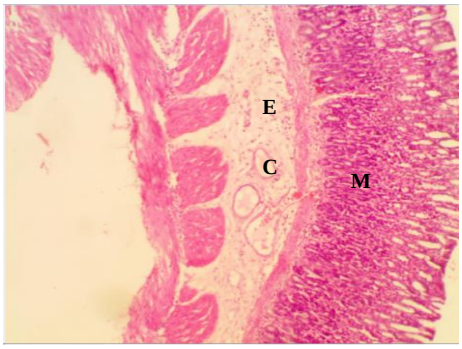
Figure 6: Histopathology of cysteamine-induced duodenal ulcer



A) Extract 100 mg/kg - No ulceration

B) Extract 200 mg/kg - Arrow points to ulcerated area

C) Ranitidine 50 mg/kg - Well regenerated mucosa



D) Omeprazole 200µg/kg - Well regenerated mucosa M, submucosa edema E and congestion C

M = Mucosa. MM = Muscularis mucosae. MP = Muscularis propriae, Arrow points to the area of ulceration. E = submucosa edema.

Figure 7: Histopathology of acetic-induced gastric ulceration

DISCUSSION

Peptic ulcer is one of the major gastrointestinal disorders affecting humans and it is consequent to an imbalance between aggressive (acid, pepsin, *H. pylori*, bile salts) and defensive factors (mucous, bicarbonate, mucosa blood flow, epithelial cell restoration and prostaglandins) [20, 31].To regain the balance, different therapeutic agents including plant extract have been employed to inhibit excess gastric acid secretion or to boost the mucosal defense mechanism by increasing mucus production, stabilizing the surface epithelial cells or interfering with the PGs synthesis [32, 33]. The present study investigated the effect of DCS leaf extract on both gastric and duodenal ulcers.

Pylorus ligation-induced ulcer model is an important procedure for the measurement of mean ulcer index in ulcerogenesis, since it shows the possible changes in different parameters describing gastric content; among which are volume of gastric juice, total acidity and pH[34]. Ulcerations caused by pyloric ligation may be due to stress-induced secretion of gastric acid from parietal cells and pepsin, both of which accumulate, leading to the autodigesion of gastric mucosa [35, 36]. Free radicals may also be associated, since studies have shown changes in the antioxidant status following pylorus ligation-induced ulceration in rats [37]. Inhibition of gastric acidity is one of the important protective factors, since overwhelming of the mucosal defense mechanisms by acid level leads to ulcer formation [38]. The dose-independent but significant reduction in ulcer index recorded by DCS, showed it to be antisecretory in action; its activity also compared effectively with the standard antisecretory drugs, ranitidine, cimetidine and omeprazole used in the various models. Parietal cells bear receptors for three secretagogues namely acetylcholine (muscarinic type receptor), gastrin and histamine (H_2 type receptor), which reflects a triumverate of neural, paracrine and endocrine control. A pilot*in vitro* study conducted to explore the possible relationship between DCS and atropine, showed it to be antagonistic; similar to atropine-acetylcholine interaction. Furthermore, the antisecretory activity of DCS could also be mediated through histamine, the primary modulator of gastric acid secretion [39] or gastrin.

Acetic acid (1 ml, 4%) is considered one of the inducers of gastric ulcers, since it generates free radicals and consequently causes lipid peroxidation [40]. Aqueous leaf extract of DCS was effective in protecting against acetic acid induced-chronic ulcer, probably through its free radical scavenging property, since the study showed it has a relatively high antioxidant capacity. Antioxidants have been reported to play a major role in repairing gastric damage [41].

Cysteamine is a low molecular weight amino thiol and natural product of coenzyme A [42]. It has been reported that cysteamine leads to progression of duodenal ulcer by elevating the levels of gastrin, thereby, increasing the rate of gastric acid secretion and alsoinhibits the alkaline mucus secretion from Brunner's glands in the proximal duodenum [43]. The antiulcer activity recorded by DCS, and which compared effectively with the standard drugs employed, could also be attributed to its ability to enhance alkaline mucus and bicarbonate secretion, as well as by stimulating epidermal growth factor.

Non-steroidal anti-inflammatory drugs such as indomethacin, induce gastric lesions due to the reduction of endogenous prostaglandin synthesis, which is known to be cytoprotective in the gastric mucosa [20]. Combination of indomethacin and histamine as used in this study produced lesions in the proximal duodenum, as acid secretion from the parietal cells is strongly stimulated by histamine, whose action is exerted through the H_2 receptor subtype [44]. Therefore this model investigated both the cytoprotective and antisecretory properties of DCS, which recorded 57.89 % inhibition, and compared well with the effect produced by cimetidine, the positive control.

Methylene blue is a synthetic drug known to uncouple ATPases; when administered to animals, it decreases blood supply to gastric mucosa, which results in oxidative stress and generation of superoxide radical ions, with subsequent erosion and ulceration of gastric mucosa [23]. In this model, DCS offered 48.75% protection against ulceration, the activity which was significant (p<0.05-0.01) and better than omeprazole.

Preliminary analysis of the chemical composition showed that DCS containsalkaloid, steroid, flavonoids, cardiac glycosides, tannins, phlobatannins, terpenoids phenol, reducing sugar and saponins,Flavonoids are thought to increase mucosal prostaglandins content, decrease histamine secretion from mast cells by inhibition of histidine carboxylase, inhibit *H. pylori* growth, act as free radical scavengers and inhibit H^+/K^+-ATPase [33, 45]. Saponins may activate mucous membrane protective factors, while tannins render the outermost layer of the mucosa less permeable to chemical irritation [46]. Furthermore, terpenoids and alkaloids have also been reported to elicit potent activity against gastric ulcers [47].One or combination of these phytoconstituents may also contribute to gastro-duodenal protective effect of DCS.

The extract of DCS was found to be relatively safe; up to 5 g/kg oral dose administered recorded neither mortality nor a sign of acute or delayed toxicity over the period of 14 days observation. The LD_{50} via intraperitoneal (i.p) route was estimated to be 126 mg/kg, which suggested that the herbal drug is liable to first-pass effect.

Histopathological presentations in this study gave credence to the protective and healing effects of DCS on gastric and duodenal ulcers.

DCS extract was also found to be rich in sodium, magnesium, zinc, and iron among other elements (Table 2). Zinc is physiologically a very important metal and acts as a cofactor for many enzymes;moreover, it has been reported to be useful in promoting healing of wounds and skin lesions [48].

CONCLUSION

Findings from this study showed that the aqueous leaf extract of *Daucuscarota sativus* protects against gastric and duodenal ulcers, probably through its antioxidant, antisecretory and cytoprotective properties. It was also found to be relatively safe orally and it compared effectively with some of the currently employed antiulcer drugs. It could therefore serve as a lead for newer antiulcer therapies in the nearest future.

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