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Anti-Ulcerogenic effect of methanol extract of *theobroma cacao* on indomethacin and aspirin-induced ulcer in rats

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Abstract
Methanol extract of *Theobroma cacao* was tested for inhibitory activity against Indomethacin and Aspirin induced ulcers. In each ulcer model, twenty male albino rats divided into five groups of four rats each were used for the study. Three out of the five groups of rats received the extract at doses of 100, 200 and 400mg/kg while the other two groups which received 5ml/kg of the extract vehicle (3% Tween 80) and 100mg/kg of ranitidine served as the normal and standard control respectively. The extract doses, Tween 80 and ranitidine were administered to the various rat groups orally. Ulcer was induced in the rats using Indomethacin (50mg/kg) and Aspirin (50mg/kg) 30 minutes after extract administration. The rats were kept under protection for a further 8 hr before being sacrificed by chloroform anesthesia. Their stomachs were isolated, cut along the greater curvature and displayed for ulcer rating on a PVC board. From values of the ulcer index obtained from the ulcer models, the rats pre-treated with the extract showed significant decrease ($p<0.05$) in ulceration when compared to rats of the normal control (pre-treated with 3% Tween 80) and those of the standard control (pre-treated with ranitidine).

Keywords: *Theobroma cacao*, rats, ulcer inhibition, Indomethacin, Aspirin

1. Introduction
Peptic ulcer disease (PUD), also known as peptic ulcer or stomach ulcer, is a break in the lining of the stomach, first part of the small intestine, or occasionally the lower oesophagus [1]. In healthy individuals, the digestive tract is coated with a mucous membrane that protects the underlying tissues against the highly corrosive digestive acid; however, if the amount of the acid is dramatically increased, or the pH of the acid is significantly reduced, or the mucus membrane layer becomes too thin or dry, the acid damages the tissue and ulceration ensues.

Gastric ulcers are a type of peptic ulcer that affects the stomach lining due to an imbalance between gastric acid and the gastric mucosa. For many decades, peptic ulcer disease was thought to be primarily a result of emotional stress and diets rich in spicy foods. However, in the mid-1980s, *Helicobacter pylori* bacteria was discovered in the stomach of many patients with gastritis and peptic ulceration and recognized to be a significant causative agent [2]. Another major cause of gastric ulceration is the regular use of non-steroidal anit-inflammatory drugs (NSAIDs), especially those that are classified as COX-1 inhibitors [3]. The growth and maintenance of stomach mucus is stimulated by certain prostaglandins, which are blocked by most NSAIDs. Aspirin and indomethacin are some of these NSAIDs.

Cocoa (*Theobroma cacao*) belongs to the genus *Theobroma*, a group of small trees which grow in the Amazon basin and other tropical areas of South and Central Africa. They are classified under the subfamily Sterculioidea of the mallow family Malvaceae. The medicinal value of cocoa plants have assumed a more important dimension in the past few decades owing largely to the discovery that, extracts from cocoa plants contain not only minerals and primary metabolites but also a diverse array of secondary metabolites with antioxidant potentials [4]. In experimental trials, cocoa phenolics have presented several beneficial effects against platelet aggregation [5] high blood pressure [6] atherosclerosis [7] hyperglycemia [8] hypercholesterolemia [9] inflammation [10] hepatocarcinogenesis [11]. DNA damage and clastogenic effect [12]. Since *Theobroma cacao* has been known to contain polyphenols which has antioxidant and anti-inflammatory properties, the polyphenols could also attenuate the irritation and inflammation in the gastric mucosa caused by ulcer genesis. This study was aimed at assessing the anti-ulcer genic properties of *Theobroma cacao* using Indomethacin and Aspirin-induced ulcer models in albino rats.

2. Materials and Methods
2.1 Plant material
Cocoa seeds from mature plants of *Theobroma cacao* were obtained from a local farm in Cross River State of Nigeria. They were dried for 10 days and were ground into a powdered form. The powdered seed (1800g) was extracted using 5 litres of methanol in the ration of 1:2 (w/v) in a conical flask. The extract obtained was dried in a rotavapour and the dried mass was weighed and recorded.

2.2 Animals
The animals used in the study were male wistar rats weighing between 100 to 140g. They were obtained from the animal house, Department of Zoology, University of Nigeria, Nsukka. The animals were acclimatized to laboratory environment for 7 days. They were fasted for 18 hr before experiment but were allowed access to drinking water. During the fasting period, the rats were placed individually in separate cages with wide-mesh wire bottoms. On the day of the experiment, the rats in each ulcer model were divided into 5 groups of 4 rats each. They received human care throughout

the experimental period in accordance with the ethical rules and recommendations of the University of Nigeria committee on the care and use of laboratory animals and the revised National Institute of Health Guide for Care and Use of Laboratory Animal (Pub No.85-23, revised 1985).

2.3 Chemicals

Methanol, Sodium chloride (NaCl), Normal saline, distilled water, Chloroform, Formaldehyde and Tween 80 were obtained from the Biochemistry laboratory of the University of Nigeria, Nsukka. They were all of analytical grade. The standard drugs used were indomethacin and aspirin.

2.4 Indomethacin-induced gastric ulcer

This determination was carried out using the method of Urushidam *et al.* [13]. Twenty adult rats randomly divided into 5 groups of 4 rats each were deprived of food for 18 hours and treated orally with 3% Tween 80 and varying doses of the plant extracts. The extracts and drugs used were freshly prepared as a suspension in 3% Tween 80 and administered orally (p.o) to the animals in 5mg/kg doses. Group 1 (normal control) was administered 3% Tween 80 (5ml/kg). Groups II, III and IV were treated with 100, 200 and 400mg/kg of the plant extracts respectively. Group V (reference group) was administered 100mg/kg of ranitidine (zantac), a standard anti-ulcer drug. Thirty minutes later, 50mg/kg of indomethacin was administered (p.o) to the rats. After 8 hours, each animal in the groups was sacrificed by chloroform anesthesia and the stomach removed and opened along the greater curvature, rinsed with copious volume of normal saline and pinned on a flat board. Erosions formed on the glandular portions of the stomach were counted and the ulcer index calculated as described by Main and Whittle [14].

2.5 Aspirin-induced gastric ulcer

Twenty adult rats randomly divided into 5 groups of 4 rats each were also used. The same procedures as in ulcer induction by indomethacin induction were also employed in this model.

2.6 Gross lesion evaluation

Ulcers found in the gastric mucosa, appeared as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach. Each specimen of gastric mucosa was thus examined for damage. The ulcer was usually counted and scored as 0=no ulcer, 1=superficial ulcer, 2=deep ulcer, 3=perforations. The sum of all the lesions (ulcer in all the animals for each group-total ulcer score) was used to calculate the ulcer index. The percent ulcer inhibition was calculated relative to control as follows:

% ulcer inhibition (% U.I) = [1-Ut/Uc] x 100

Where Ut represents the ulcer index of the treated group and Uc represents the ulcer index of the control group.

2.7 Histological lesion evaluation

Specimens of the gastric walls from each rat were fixed in 10% buffered formalin and processed in a paraffin tissue processing machine. Sections of the stomach were made at a thickness of 5 µ and stained with hematoxylin and eosin for histological evaluation.

3. Results

3.1 Indomethacin induced ulcer

The rats pre-treated with the *Theobroma cacao* extract showed a significant decrease in gastric ulcer formation compared to the control rats pre-treated with 3% Tween 80 and Ranitidine. The more the concentration of the administered extract, the more the positive result achieved. The inhibition of indomethacin-induced ulcer by the extract was observed to be dose-dependent i.e. increased with increase in extract concentration and was more than that observed in the ranitidine-treated group (Table 1).

Table 1. Effect of methanol extract of *T. cacao* on indomethacin-induced ulcer in rats.

Treatment	Dose (mg/kg)	Ulcer index	% Ulcer inhibition
Group 1 (Control)	5ml/kg of 3% Tween 80	2.05 ± 0.41	-
Group 2	100	1.13 ± 0.23	45.12
Group 3	200	0.83 ± 0.21	59.76
Group 4	400	0.73 ± 0.28	64.63
Group 5 (Reference)	100mg/kg of Ranitidine	1.33 ± 0.38	35.36

3.2 Aspirin-induced ulcer

Groups treated with *Theobroma cacao* extract showed a significant dose dependent decrease in gastric ulcer formation compared to the control rats pre-treated with 3% Tween. The ulcer inhibition obtained in these groups were comparable to that obtained in the ranitidine treated group (Table 2).

Table 2 Effect of methanol extract of *T. cacao* on aspirin-induced ulcer in rats.

Treatment	Dose (mg/kg)	Ulcer index	% Ulcer inhibition
Group 1 (Control)	5ml/kg of 3% Tween 80	2.85 ± 0.39	-
Group 2	100	1.63 ± 0.22	42.81
Group 3	200	0.95 ± 0.16	66.67
Group 4	400	0.93 ± 0.38	67.37
Group 5 (Reference)	100mg/kg of Ranitidine	1.33 ± 0.27	53.33

3.3 Gross appearance of the gastric mucosa

The stomachs were observed using a magnifying hand lens x10, and the effects of 3% Tween, Ranitidine and the extracts were noted. The group treated with 400mg/kg extract dose showed the highest ulcer-inhibitory effect in both Indomethacin and Aspirin-induced ulcer models

3.4 Histological gross lesion evaluation

In both models of Aspirin-induced and Indomethacin-induced ulcer, the control rats pre-treated with 3 % Tween 80 suffered massive depletion of the gastric mucosa, oedema and haemorrhage. Rats that received ranitidine (100mgkg) and the extract (100 and 200mg/kg) showed reduced damage to the gastric mucosa. Those pre-treated with 400mg/kg of the extract had intact gastric epithelium with no observable lesions/ulcerations.

4. Discussion

Cocoa has been considered from long time, as a food-rich in polyphenols. Flavonoids and phenolic acids are the main types of polyphenols seen in the crop. In cocoa and chocolate, the monomeric modules of the main flavonoids are flavan-3-ols, epicatechin and catechin, and polymers of these are proanthocyanidins, which are called procyanidins [15]. Dreosti, [16] reported that the rate of total polyphenols in raw cocoa has reached to be 60% in monomeric (epicatechin and catechin) and oligomeric (procynidins) forms. The chemical structure of polyphenols can determine the bioavailability in humans, with changeable rate in different individuals [17]. Cocoa and its compounds have drawn recently a lot of attention because of its contributory role as a chemo preventive agent. Over the years, researchers have studied this important crop with the aim of establishing its potential in the area of preventive and curative medicine. In several studies, the highly anti-oxidative effect of cocoa has been demonstrated, comparing with other products, a special characteristic related to its high content of procynidins [18, 19, 20], which in turn prevent the oxidation of cholesterol-LDL [21]. In experimental trails, cocoa phenolics have presented several beneficial effects against platelet aggregation [5] high blood pressure [6] atherosclerosis [7]. Hyperglycemia [8] and hypercholesterolemia [9]. inflammation [10] hepatocarcinogenesis [11]. DNA damage and clastogenic effect [12]. So many other plants have anti-ulcer potential like cocoa. Vinothapooshan and Sundar [22] reported that the anti-ulcer activity elucidated by *Mimosa pudica* could be mainly due to the modulation of defensive factors through an improvement of gastric cytoprotection and partly due to acid inhibition. The antiulcer activity of *Abutilon indicum*in rats could be attributed to the presence of flavonoids (quercetin), alkaloids, tannins, saponin glycosides and phenolic compounds. Flavonoids are among the cytoprotective materials for which antiulcerogenic efficacy has been extensively confirmed [23]. With a percentage ulcer inhibition of 48.76 at 250mg/kg, *Abutilon indicum* shows a lesser anti-ulcer potential than *Theobroma cacao* against indomethacin-induced ulcer (59.76 at 200mg/kg). Since two other plants containing polyphenols, *Syzygium aromaticum* and *Vinca minor* have shown anti-ulcer potential [24-25]. We can conclude that the anti-ulcerogenic property of *T. cacao* is as a result of its rich polyphenol content. The result of the study shows that cocoa polyphenols also have potent antiulcerogenic properties.

5. References

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