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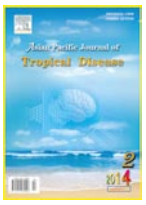

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Anti-ulcer activity of African walnut *Tetracarpidium conophorum* nuts against gastric ulcers in rats

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ABSTRACT

Objective: To determine the anti-ulcer activity of methanol extract of *Tetracarpidium conophorum* (Mull. Arg.) (METC) nuts in albino Wistar rats.

Methods: METC was investigated in pylorus ligation and ethanol induced models in experimental animals. Parameters such as gastric volume, pH, total and free acidity, and ulcer index were used as indicator for antiulcerogenic activity in both models. METC at doses of 250 and 500 mg/kg orally was used to determine whether the extract could produce significant protection of the gastric lesions by pylorus ligation and ethanol.

Results: The extract at dose levels of 250 and 500 mg/kg exhibited significant ($P<0.05$) decrease in the gastric volume, total and free acidity while the pH of gastric juice was significantly ($P<0.05$) increased in both models.

Conclusions: The result showed that METC possesses anti-ulcer as well as cytoprotective properties which could be attributed to the presence of secondary metabolites.

1. Introduction

Peptic ulcer disease is one of the disease conditions that affect many people around the world especially in the developing world[1]. Under peptic ulcer condition, there is a discontinuity in entire thickness of the gastric and duodenal mucosa and persist as a result of acid and pepsin in the gastric juice[2]. People suffer from peptic ulcer present with intermittent epigastric pain. Gastric ulcer produces a 'food pain' occurring within half an hour of a meal and the patient develops anorexia and is apt to lose weight, whereas, duodenal ulcers produce a 'hunger pain' which occurs 2-3 h after meal and the patient is apt to over eat and put on weight[3]. Gastric ulcer occurs as a result of an imbalance between the aggressive factors (acids, pepsin, bile salts, and *Helicobacter pylori*) and the defensive factors (mucus-bicarbonate secretion and prostaglandin)[4]. A lot of drugs are available for the treatment of peptic ulcer disease. These include prostaglandins, proton pump inhibitors, histamine receptor antagonists and mucoprotectives[5]. Adverse drug reactions from these drugs demands for the use of alternative or herbal medicines[6]. The use of herbal medicines has been reported in the treatment of gastric ulcer disease (GUD) condition[7]. *Tetracarpidium conophorum* (*T. conophorum*) (African walnut) nuts have a bitter taste and are used in Nigeria ethnomedicine for the treatment of stomachache[8]. Phytochemical screening of plants have revealed that many phytocompounds like terpenoids, tannins and flavonoids possess anti-ulcer, fibrinolytic, anti-diarrhoea and anti-neoplastic activities[9,10]. It is neccessary to evaluate the potential of herbal medicine and therapy to prevent drug-herbal medicine interaction during patient management[11]. The aim of the present study is to determine the anti-ulcer activity of the methanol extract of *T. conophorum* (METC) in pylorus ligation and ethanol induced models.

2. Materials and methods

2.1. Plant collection and extraction procedure

The nuts of *T. conophorum* (African walnut) were purchased in June of 2013 from Choba market, Port

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Harcourt. Voucher specimen was archived in the herbarium of Department of Pharmacognosy at University of Port Harcourt, register number TCAW 5647. The nuts were deshelled and air-dried at room temperature, and milled into powder, resulting in a mass of 800.43 g of the plant material. The powdered material (500 g) was defatted with 800 mL of *n*-hexane and the resulting marc was subjected to Soxhlet extraction with 1.5 L of 98% methanol. The extract was evaporated under reduced pressure at low temperature (40 °C) to remove the methanol.

2.2. Phytochemical analysis

The phytochemical screening of the METC was carried out using standard phytochemical procedures and tests[12].

2.3. Animals

Wistar rats of both sexes (200-250 g), obtained from the animal house of University of Port Harcourt, were housed in groups of 5 to 6 in a 12 h light/dark cycle at room temperature and were fasted for 24 h with free access to water before the experiment. All the animals were used according to the guidelines of the Committee on Care and Use of Experimental Animal and Environmental Ethics from University of Port Harcourt, Nigeria (UPAEE/2014).

2.4. Chemicals

Methanol and *n*-hexane were purchased from Sigma-Aldrich (St. Louis, MO, USA). Ranitidine was acquired from Juhel Pharmaceuticals Company Ltd (Awka, Nigeria).

2.5. Pyloric ligation induced gastric ulceration

The animals were fasted for 24 h and randomly divided into 4 groups of six animals each. METC and ranitidine were prepared in 10% dimethyl sulphoxide as aqueous suspension and administered orally to the rats. After 1 h of vehicle administration, the animals received distilled water 10 mL/100 g (control) in Group I. Group II animals received ranitidine (50 mg/kg) and were served as positive control. Groups III and IV animals received 250 and 500 mg/kg body weight of METC, respectively. The METC doses chosen were safe from a previous study of the acute toxicity[13]. After 1 h of treatment with METC or ranitidine, the rats were anaesthetized using chloroform. Their abdomen were opened by a small midline incision below the xiphoid process, according to the method described by Sen *et al.*[14], avoiding traction to the pylorus. The stomach was closed up carefully by interrupted sutures. The animals were allowed to recovery and stabilization and were deprived of water during postoperative period. The animals were then sacrificed by an over dose of chloroform inhalation after 4 h of pyloric ligation to observe gastric lesions.

2.5.1. Gastric acid and pH measurements

The abdomen of each animal was carefully opened, cardiac end of the stomach was dissected out and gastric contents were collected, measured and centrifuged at 2 500 r/min for 10 min. The volume of the supernatant was determined. The pH of the supernatant was measured using a pH meter[15,16]. Total and free acidity of the gastric juice was determined by titrating 1 mL of the gastric juice in 10 mL of distilled water with 0.01 mol/L NaOH using phenolphthalein and dimethylamino-azobenzene (Töpfer's reagent) as indicators respectively and were expressed as mEq/L of the gastric juice[17-19], using the formula:

$$\text{Acidity (mEq/L)} = \frac{\text{Volume of NaOH} \times \text{normality} \times 100}{0.10}$$

2.5.2. Macroscopical evaluation of stomach

The stomachs of the rats were opened along the greater curvature and rinsed with 0.9% saline to remove gastric contents and blood clots and then were examined for lesions in the glandular part under 10 times magnified lens to access the formation of ulcer. The number of ulcer was estimated using the ulcer index as previously reported[20,21], using the following scale. Normal coloured stomach=0; Red colouration=0.5; Spot ulcer=1.0; Hemorrhagic streak=1.5; Deep ulcer=2.0; Perforation=3.0.

The mean score for each animal was expressed as ulcer index. Percentage inhibition of ulceration was used estimated using the formula:

$$\text{Protection of ulceration (\%)} = \frac{\text{Ulcer index}_{\text{control}} - \text{Ulcer index}_{\text{test}}}{\text{Ulcer index}_{\text{control}}} \times 100$$

Estimation of ulcer index (U_I) was estimated using the following formula[22]:

$U_I = U_N + U_S + U_P \times 10^{-1}$

Where U_N is the average number of ulcers per animal; U_S is the average number of severity score; U_P refers to the percentage of animal with ulcers.

2.6. Ethanol-induced gastric ulcer

Ulcer was induced in the rats by oral administration of ethanol. The animals were fasted for 36 h before administration of ethanol. The animals were randomly divided into 4 groups with six animals each. After 1 h of ethanol administration, the Group I animals received 0.5 mL/100 g of ethanol (control). Group II animals received ranitidine 50 mg/kg and were served as positive control. Groups III and IV animals received 250 and 500 mg/kg body weight of METC similar to ethanol induced model as previously reported[21,23]. The gastric ulcers were induced in rats by administering 0.5 mL/100 g of 90% ethanol orally after 1 h of treatment with METC and ranitidine. The animals were anaesthetized 1 h later with chloroform inhalation and the stomach was incised and ulceration scored. Scoring was done as described in the pyloric ligation induced ulcer model[20,21].

2.7. Statistical analysis

The results are expressed mean±SEM of at least triplicate determinations (n=3). Statistical comparisons were performed

by One-way analysis of variance using GraphPad Prism 5 software followed by Dunnett's *post hoc* least significant difference (LSD) test. Comparing with control group, $P<0.05$ was considered to be significantly different.

3. Results

3.1. Preliminary phytochemical screening

The qualitative phytochemical result of the METC showed the presence of saponin, alkaloid, tannin and steroid in trace amount while flavonoids and terpenoids were abundantly present as previously reported[13].

3.2. Pyloric ligation induced gastric ulceration

The effects of METC on pyloric ligation induced ulceration are shown in Table 1. METC at the tested doses produced a reduction in the ulcer index, gastric volume, total and free acidity but increased the values of pH significantly ($P<0.05$) in comparison to the control group. The percentage about inhibition of ulceration was found to be 73% and 56% at the doses of 250 and 500 mg/kg, respectively in comparison to control whereas ranitidine as a standard drug showed inhibition of ulcer 81%.

3.3. Gastric ulceration test

The effects of METC on gastric lesion induced by ethanol (0.5 mL/100 g body weight) are shown in Table 2. Pretreatment with ethanol showed presence of superficial and deep ulcers and in some cases perforations in the control animal group (6.75±0.61). METC showed a reduction in the ulcer index at all the tested doses: (3.17±1.39) at 250 mg/kg (53% ulcer inhibition), (1.75±0.92) at 500 mg/kg (74% ulcer inhibition), compared to the control group. The animals that received ranitidine (50 mg/kg) showed significant ($P<0.05$) decrease in gastric lesion with (0.92±0.89) ulcer index and 86% ulcer inhibition compared to control. Treatment with METC significantly ($P<0.05$) reduced the volume of gastric content to (4.40±0.01) and (3.60±0.11) at the dose of 250 mg/kg and 500 mg/kg, respectively. The pH was significantly ($P<0.05$) increased to (5.14±0.05) and (5.51±0.15) at the tested dose levels.

4. Discussion

Gastric ulcer condition occurs when the aggressive factors outweigh the endogenous defence mechanisms. Various anti-ulcer drugs are used to restore this imbalance by their action on proton pump, *Helicobacter pylori* or its antacid properties[24]. Pylorus induced ulceration is due to the stress induced on hydrochloric acid producing mechanism in the body system, resulting in ulceration of the gastric mucosa because of accumulation of gastric acid in the stomach[25,26]. Ethanol induced gastric ulcer was employed to study the cytoprotective effect of METC. The mechanism of ethanol induced ulceration, results from release of superoxide anions and hydroperoxy free radicals which lead to an increased lipid peroxidation from ethanol metabolism. Increases in lipid peroxide content and oxygen-derived free radicals result in marked changes in cellular levels and cause membrane damage, cell death, exfoliation and epithelial erosion[27]. METC showed a marked reduction in the ulcer parameters studied on the test animal in a dose dependent manner with a significant increase in both the pH and percentage ulcer inhibition. Phytochemical analysis of the METC showed that it contains trace amount of saponins, alkaloids, and steroids while flavonoids and terpenoids were found abundantly. It should be stated that the ability of METC to reduce acidity might be due to the presence of some phytocompounds such as tannins, terpenoids and flavonoids. Flavonoids are chemical constituents that have been indicated for the cytoprotective properties as well as

Table 1

Effect of METC on various parameters in pyloric ligation induced gastric ulcer.

Groups	Treatments	Dose (mg/kg)	Ulcer index	Protection (%)	Gastric juice		Gastric acidity (mEq/L)	
					pH	Volume (mL)	Free	Total
I	Control (distilled water)	10 mL/100 g	6.25±0.61	–	3.50±0.22	12.00±0.25	58.75±0.18	102.50±0.25
II	Ranitidine	50	1.17±0.86**	81	4.01±0.15	8.20±0.15*	30.04±0.02*	57.40±0.15*
III	METC	250	2.75±1.31	73	5.23±0.21*	7.60±0.25*	25.77±0.10*	52.30±0.15*
IV	METC	500	1.67±1.39*	56	5.80±0.12*	7.20±0.15*	14.04±0.25*	25.00±0.13*

Values are expressed as mean±SEM (n=6). *: $P<0.05$; **: $P<0.01$ (shown significant difference and ANOVA followed by Dunnett's *post hoc* LSD test compared with control group.

Table 2

Effect of METC on various parameters in ethanol induced gastric ulcer.

Groups	Treatments	Dose (mg/kg)	Ulcer index	Protection (%)	Gastric juice		Gastric acidity (mEq/L)	
					pH	Volume (mL)	Free	Total
I	Control (ethanol)	0.5 mL/100 g	6.75±0.61	–	3.72±0.10	8.00±0.15	30.30±0.11	57.20±0.12
II	Ranitidine	50	0.92±0.89**	86	6.02±0.11*	2.40±0.15**	7.40±0.10**	12.60±0.15*
III	METC	250	3.17±1.39	53	5.14±0.05*	4.40±0.01*	16.10±0.12*	30.70±0.10*
IV	METC	500	1.75±0.92*	74	5.51±0.15*	3.60±0.11*	13.10±0.10*	25.80±0.15*

Values are expressed as mean±SEM (n=6). *: $P<0.05$; **: $P<0.01$ (shown significant difference and ANOVA followed by Dunnett's *post hoc* LSD test compared with control group.

wound healing activity.

In conclusion, METC has anti-ulcer activity. This activity could be attributed to the cytoprotective property of the nut extract and inhibition of the gastric acid. Further studies are needed to elucidate the mechanism of action of gastric protection by the extract of *T. conophorum*.

Conflict of interest statement

We declare that we have no conflict of interest.

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