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Experimental evaluation of antiulcer and spasmolytic potentials of leaves of *Cajanus cajan*

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ABSTRACT

The antiulcer effect of methanol leaf extract of *Cajanus cajan* (*L.*) Millsp. (Fabaceae) was evaluated using ulcers experimentally induced by indomethacin and ethanol in rodents. The effects of the extract on rodent gastrointestinal motility, and contractions of isolated intestinal tissues induced by acetylcholine (1.6 µg/ml) and histamine (3.2 µg/ml) were also studied. The results showed that the extract significantly inhibited the development of gastric lesions induced by indomethacin and ethanol. It also inhibited intestinal propulsion and antagonized contractions evoked by acetylcholine and histamine. Acute toxicity tests showed an oral LD_{50} greater than 5000 mg/kg in mice. The presence of saponins, tannins, reducing sugars, terpenoids and resins in the extract was detected by general phytochemical tests. These findings demonstrate that the plant possesses pharmacological properties which lend credence to its ethnomedicinal use as an antiulcer and antidiarrhoeal agent.

KEYWORDS

Cajanus cajan;
Antiulcer;
Antidiarrhoeal;
Spasmolytic.

INTRODUCTION

Peptic ulcer, a benign lesion of gastric or duodenal mucosa, occurs due to an imbalance between the aggressive (acid, pepsin and *Helicobacter pylori*) and the protective (gastric mucus and bicarbonate secretion, prostaglandin and innate resistance of the mucosal cells) factors. Different therapeutic agents including plant extracts that inhibit the aggressive factors or boost the mucosal defensive mechanism are used to re-establish the balance and promote healing. One of such plants is *Cajanus cajan* (*L.*) Millsp. (Fabaceae), an erect woody and annual or short- lived perennial shrub or small tree that is widespread and cultivated throughout the tropics and subtropics. It is commonly known as "Pigeon pea" (English), "Guandu" (Brazil), "Fio-fio" (Ibo - Nigeria) and "Caja" or "Puspo-poroto" (Peru). The seeds (pigeon peas) are popular food in developing countries. In Africa, Asia and South America different parts of the plant are used in the management of various disorders such as ulcer, diarrhea, pain, diabetes, cough and sores. The plant, often grown as a shade crop, is commonly used all over the world for the treatment of diabetes, dysentery, hepatitis, measles, and as a febrifuge to stabilize the menstrual period[1-4]. In traditional Chinese medicine, the leaves have been widely

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used to stop bleeding, relieve pain, kill worms[5], treat wounds, bedsores and malaria, as well as diet-induced hypercholesterolemia[6-9]. The hypoglycemic[10], antioxidant[11] and protective effects of the leaf extracts against hypoxic-ischemic brain damage and alcohol-induced liver damage have also been documented[12,13]. Pigeon pea leaves are rich in flavonoids and stilbenes considered responsible for some of its therapeutic effects[14-16]. Antiplasmodial compounds- betulinic acid (roots) and longistylin A and C (leaves) have been isolated from the plant[14].

This study evaluated the antiulcer and spasmolytic potentials of the methanol leaf extract.

MATERIALS AND METHODS

Materials

Solvents and reagents

Methanol, absolute ethanol (Sigma, Germany), Tween 20

Drugs

Indomethacin, atropine, histamine, acetylcholine, cimetidine (Sigma, Germany), sucralfate (Chugal Pharma Ltd., London).

Animals

Adult New Zealand rabbits (1.5 – 3.0 kg), guinea pigs (350 – 400 g), Swiss albino rats (150 - 250 g) and mice (19 - 35 g) of either sex bred in the Laboratory Animal facility of the Department of Pharmacology and Toxicology, University of Nigeria, Nsukka were used for the study. The animals were maintained freely on the appropriate respective diet and allowed 2 weeks for acclimatization before use. All animal experiments were in compliance with National Institute of Health Guide for Care and Use of Laboratory Animals (Pub No. 85 – 23, revised 1985).

Preparation of extract and phytochemical analysis

Fresh leaves of *Cajanus cajan* were collected in Nsukka, Enugu State, Nigeria in April. The plant was identified and authenticated at the International Centre for Ethnomedicine and Drug Development (InterCEDD), Nsukka, Enugu State, where a voucher specimen is deposited (specimen number: INTERCEDD/915) The leaves were cleaned, dried under shade for 5 days and reduced to coarse powder using a mill. The leaf powder (700 g) was extracted with methanol by maceration at room temperature (28 ± 1°C) for 48 h with intermittent shaking. The filtrate was concentrated in a rotary evaporator at 50°C to yield 85.45 g of the methanol extract (CCM; 12.2% w/w), which was stored in a refrigerator (–4°C). The phytochemical constituents of CCM were identified using standard procedures[17,18].

Methods

Acute toxicity tests

The acute toxicity and lethality of CCM was evaluated using the method described by Lorke (1983). Briefly, nine rats randomly divided into three groups (n=3) were orally administered 10, 100, and 1000 mg/kg of the extract respectively and observed for deaths within 24 h. Since no death was recorded, higher doses of 1,600, 2,900 and 5000 mg/kg of the extract were administered to a fresh batch of animals (n=1) and the number of deaths in 24 h recorded[19].

Indomethacin – induced ulcer

Rats fasted for 24 h but allowed free access to water were used to determine the least effective dose of indomethacin that would produce 100% gastric ulceration. Various doses of indomethacin (40, 60 and 100 mg/kg p.o) were administered, and it was observed that, 100 mg/kg produced gastric ulceration in all rats in 4 h. Consequently, a fresh batch of rats fasted for 24 h were randomly divided into four groups (n = 5) to receive oral administration of one of 400 or 800 mg/kg of CCM suspended in 10% v/v Tween 20. Control animals received either the vehicle (5 ml/kg) or cimetidine (100 mg/kg). Thirty minutes later, ulcer was induced with indomethacin (100 mg/kg; p.o). Four hours after indomethacin administration, the animals were sacrificed with overdose of chloroform anaesthesia and their stomachs dissected out. The stomachs were opened along the greater curvature, rinsed under a stream of water and pinned flat on a cork–board. The stomachs were coded to prevent observer's bias and studied. Erosions formed only on the glandular mucosa were counted and each one given a severity rating on a 1 - 3 scale where 0 = normal, 1.0 = less than 1 mm, 2.0 = 1 to 2 mm, 3.0 = > 2 mm[20]. Mean score for each group was calculated and expressed as ulcer index (UI).

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The level of protection (%) of the treated groups against ulcer formation was calculated using the relation; $[(UI_C - UI_T)/ (UI_C)]$ 100, where UIc = ulcer index of control group and UI_T = ulcer index of the treated group[21].

Ethanol–induced gastric lesions

Ulceration was induced as described by Robert (1979)[22]. Twenty albino rats fasted for 24 hours prior to the experiment but allowed free access to water, were randomly divided into 4 groups (n = 5) to receive oral administration of one of 400 or 800 mg/kg of CCM suspended in 10% v/v Tween 20. Control animals received either the vehicle (5 ml/kg) or sucralfate (1000 mg/kg). Thirty minutes later, ulcer was induced by oral administration of 1 ml absolute ethanol to all the animals. The animals were sacrificed 1 h later, and the stomachs removed and opened along the greater curvature. The stomach was rinsed under a stream of water and fixed with 10% formaldehyde in saline, pinned flat on a corkboard and observed with a hand lens (x10). The number and severity of erosions were scored according to an arbitrary scale (Adami *et al*., 1964)[23], where 0 = no ulcer; 1 = haemorrhagic and slight ulcer, length < 2 mm; 2 = one haemorrhagic and slight ulcer, length < 5 mm; 3 = more than one grade 2 ulcers; 4 = one ulcer of length < 5 mm and diameter < 2mm; 5 = one to three ulcers of grade 4; 6 = four to five ulcers of grade 4; 7 = more than six ulcers of grade 4; 8 = complete lesion of the mucosa with haemorrhage. Mean score for each group was calculated and expressed as ulcer index (UI). The level of protection (%) of the treated groups against ethanol ulcers were calculated using the relation; $[(UI_C - UI_T)/ (UI_C)]$ 100, where UIc = ulcer index of control group and UI_T = ulcer index of the treated group.

Gastrointestinal motility test

The effect of CCM on small intestinal propulsion was determined in mice using the charcoal meal method[24], with some modifications. Twenty mice fasted for 24 h but allowed free access to water, were randomly divided into four groups (n = 5) to receive oral administration of one of 400 or 800 mg/kg of CCM suspended in 10% v/v Tween 20. Control animals received either the vehicle (5 ml/kg) or atropine (10 mg/kg). Thirty minutes later, charcoal meal (0.5 ml of 5% activated charcoal in 10% aqueous solution of tragacanth powder) was administered orally to each animal. The animals were sacrificed 30 min later, and the abdomen opened. The small intestine was carefully inspected and ligated at both the pyloric sphincter and where the charcoal meal stopped, to avoid disruption of the charcoal meal during handling. The distance traversed by the charcoal meal from the pylorus, and the length of the whole small intestine were measured. The extent of intestinal propulsion (%) and level of inhibition of intestinal propulsion (%) was calculated for each animal and the mean for each group determined.

Intestinal propulsion (%) = 100 [DT/TL]

Inhibition of intestinal propulsion (%) = 100 [TL-DT/TL], where DT = Distance traversed by the charcoal meal; TL = Total length of small intestine.

Studies on the isolated guinea pig ileum

Guinea pigs were sacrificed by cervical dislocation and bled, and a segment of the ileum was removed after discarding the portion nearest to the ileocaecal junction. The ileal strips (approximately 2 cm in length) were mounted vertically under resting tension of 0.5 g in 20 ml organ baths. The tissue bathing fluid was Tyrode solution of the following composition (g/L) NaCl (8), KCl (0.2), $CaCl_2$ (0.2), $NaHCO_3$ (1.0), $MgCl_2$ (0.1), NaH_2PO_4 (0.05), glucose (1.0); which was maintained at 37°C and aerated with air. The tissue was allowed to equilibrate for 60 min during which the bathing fluid was changed every 10 min. Responses of the isolated ileum to graded concentrations of CCM, histamine and acetylcholine were recorded isometrically on an Ugo Basile Unirecorder (7050) through isometric transducer (7004). Drug tissue contact time was 1 min, and a 3 min time cycle was maintained.

Also the effects of CCM on histamine- (3.2 µg/ml) and acetylcholine- (1.6 µg/ml) induced contractions of the guinea pig ileum were recorded. The extract was added to the tissue bath and allowed to act for 3 min; subsequently, the standard agonists were added and allowed to act for 1 min before washing off the drugs. The experiments were done in triplicates using ileum from different animals.

Studies on isolated rabbit jejunum

Rabbits were sacrificed by cervical dislocation and bled, the stomachs were removed and segments of the

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jejunum (about 2 cm long) removed and dissected free of mesenteric attachments. The jejunal tissues were mounted vertically under resting tension of 0.5 g in 20 ml organ baths. The tissue bathing fluid was Tyrode solution maintained at 37°C and aerated with air. The tissue was allowed to equilibrate for 60 min during which the bathing fluid was changed every 10 min. Responses of the isolated tissue to graded concentrations of CCM, and acetylcholine were recorded isometrically on an Ugo Basile Unirecorder (7050) through isometric transducer (7004). Drug tissue contact time was I min, and a 3 min time cycle was maintained.

Also the effects of CCM on acetylcholine (1.6 µg/ml) - induced contractions of the rabbit jejunum were recorded. The extracts were added to the tissue baths and allowed to act for 3 min; subsequently, the standard agonists were added and allowed to act for 1 min before washing off the drugs. The experiments were done in triplicates using jejunum from different animals.

Statistical analysis

Data obtained was analyzed using One-Way analysis of variance (ANOVA) and further subjected to LSD post hoc test for multiple comparisons. The results were presented as Mean ± SEM. Differences between means of treatment and control groups were accepted significant at $P<0.05$.

RESULTS

Phytochemical constituents of CCM

The phytoconstituents detected in CCM were saponins, tannins, terpenoids and resins (TABLE 1).

TABLE 1: Phytochemical constituents of CCM

Phytochemical constituents	CCM
Alkaloids	-
Carbohydrates	-
Flavonoids	-
Glycosides	-
Reducing sugars	++
Resins	++
Saponins	+
Tannins	+++
Terpenoids	++

+++= Conspicuously present, ++= moderately present, += present, - = absent

Acute toxicity and lethality

Oral administration of up to 5, 000 mg/kg of CCM caused no death in treated mice. Hence, the oral LD_{50} of CCM in mice was estimated to be greater than 5, 000 mg/kg.

Effect of CCM on indomethacin induced ulcer

The CCM significantly ($P<0.05$) inhibited the formation of indomethacin-induced gastric lesions in a dose-related manner. The extract (800 mg/kg) afforded 85% protection against indomethacin-induced ulcer in treated animals (TABLE 2).

TABLE 2: Effect of CCM on indomethacin-induced gastric ulcers

Treatment	Dose (mg/kg)	Ulcer index	Protection (%)
CCM	400	9.00 ± 4.94	41.94
	800	2.25 ± 1.31*	85.48
Cimetidine	100	0.25 ± 0.25	98.39
Control	-	15.50 ± 3.40	-

n = 5; *$P<0.05$ compared to control (*ANOVA; LSD post hoc*); CCM = *C. cajan* Methanol extract; Protection (%) was calculated relative to the control.

Effect of CCM on ethanol induced ulcer

Oral administration of CCM elicited a significant ($P<0.05$) dose-related inhibition of ethanol-induced gastric ulcers. The extract (800 mg/kg) afforded 81% protection against ethanol-induced ulcer, and was more potent than sucralfate (TABLE 3).

TABLE 3: Effect of CCM on ethanol-induced gastric ulcers

Treatment	Dose (mg/kg)	Ulcer index	Protection (%)
CCM	400	4.75 ± 1.03	13.64
	800	1.00 ± 0.70*	81.82
Sucralfate	1000	4.75 ± 1.03	13.64
Control		5.50 ± 0.28	-

n = 5; *$P<0.05$ compared to control (*ANOVA; LSD post hoc*); CCM = Methanol extract; Protection (%) was calculated relative to the control.

Effect of CCM on gastrointestinal propulsion

The CCM reduced the distance traversed by charcoal meal. The inhibition of small intestinal transit was dose-related (TABLE 4).

Effect of CCM on isolated guinea pig ileum

The CCM neither relaxed nor contracted the iso-

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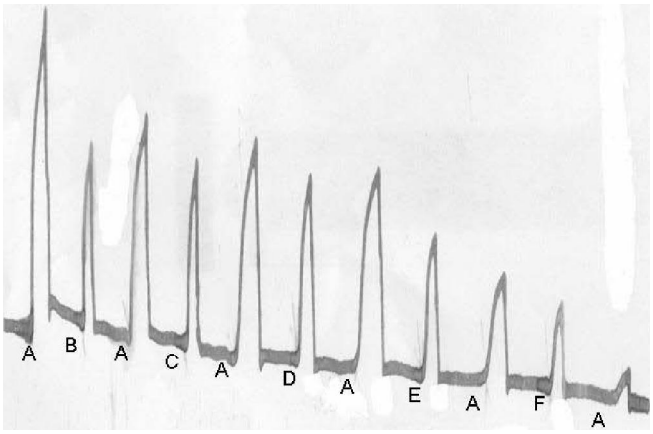
TABLE 4: Effect of CCM on gastrointestinal propulsion

Treatment	Dose (mg/kg)	Total length of intestine (cm)	Distance traversed by charcoal meal (cm)	Intestinal Propulsion (%)	Inhibition of propulsion (%)
CCM	400	44.64 ± 1.94	27.58 ± 5.49	60.98 ± 11.02	39.02 ± 11.02
	800	42.96 ± 1.63	25.06 ± 3.68	58.15 ± 7.77	41.85 ± 7.77
Atropine	10	45.28 ± 1.41	17.18 ± 3.44	37.41 ± 6.66	62.59 ± 6.66*
Control	-	39.88 ± 0.96	31.92 ± 1.47	80.38 ± 4.93	19.62 ± 4.93

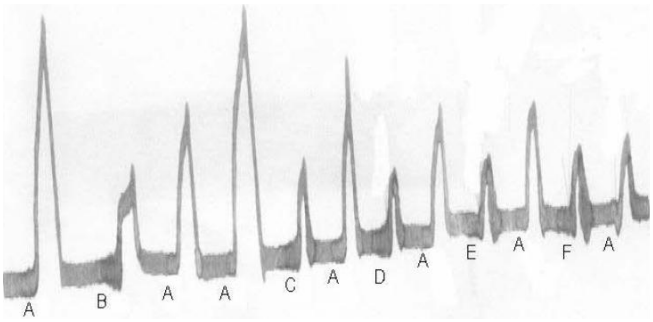
***n* = 5; **P*<0.05 compared to control (*ANOVA; LSD post hoc*); CCM = *C. cajan* methanol extract; Intestinal propulsion (%) and Inhibition (%) was calculated for each animal and the mean for each group recorded**

lated guinea pig ileum. However, it potently inhibited acetylcholine (1.6 μg/ml)- and histamine (3.2 μg/ml)-induced contractions of the ileum with IC_{50} values of 500 and 180 μg/ml respectively (Figures 1, 2 and 5).



A = Ach 1.6 μ g/ml; B,C,D,E,F = Ach 1.6 μ g/ml + CCM 0.05, 0.1, 0.2, 0.4 or 0.8 mg/ml

Figure 1 : Effect of CCM on acetylcholine-induced contractions of the isolated guinea pig ileum



A = histamine 3.2 μ g/ml; B,C,D,E and F = histamine 3.2 μ g/ml + CCM 0.05, 0.1, 0.2, 0.4 or 0.8 mg/ml

Figure 2 : Effect of CCM on histamine-induced contractions of the guinea pig ileum

Effect of CCM on rabbit jejunum

The CCM inhibited the rhythmic contractions of the isolated rabbit jejunum. It also reduced jejunal contractions induced by acetylcholine (1.6 μg/ml), with IC_{50} of 150 μg/ml (Figures 3, 4 and 5).

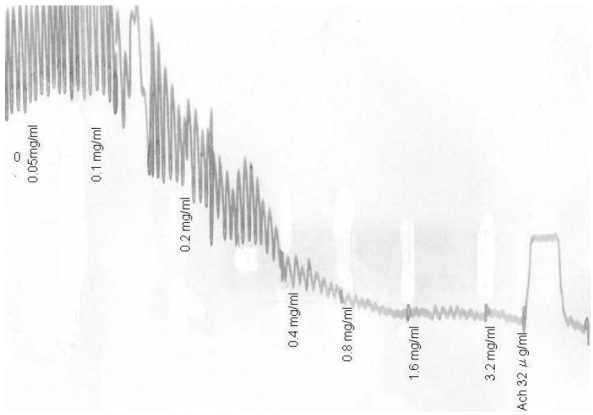
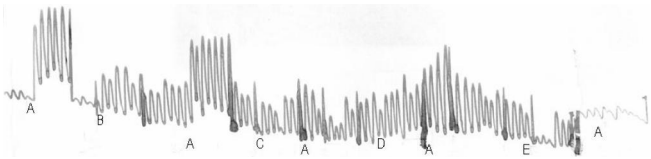


Figure 3 : Effect of CCM on rhythmic contractions of the isolated rabbit jejunum



A = Ach 1.6 μ g/ml; B,C,D and E = Ach 1.6 μ g/ml + CCM 0.05, 0.1, 0.2 or 0.4 mg/ml

Figure 4 : Effect of CCM on Ach-induced contractions of the isolated rabbit jejunum

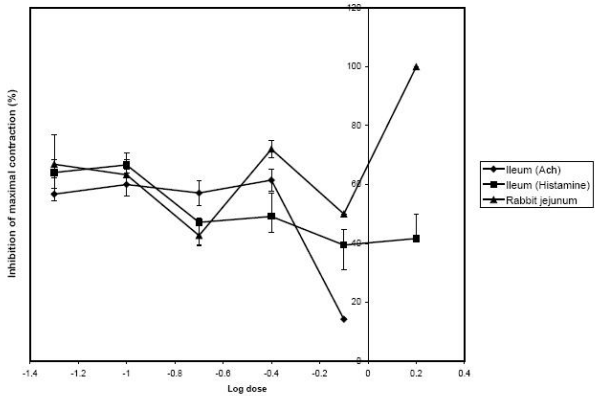


Figure 5 : Effect of CCM on agonist-induced contractions of intestinal tissues

DISCUSSION

This study has shown that leaves of *C. cajan* pos-

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sess antiulcer and spasmolytic properties. The extract protected rats against gastric ulcers induced by indomethacin and ethanol, inhibited gastrointestinal motility by reducing small intestinal propulsion and exhibited spasmolytic effects by inhibiting jejunal contractions and antagonizing contractions of the isolated intestinal tissues evoked by acetylcholine and histamine.

Indomethacin-induced gastric mucosal damage results from suppression of prostaglandin synthesis via the arachidonic pathway. Hence, inhibition of indomethacin-induced ulcer formation is likely a consequence of enhanced prostaglandin synthesis. Prostaglandins are known to serve cytoprotective functions in the stomach by maintaining gastric microcirculation[25,26] and causing gastric secretion of bicarbonate[27] and mucus[28]. This suggests that the leaf extract may protect against gastric mucosal damage by enhancing gastric cytoprotective mechanisms. Consistent with this is the protection it offered against ethanol challenge. Ethanol-induced ulcers, which are predominant in the glandular part of the stomach, may result from factors ranging from direct toxic action of ethanol and reduction of the secretion of bicarbonate to depletion of gastric wall mucus[29,30]. Ethanol reduces endogenous glutathione and prostaglandin levels and increases the release of histamine, influx of calcium ions and generation of free radicals via increased lipid peroxidation[31-34] which culminate in damage to cell and cell membranes. Hence, like in indomethacin ulcer, inhibition of ethanol-induced gastric mucosal lesions indicates cytoprotective effect due possibly to enhancement of mucosal defensive factors. Although the precise mechanism of cytoprotection remains to be investigated, the protective effect of the extract against ulcer formation in both models points to the cytoprotective potential and the ability to enhance mucosal defensive factors.

Studies on gastrointestinal motility showed that the extract inhibited peristaltic propulsive movement indicative of inhibition of gastrointestinal motility. This is consistent with the results of isolated intestinal tissue studies where CCM neither relaxed nor contracted the guinea pig ileum, but potently inhibited acetylcholine- and histamine-induced contractions suggestive of non-specific spasmolytic activity. Thus, the spasmolytic effect of the extract may contribute, albeit in part, to the anti-ulcer properties of the plant since inhibition acetylcholine- and histamine-induced contractions may derive from suppression of processes mediated by these spasmogens including those associated with ulcer induction. Acetylcholine and histamine are implicated in ulcer pathogenesis since both neurotransmitters stimulate and regulate gastric acid secretion[35]. Antagonism of their actions is apt to provide beneficial effect in ulcer therapy by further reducing gastric acid secretion. In addition to ameliorating ulcer, reduced intestinal motility may account for the antidiarrheal activity of this plant. The plant extract can be generally regarded as safe[19] since the high LD_{50} value suggests remote risk of acute intoxication.

The phytochemical constituents responsible for these pharmacological activities are yet to be identified. Although preliminary phytochemistry studies revealed the presence of several constituents, further studies are needed to isolate and characterize the antiulcer and or spasmolytic constituents.

In conclusion, findings from this study showed that leaves of *C. cajan* possess antiulcer and antidiarrheal properties. The antiulcer effect is attributable to cytoprotection through enhancement of mucosal barrier while reduction of gastrointestinal motility and spasmolytic effects provide a pharmacological basis for the usefulness as antidiarrhoeal remedy.

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