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Original Research

Preliminary study on the gastrointestinal activities of methanol extracts of *Persea americana* seed (Mill)

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The gastrointestinal activities of the methanol extract of the seed of *Persea americana* were studied in rats and compared to that of cimetidine. Ulcers were induced in rats using indomethacin and stress .The extract was found to have significant ($p<0.05$) antiulcer activity in both models studied. The extract enhanced gastrointestinal motility and contained phytochemical constituents as alkaloids, glycosides, resins, saponins, steroids, carbohydrates and tannins.

Keywords: *Persea americana*, ulcerogenic agents, gastrointestinal motility.

INTRODUCTION

Persea americana Mill (Lauraceae), originated from Central America but widely cultivated in the tropical and subtropical zones of the world (1,2). It is commonly called avocado pear and evergreen with height range of 9-18 m. It bears green fruits that mature into fresh butter-flavored greenish bulbs that encase the hard medicinal seeds.

The fruit, like most other parts of the plant, has been used for medicinal and food purposes from the very ancient times. The fruit has antimicrobial, vermifugicidal, rodenticidal and anti-dysentery activities (2). The aqueous extract of the leaf has been found to induce marked fall in mean arterial blood pressure of anaesthetized normotensive rats (1) and showed strong inhibitory effect on acyclovir mutant herpes simplex virus (3). The leaf and fresh shoot extract showed cancer reduction ability in laboratory animals (4). A compound isolated from the fruit of *P.americana* has been found to be growth deterrant, insecticide and anorexic to *Spodeptera exigua* (5).

In Nigeria some herbalists claimed to use the dried seed powder of *Persea americana* in the treatment of epigastric pain common with ulcer patients. The present study was undertaken to evaluate the activity of methanol extract of *Persia americana* seed on the gastrointestinal motility, and ulcerogenic effects of indomethacin and stress, using animal models.

MATERIALS AND METHODS

Collection of plant materials: The seeds from the matured fruits of *Persea americana* were collected from Abavo, Delta State, Nigeria in the December of 2002 and were identified by Mr. C.O.Ezugwu, of the Department of Pharmacognosy, University of Nigeria, Nsukka.

Preparation of Extract: The seeds were removed from the matured Avocado fruits, sliced and air dried for two weeks. The dried flakes were coarsely pulverized and 0.5 kg of the powder was soaked in 1 L of 99 % methanol (Fisher Chemicals, USA) for seven days. The procedure was repeated twice and the extract was evaporated under reduced pressure. The yield was 36.6 % w/w for methanol.

Phytochemical tests: The phytochemical analysis of the methanol extract was done in accordance with standard methods proposed by Evans (6).

Acute toxicity test: The acute toxicity, LD_{50}, of the methanol extract was determined in mice using oral route in accordance with Lorke method (7).

Animals: White albino mice (12-24 g body weight) and rats (65-200 g body weight) used were all bred in the Animal house of the department of Pharmacology and Toxicology, University of Nigeria, Nsukka.They were left to acclimatize in the research laboratory for 5 days before being used for the study, and had access to water and food.

Drugs: Drugs used were indomethacin (Ranbaxy, India), carbacol (Koch light, Germany), acetylcholine (Koch-light, Germany) and activated charcoal (Merck, Germany).

Experimental procedures

In vivo gastrointestinal motility study in mice: The model adopted in this study was that reported by Koezuka et al (8). The animals were randomly divided into four groups of five animals per group and were all fasted for 24 h before the commencement of the experiment.

The first group received 500 mg/kg extract, the second group received 1000 mg/kg extract, group three received 1 mg/kg carbacol and group four received 5 ml/kg 3 %Tween- 85. All the drugs used were dissolved in 3% Tween 85 before administration through ip route. Five minutes after intraperitoneal (ip) drug administration, 0.5 ml of 5%w/v charcoal in mucilage of tragacanth was administered orally to all the groups.

The animals were sacrificed 20 min after the charcoal meal and their abdomen opened. The intestinal propulsion was determined by measuring the charcoal meal movement from the pyloric sphincter to the caecum of each animal fed, and this was expressed as percentage of the total length of the small intestine. The percentage motility of charcoal meal in comparison to the entire length of the gastrointestinal tract from the pyloric sphincter to the ileocaecum was used as the index of GIT motility and calculated through *equation 1*

%GM= A/Bx100----------Equation 1

where **GM** =gastrointestinal motility

A =distance moved by charcoal meal head from the pyloric sphincter

B=mean length of gastrointestinal tract from pyloric sphincter to ileocaecum.

Anti ulcer studies:

Indomethacin-induced gastric ulcer. The method of Main and Whittle (9) was used. Twenty albino rats were randomly divided into four groups and fasted for 24 h before the commencement of the experiment. All the animals had access to water *ad libitum.*

The first group of the animals received 5 ml/kg of 3% Tween 85 to serve as the negative control; the second group received 500 mg/kg of extract, the third group received 1000 mg/kg of extract, and the fourth group received 100 mg/kg cimetidine as the positive control. Ulcers were induced using Indomethacin (30 mg/kg) 30 min after the drugs were administered. All the drugs were administered orally.

After 8 h the animals were sacrificed, their stomachs removed and opened along the greater curvature to examine the ulcer craters formed under a magnifying glass (x10). The ulcer craters formed were counted and assigned severity rating score as follows: <1 mm=1, >1 mm<2 mm=2,and >2 mm=3.

Stress-induced ulcer. The animals were randomly grouped into four with five rats per group and fasted for 24 h but had access to water *ad libitum.* The first group of the animals received 5 ml/kg of 3% Tween 85 to serve as the negative control; the second group received 500 mg/kg of extract, the third group received 1000 mg/kg of extract, and the fourth group received 100 mg/kg cimetidine as the positive control. All the drugs were administered orally and 30 min after the drugs were administered, the animals were immobilized in cylindrical cages containing cold water (8-10 ^{0}C). The animals were sacrificed after 8 h and their stomach removed and opened along the greater curvature to examine the ulcer crates formed under magnifying glass (x 10) and ulcer index calculated as above.

RESULTS

Phytochemical constituents of methanol extract:

The extract of *P. americana* seed gave positive chemical reactions for alkaloids, glycosides, saponins, resins, tannins, steroids, terpenoids, proteins and carbohydrates.

Acute toxicity test:

The acute toxicity test (LD_{50}) of methanol extract was above 5000 mg/kg. No animal showed any sign of toxicity or death after 24 h of observation even at 8.3 g/kg.

***In vivo* gastrointestinal motility study in mice:**

The different doses of the extract, 500 mg/kg and 1000 mg/kg, caused significant ($P<0.05$) increase in the percentage gastrointestinal motility when compared to 3% Tween 85 in a dose dependent pattern (Table 1).

Table1: Effect of methanol extract of *Persea americana seed* on gastrointestinal motility

Group	Treatment dose (mg/kg)	Distance Traveled (cm)	Percentage motility
Extract	500	31.75 ± 6.8	13.40*
Extract	1000	33.13 ± 5.7	18.33 * *
Carbachol	1	37.96 ± 7.4	35.57*
3% Tween 85	5ml/Kg	6.03 ± 1.7	8.72

Animals per group (n) =5; * Significant (p<0.05) vs. Tween 85 control; ** Significant (p<0.05) vs. Carbachol

Anti ulcer activity:

Indomethacin induced ulcer. Indomethacin induced ulcer (30 mg/kg) in 100% of the animals in the control group on 3% Tween 85. The ulcer index was 10.33 ± 2.4.The administration of methanol extract (500 mg/kg and 1000 mg/kg) and cimetidine significantly (P<0.05) reduced the ulcerogenic effect of indomethacin to 3.00 ± 0.57, 3.67 ± 0.88 and 2.67 ± 0.90 respectively (Table 2).

Table 2: Effect of the methanol extract of *Persea americana* on indomethacin-induced ulcers

Drug	Dose (mg/kg)	% of rats having ulcers	Ulcer index
Extract	500	29.04	3.00± 0.57*
Extract	1000	35.53	3.67 ± 0.88*
Cimetidine	100	25.84	2.67 ± 0.90
3%Tween85	5ml/kg	100	10.33 ±2.4

*Significant (p<0.05) vs. Cimetidine; SE= standard error. Number per group (n) =5

Stress induced ulcer. Stress induced ulcer in 100% of the animals in the control group .The ulcer index was 35.33 ± 0.88. The administration of methanol extract, 500 mg/kg and 1000 mg/kg caused reduction of the ulcer index to 22.33 ± 3.48 and 20.00 ± 5.13 respectively while cimetidine caused significant (p<0.05) reduction of the ulcer index to 17.66 ± 6.11 (Table 3).

Table 3: Effect of the extract of *Persea americana* on stress induced ulcers

Drug	Dose (mg/kg)	% of rats having ulcers	Ulcer index
Extract	500	63.20	22.33 ± 3.48*
Extract	1000	56.61	20.00 ± 5.13*
Cimetidine	100	50.01	17.7 ± 6.10
Tween 85	5ml/kg	100	35.3 ± 0.88

*Significant (p<0.05) vs. cimetidine; SE= standard error Number per group (n) =5

Data analysis: All analysis was done using the student's "t" test.

DISCUSSION

The result of the LD_{50} study, that was greater than 5000 mg/kg, showed that methanol extract of *Persea americana* is relatively safe (7) and contained alkaloids, tannins, resins, glycosides, proteins, carbohydrates, steroids, terpenoids and saponins.

Gastric ulcer is known to be related to several pathologic mechanisms including delayed gastric emptying which may prolong the contact time between the gastric acid and the gastric mucosa (10). The methanol extract facilitated gastric motility through intestinal contractions; this may have contributed to its antiucler activity by reducing the contact time between the gastric acid, exogenous ulcerogenic materials and the gastric mucosa.

In the cytoprotective activity against drug -induced and stress-induced types of ulcer, the methanol extract showed significant protection comparable to cimetidine considering their respective ulcer indexes. In ulcerogenesis, intramucosal acidosis and alteration of the normal mucosa protective mechanisms such as diminished secretions of mucus and bicarbonate (11) are very important. Indomethacin, in the induction of ulcer, is known to reduce the mucus production through its inhibition of prostaglandin (10) synthesis. The methanol extract may have protected the animals from the indomethacin and stress ulcerative effects through the formation of an ulcer-adherent complex with the proteinaceous exudates and prostaglandin-mediated cytoprotective mechanism of action at the ulcer site (12) since the extract contained tannins and saponins that can render the mucosa layer less permeable to chemicals and mechanical injuries. However, the mechanism cannot be stated with exactness until the specific constituents are isolated, purified and identified, and further investigation done.

In conclusion, orally administered methanol extract of *Persea americana* seed has proved effective against experimentally induced ulcer as studied with indomethacin and stressed rats. It is relatively safe and can cause increased gastrointestinal motility. But further work need to be done to isolate and characterize the active constituents and determine the exact mechanisms of action.

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