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Antiulcer Activity of Stem Extract of *Telfairia Occidentalis* in Rodents

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Abstract
The vegetable, *Telfairia occidentalis* Hook (*Cucurbitaceae*), is useful in Ibibio traditional medicine system for the treatment of various types of diseases such as malaria and gastrointestinal disorders. Antiulcerogenic activity of the stem extract was evaluated against ulcers experimentally-induced in rats. Three doses (200, 400 and 600 mg/kg) of the stem extract of *Telfairia occidentalis* were used to investigate the antiulcer activity in three experimental models; indomethacin, ethanol and histamine-induced ulcers. The animals weighing 130-155 g were fasted overnight before being randomized into different experimental groups and treated with extract and ulcerogens. In each model, the animals were sacrificed after the required period of time and their stomachs removed and examined for ulcerations. Ulcers scores were awarded and preventive ratio calculated accordingly. The results showed that the stem extract (200-600 mg/kg) exerted dose-dependent and significant ($p<0.05$-0.001) antiulcer potential against ulcers induced by indomethacin, ethanol and histamine. These findings show that the stem extract of *Telfairia occidentalis* exhibited antiulcer property which is traceable to the actions of the phytoconstituents and thus a useful remedy for ulcers.

Keywords: *Telfairia occidentalis,* antiulcerogenic, gastrointestinal disorders, ulcer

Introduction
The vegatable, *Telfairia occidentalis* Hook (*Cucurbitaceae*) commonly called fluted pumpkin, is one of the widely consumed vegetables in Nigeria [1], notably in the preparation of varieties of dishes especially in the southern part of the country using different parts of the plant; leaves, stem and seeds [2]. Traditionally, these parts (seeds, leaves and stem) are also useful as medicine for the treatment of various ailments and diseases including gastrointestinal disorders such as gastric ulcer [3,4]. The plant is known for many biological activities such as antiplasmodial activity, which has been reported on the seed, leaves and roots [5,6]. Okokon *et al* [7] reported that the stem extract has effects on parasitaemia, oxidative stress, lipid profile, haematological parameters, liver function indices and liver histology of *Plasmodium berghei* infected mice. Documented reports also showed that the stem extract has analgesic [8], antioxidant and *in vivo* inhibitory effect on alpha amylase and alpha glucosidase of rats [9]. The anti-inflammatory effects of the leaf [10] and seed [11] extracts have also been reported and the aqueous extract of the leaves has been shown to possess antiulcer activity [3,4]. The stem extract whose fractions is rich in some polyunsaturated fatty acids such as hexadecanoic acid, methyl ester, 9,12-octadecadienoic acid methyl ester (linoleic acid), 9,12,15-octadecatrienoic acid, methyl ester (linoleic acid) and 9-octadecenoic acid also contain some secondary metabolites like alkaloid, terpenes, saponin, flavonoid and tannin in the crude extract [9]. There is no report on the antiulcerogenic activity of the stem extract of *T. occidentalis* in literature, hence this study aimed to investigate the antiulcerative activity of the stem extract of *T. occidentalis* in rats.

Materials and Methods
Plant materials
Matured fresh stems of *Telfairia occidentalis* were obtained from farms in Uyo metroplis in Uyo LGA, Akwa Ibom State, Nigeria. Identification and authentication of the plant, *Telfairia occidentalis,* was carried out in the Department of Botany and Ecological studies, University of Uyo, Uyo, Nigeria by a taxonomist and a voucher specimen (FPH 20a) was deposited at the herbarium of the Department of Pharmacognosy and Natural Medicine, Faculty of Pharmacy, University of Uyo.

Extraction
Fresh mature stems of *Telfairia occidentalis* were cleaned by washing in water, reduced to smaller pieces and shade-dried for fourteen days. Pulverization of the dried stems to powder was done using electric grinder and 2 kg of the powder was soaked for 3 days in 50% ethanol (7.5 L) at room temperature (28 ± 2 ˚C). Filtration of the soaked powdered material was done after 72 hours and the liquid

filtrate concentrated to dryness in *vacuo* at 40 ˚C using a rotary evaporator (BuchiLab Switzerland). The weighed extract was stored in a refrigerator at -4 ˚C, until used for the proposed experiments.

Experimental animals

Albino wistar rats weighing 130-155 g and mice weighing 20-35 g (male and female) were obtained from the University of Uyo's Animal house and used in the study. Standard plastic cages and a well-ventilated room were used to accommodate the animals for a period of 10 days to acclimatize before the study. Standard pelleted Feeds were used to feed the rats and they were allowed free access to water. The animal experiments were conducted according to care and use of animals in conformity with the National Institute of Health Guide for the Care and Use of laboratory Animals (NIH Publication, 1996). This study was approved by the University of Uyo's Animal Ethics Committee (UU/CHS/AE/24/054).

Determination of median lethal dose (LD_{50})

Oral route was used to determine the median lethal dose (LD_{50}) of the extract applying a modified method of Lorke [12], which was conducted in two phases. Three doses of the extract (10,100 and1000 mg/kg) was employed in the first phase and administered to 3 groups of three mice each. Four more doses (2000, 3000, 4000 and 5000 mg/kg) were employed in the second phase when no mortality was recorded in the first phase and administered orally to the respective groups of 3 mice each. Toxic signs such as writhing, decreased motor activity, decreased body/limb tone, decreased respiration and death were monitored and observed in the treated animals. Mortality was recorded in each group within 24 hours. The LD_{50} was estimated as geometrical means of the highest dose causing 0% (a) and the lowest dose causing 100% mortality (b) using Eq. 1.

$LD_{50} = \sqrt{ab}$….Eq. 1

Evaluation of antiulcer activity:

Indomethacin-induced ulcer

In this study, overnight fasted adult male albino rats were shared randomly into 5 groups of six rats each. The experiment was conducted according to previously reported method and water was withdrawn from the rats 2 hours before they were treated with the extract and drugs [13]. Indomethacin (Sigma, 60 mg/kg p.o. dissolved in 5% Na_2CO_3) was administered to the control animals in group 1; *Telfairia occidentalis* stem extract (200, 400 and 600 mg/kg p.o.) prepared as aqueous suspension was given to rats in groups 2-4 respectively; Cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80), the standard drug, was given to rats in group 5. Indomethacin (60 mg/kg, p.o) was administered to rats in groups 2 - 5, one hour after pretreatment with the extract and drug. The animals were thereafter subjected to light diethyl ether anaesthesia and sacrificed by cervical dislocation, four hours post indomethacin administration. The stomach of each animal was harvested and opened along the greater curvature. The stomachs were thereafter fixed in 10% buffered formalin. Hand lens was used to examine all the fixed stomachs macroscopically and each ulcer lesion present was scored [13, 14]. Standard methods [11] were used to estimate the ulcer index (UI, degree of lesion or ulceration) and preventive ratio (PR, protective potential) of each group of the treated animals.

Ethanol-induced gastric ulceration

A procedure similar to that used in indomethacin-induced ulcer was employed in this model. However, ethanol (97%) (2.5 mL/kg) was used as ulcerogen to induce ulcer and propranolol (40 mg/kg p.o.),was used as the standard drug [13]. Propranolol, a non-selective beta adrenoceptor antagonist has been shown to have protective effect on ethanol-induced gastric ulceration in rodents [15, 16].

Histamine-induced gastric ulceration in rats

In this model, a similar procedure to that used in indomethacin-induced ulcer model was employed. The ulcerogen used to induce ulcer was histamine acid phosphate (Sigma, 100 mg/kg i.p. dissolved in distilled water) with cimetidine (100 mg/kg p.o. dissolved in 50% Tween 80) as the standard drug. The animals were left for 18 hours post histamine acid phosphate (100 mg/kg, i.p) administration for the development of ulcer [13, 14]

Statistical analysis

Data obtained from these experiments were subjected to statistical analyses using one way analysis of variance (ANOVA) followed by Tukey Kramer's multiple comparison post-test (Graph pad prism software Inc. La Jolla, CA, USA). Values were expressed as mean ± SEM and significant values relative to control were considered at $p<0.05$.

Results

Determination of Median lethal dose (LD_{50})

No mortality was recorded following administration of T. *occidentalis* stem extract (10 - 5000 mg/kg) orally to the animals groups. Moreover, there were no observable physical signs of toxicity resulting from the administration of the stem extract to mice. The LD_{50} of *T. occidentalis* stem extract was calculated to be ≥5000 mg/kg.

Indomethacin-induced gastric ulceration

T. occidentalis stem extract (200 - 600 mg/kg, p.o.) exhibited a significant ($p<0.05$-0.001) dose-dependent lowering of ulcer indices in indomethacin-induced gastric ulceration in rats following its pretreatment to rats relative to control. (Table 1). Only pinpoint wounds were observed to be present in the stomach of all extract pretreated rats groups without any visible severe wound, which was only present in the stomach of rats in the control group. However, the activity exhibited by the extract was lower relative to that of

cimetidine. The preventive ratios were 96.29, 36.36, 50.03 and 77.30 respectively for cimetidine, 200, 400 and 600 mg/kg of the extract (Table 1).

Ethanol-induced gastric ulceration

T. occidentalis stem extract (200- 600 mg/kg) pretreatment to rats significantly ($p<0.05$-0.001) and dose-dependently protected the pretreated animals against ethanol–induced ulcer as shown in Table 2. The extract exhibited significant ($p<0.05$-0.001) lowering of ulcer indices relative to control especially in highest dose (600 mg/kg) pretreated group. No severe wound present in the stomach of the rats in control group was observed in those of rats pretreated with the stem extract as only pinpoint wounds were observed to be present in the stomachs of all extract pretreated rats groups. The standard drug, propranolol, exhibited the highest protection (91.30%), while the extract; 200, 400 and 600 mg/kg respectively had protective indices of 30.43, 60.86 and 73.91% (Table 2).

Histamine–induced ulceration.

Significant ($p<0.05$-0.001) dose-dependent antiulcerogenic activity against histamine-induced gastric ulceration was observed following pretreatment of rats with *T.occidentalis* stem extract (200- 600 mg/kg) as shown in Table 3. Severe wounds present in the stomachs of rats in control group were absent in the stomachs of the extract/drug pretreated groups. The preventive ratios were 48.86, 71.59 and 88.63% respectively for 200, 400 and 600 mg/kg of the extract (Table 3).

Table 1: Effect of ethanol stem extract of *Telfairia occidentalis* on indomethacin-induced ulcer

Treatment	Dose (mg/kg)	Ulcer indices	Preventive ratio
Control Indomethacin	60	13.75 ±1.25	-
Cimetidine	100	0.51 ±0.01^c	96.29
Crude extract	200	8.75±1.25^a	36.36
	400	6.87±1.87^b	50.03
	600	3.12± 0.62^c	77.30

Data are expressed as Mean ± SEM, Significant at $^a p < 0.05$,$^b p<0.01$,$^c p<0.001$, when compared to control. (n=6).

Table 2: Effect of stem extract of *Telfairia occidentalis* on ethanol-induced ulcer

Treatment	Dose (mg/kg)	Ulcer indices	Preventive ratio
Control normal	60	5.75 ±0.25	-
Propranolol	40	0.50 ±0.28^c	91.30
Crude extract	200	4.00±0.40 ^{a}	30.43
	400	2.25 ± 0.25^b	60.86
	600	1.50 ± 0.28^c	73.91

Data are expressed as Mean ± SEM, Significant at $^a p < 0.05$,$^b p<0.01$,$^c p<0.001$, when compared to control. (n=6).

Table 3: Effect of stem extract of *Telfairia occidentalis* on histamine-induced ulcer

Treatment	Dose (mg/kg)	Ulcer indices	Preventive ratio
Control normal	60	4.40±0.01	-
Cimetidine	100	0.25 ±0.14^c	94.31
Crude extract	200	2.25±0.25^a	48.86
	400	1.25 ± 0.25^c	71.59
	600	0.50 ± 0.20^c	88.63

Data are expressed as Mean ± SEM, Significant at $^a p < 0.05$,$^b p<0.01$,$^c p<0.001$, when compared to control. (n=6).

Discussion

The antiulcerogenic activity of *T. occidentalis* stem extract was investigated in rats against indomethacin, ethanol and histamine–induced ulcers. Indomethacin causes wound on the glandular part (mucosal) of an empty stomach [17-19] via inhibition of prostaglandin synthetase activity, thereby inhibiting prostagladins synthesis [20] and exposing the stomach to injury and ulceration. Stimulation of bicarbonate and mucus secretion, maintenance of mucosal blood flow and regulation of mucosal turn over and repair [21, 22] are physiological functions of prostaglandins. The stem extract significantly reduced the ulcer indices of the pretreated rats in the indomethacin–induced ulcer model as observed in this study, demonstrating the extract's potential in mobilizing and enhancing prostaglandins synthesis. Ethanol reportedly inflicts injury to the stomach by depleting of gastric mucus, altering gastric secretion and permeability of mucosa, and stimulating free radical generation [23], resulting in mucosa damage. This has been attributed to superoxide anion and hydroperoxy free radicals generation which is prominent during ethanol metabolism and have been implicated in the pathogenesis of acute and chronic ulceration in the gastric mucosa [24]. The stem extract was observed to have lowered the effect of ethanol in this study as seen in the significantly lowered ulcer indices. This suggests cytoprotective and free radical scavenging potentials of the stem extract as earlier reported on the stem extract [9]. Ethanol can also damage gastric mucosa by mobilising the production of leukotriene C4 (LTC4) [25], which is suggestive of another probable mode of action by the extract to inhibit ethanol activity through suppression of lipoxygenase activity [19].

Stimulation of gastric secretion and vasospastic activity of histamine have been reported to be responsible for histamine-induced ulcerations [26]. The extract under study was observed to inhibit histamine-induced ulcer which suggest the stem extract's potential to suppress histamine-induced vasospastic and gastric secretion activities. Secondary metabolites such as tannins, flavonoids, alkaloids, terpenes, triterpenes, and polyunsaturated fatty acids (PUFAs), have been reported to be present in the stem extract among others [9]. Flavonoids reportedly protect the gastric mucosa from damage through elevation of neutral glycoproteins levels [27, 28], stimulation of mucosal prostaglandin synthesis and inhibition of histamine secretion from mast cells via histidine decarboxylase inhibition. Flavonoids also act to scavenge free radicals through their antioxidant activities thereby protecting the gastrointestinal tract from injuries [29]. Other phytoconstituents like some types of saponins, exhibit antiulcer potentials by forming protective mucus on the gastric mucosa by selectively inhibiting $PGF_{2\alpha}$ [30, 31]. Besides, Polyunsaturated fatty acids (PUFAs) such as hexadecanoic acid and others found to be present in this stem extract are potent active antioxidant compounds [32-34] and are likely to scavenge generated free radicals such as those involved in ethanol-induced ulcerations. The antioxidant activities of these phenolic compounds and PUFAs as earlier reported by Enin *et al.* [9] as constituents of the stem extract may be accountable for the exhibited antiulcer activity observed in this study.

Conclusion

The findings of this investigation demonstrate that *T. occidentalis* stem extract may possess antiulcer property which may be attributed to the antioxidant potentials of its constituents.

Conflict of Interest

The authors have not declared any conflict of interests.

Authors' Contributions

JEO,UEA - Research concept and design; JEO,UEA- Animal studies, JEO,UUF- Data analysis and interpretation; JEO,CCO,UUF- Writing the article. JEO, CCO, UUF and UEA read and approved the final manuscript.

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