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Original Research Article

Gastro-Protective Mechanisms of Aqueous Leaf Extract of *Telfairia ocidentalis* in Ethanol Induced - Gastric Ulceration in Spraque Dawley Rats

Williams A. Adisa[*1]., Azubuike A. Nwankwo[2]., Eme E. Osim[3]., Deliverance Brotobor[4]

[1]*Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State, Nigeria,*
[2]*Department of Physiology, Faculty of Basic Medical sciences, College of Medicine, Abia State University, Uturu, Abia State Nigeria,*
[3]*Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, University of Calaba, Calaba, Cross River State, Nigeria.*
[4]*Department of Nursing Science, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma, Edo State,Nigeria.*

ABSRTACT

In terms of both morbidity and mortality gastric ulcer is a major health challenge and can induce upper gastrointestinal bleeding if not treated. This study evaluated the effects of aqueous leaf extract of *Telfairia occidentalis* on ethanol induced gastric ulcer in male *spraque dawley* rats. Twenty five adult male rats of comparable weight were divided into 4 groups of 5 rats each. Group A served as normal control, group B served as the test control and was ulcer induced with single oral administration of 5ml/kg body weight 75% absolute ethanol, group C received (500mg/kg) of *T occidentalis*, while group D was ulcer induced with single oral administration of 5ml 75% ethanol solution after pre-treatment with (500mg/kg) of *T occidentalis*. Another set of rats were distributed into various groups of 5 rats each and administered N-nitro-Larginine methyl ester 70 mg/kg, 1H[1,2,4] oxadiazolo [4,3-a]quinoxaline-1-one 10 mg/kg, glibenclamide, N-ethylmaleimide 5 mg/kg, and verapamil 5 mg/kg respectively. This was followed by admiration of *T occidentalis* extract and 5mL/kg body weight 75% absolute ethanol for gastric ulcer induction. Comparatively, the results of the study suggest that pre-treatment with *T. occidentalis* increased gastric wall mucus content, superoxide Dismutase, catalase, and prostaglandin but reduced gastric ulceration, oxidative stress, IL-8, and has apparent protective effect on gastric macroscopic architecture. Judging by the findings of this study, *T occidentalis* is highly gastroprotective, and possibly involving NP-SH, K_{ATP}, and Ca^{2+} channel to mediate its gastroprotective mechanism against ethanol induced gastric ulcer.

Keywords: *Telfairia occodentalis*, gastric ulceration, oxidative stress makers, endogenous antioxidant, ethanol.

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Introduction

In terms of both morbidity and mortality Gastric ulcer is a major health challenge and can induce upper gastrointestinal bleeding if not treated.[1,2] The etiology of gastroduodenal ulcers is linked to various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, -mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents.[3] There are also reports that peptic ulcer occurs as a result of oxidative stress, induced by the generation of free radicals such as superoxide anions, hydrogen peroxide, hydroxyl radical and nitric oxide, causing cellular damage by modifying macromolecules such as DNA, carbohydrates, proteins and lipids thereby damaging them.[4] Several pharmaceutical products are being used in the treatment of gastro-duodenal ulcer and peptic diseases, to decrease mortality and morbidity rates, but they are not completely effective in view of their many adverse effects.[5] There has also been growing interest in alternative therapies and the use of natural products, especially those derived from plants.[5] Plant extracts are some of the most attractive sources of new drugs and have been shown to produce promising results for the treatment of gastric ulcer.[6,7]

*Corresponding author. mail: williamsadewumi@yahoo.com
Tel: +2348056049158

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Telfairia occidentalis is one of such plants that have been extensively studied and reported to have great potential and is of interest in this study. *Telfairia occidentalis* occurs in the forest zone of West and Central Africa. It is a popular vegetable all over Nigeria because of its nutritional and medicinal values. It originated in south-east Nigeria and was distributed by the Igbos.[8] A good number of researchers in the field of medical sciences have reported free radical scavenging capability and antioxidant property in *Telfairia occidentalis*. It has also been found to suppress or prevent the production of free radical and scavenge already produced free radical, lower lipid peroxidation status and elevates antioxidant enzymes (such as superoxide dismutase and Catalase) both in vitro and *in* vivo.[5,9,10,11] Although several studies have been conducted on the medicinal potentials of *Telfairia occidentalis*, its pharmacological effects on gastrointestinal system is yet to be adequately explored. Our previous study,[9] revealed ameliorative effect of *Telfairia occidentalis* on indomethacin induced gastric ulcer; hence this research to investigate further; the effect and possible mechanism of action of *Telfairia occidentalis* leave extract in ethanol induced gastric ulcer in rats.

Materials and Methods

Telfairia occidentalis leaves were obtained from a local market in Ibadan and was authenticated as such at the Herbarium Unit of the International Institute for Tropical Agriculture, with batch no: FHI.111998. Absolute ethanol was purchased from Mega Chemicals while animal feed produced by Grand Cereals Ltd, a subsidiary of UAO Nigeria PLC, Jos, Plateau State) was purchased from an open shop in Ekpoma, Edo State Nigeria.

Drugs and all other chemicals (indomethacin, sodium bicarbonate used to dissolve indomethacin, tween, phenolthalin, Sodium hydroxide, distilled water, N-nitro-Larginine methyl ester (L-NAME), oxadiazolo quinoxaline, (ODQ), glibenclamide, NEM and verapamil) were ordered from Sigma Aldrich (Germany). The solution needed was prepared in the physiology laboratory where the experiment was conducted and all chemicals were of analytical grade.
The instruments (dissecting set, titration apparatus, magnifying lens, weighing balance, and centrifuge) used in this study were obtained from the Department of Physiology, Faculty of Basic Medical Sciences, College of Medicine, Ambrose Alli University, Ekpoma. Others such as plastic basket, Wattman No. 1 filter paper, orogastric cannula, plates for food and water, syringes, centrifuge bottles and hand gloves were obtained from a local store.

Preparation of extract
The extract preparation was as described by Ekpenyong *et al*.[12] with little modifications. The fresh leaves were rinsed to remove sand and other debris and pulverized into powder using electric blender to give a gram weight of 865g. This was soaked in 2 liters of distilled water and allowed to stand overnight for about 12 hours. The mixture was sieved with Wattman No. 1 filter paper. The filtrate was dried by heating in water bath at 40°C to obtain a solid extract. The solid extract was weighed with an electronic weighing balance and the stock solution was prepared. The stock solution was prepared by dissolving 15g of extract in 10ml of water to give concentration of 1500mg/ml. The solution was well labeled and preserved in a refrigerator at 4°C until required for use.

Acute toxicity test
Lorke's method with a few modifications was used to evaluate the acute oral toxicity of *T. occidentalis* using twelve rats. The method involved two phases, phase l and 2. In phase 1, three treatment groups with three rats each were used. They were administered 10 mg/kg, 100 mg/kg and 1000 mg/kg of the extract respectively per group. They were observed for mortality or general signs of toxicity for 24 hours. In phase 2, three treatment groups of one animal each were treated with 1500 mg/kg, 3000 mg/kg and 5000 mg/kg of the extract respectively, and observed for 24 hours. The median lethal dose of the plant was greater than 5000 mg/kg body weight. The aqueous extract did not cause death or signs of toxicity in the rats. This shows that *Telfairia occidentalis* has a wide safety margin.

Experimental Animals
Male *spraque dawley* rats weighing between 150g to 200g were procured from Animal Farm. The animals were fed standard diet (Grower's mash) and water given *ad libitum.* They were housed in well-ventilated cages (each with a dimension of 45cm x 45cm x 45cm) under standard environmental conditions in a well-ventilated room under a 12/12 hours light/dark cycle and allowed two weeks of acclimatization.

Gastroprotective Study Grouping
All animals were fasted for 18-24 hours in all studies and had free access to water up to 1 hour before the commencement of each study.[13] The animals were divided into 4 groups of 5 rats each as follows:
Group A: Received no treatment.
Group B: Served as the test control. Ulcer was induced in this group with single oral administration of 5ml/kg body weight 75% absolute ethanol without treatment and then sacrificed after one hour.[14]
Group C: Served as the test. The animals in this group received (500mg/kg) of aqueous leave extract of *Telfairia occidentalis* for 7 days without ulcer induction.
Group D: Served as pre-treated test group. In this group of animals, ulcer was induced with single oral administration of 5ml 75% ethanol solution after pre-treating the animal with (500mg/kg) of aqueous leave extract of *Telfairia occidentalis* daily for 7 days.[14] One hours after the induction of ulcer the animals were scarified.

Duration of Study
The study (from animal procurement to the end of actual experiment) lasted for about twenty weeks. However, actual animal experiment lasted for about one, based on the fact that gastric epithelium is renewed every 2 – 4 days.[15]

Experimental Procedure
Following two weeks of acclimatization, animals in each groups received their corresponding treatments. Thereafter; all animals were scarified via cervical decapitation. All suspension was given orally according to their body weight by oro-gastric iron cannula and was prepared within 15 to 30minutes before use.

Sample Collection
At the end of the treatments, animals were sacrificed and the stomach harvested following standard laboratory procedures. The stomachs were obtained for the determination of gastric acid, ulcer indices, macroscopic, histological evaluation, oxidative stress, antioxidant enzymes, PGE2, gastric wall mucus, IL-8, (interleukin) and TNF-α (tumor necrosis factor-α). The research protocols were carried out at the Ambrose Alli University, Ekpoma according to the rules in Nigeria governing the use of laboratory animals as acceptable internationally.[16]

Sample Analysis
a. Evaluation of gastric wall mucus
The gastric wall mucus was evaluated according to.[17] The glandular portion of stomach was weighed and transferred immediately to 10 ml of 0.1% w/v Alcian blue solution (0.16 M sucrose solution buffered with 0.5 ml of sodium acetate at pH 5). The tissue was kept in Alcian blue for 2 hours, and the excess colour was eliminated by consecutively rinsing it twice with 10 ml of 0.25 M sucrose. Dye combined with gastric wall mucus was extracted with 10 ml of 0.5 M magnesium chloride, which was irregularly shaken for 1 minute at 30-minute interval for 2 hours. Four milliliters of blue concentrate was then strongly shaken with an equivalent volume of diethyl ether. The achieved emulsion was spined at 3000 $\times g$ for 10 minutes, and the absorbance of the fluid layer was recorded at 580 nm. The amount of Alcian blue concentrated for every gram of wet glandular tissue was afterwards calculated.

b. Evaluations of gastric acidity in stomach
The stomach contents were collected into a centrifuge bottle after opening the stomach along the greater curvature. The stomach was then rinsed with 5ml of Normal Saline and the mixture mixed properly and then centrifuged at 5000 revolutions per minute for 3 minutes. Titration was then performed on the supernatant by obtaining 2mls (Volume of Acid - V_A) into a conical flask and 1 drop of phenolphthalein indicator was then added to the supernatant and shake to mix properly. The resultant mixture (2ml of supernatant + 1 drop of phenolphthalein) was titrated against 0.0025M NaOH (Concentration of the Base - C_B) obtained from 1M NaOH (stock solution). The colour change was noted at the end point and the volume of base added to cause the colour change was recorded (Volume of Base - V_B). This was repeated twice on each animal sample and the average was taken and recorded. Gastric acidity (C_A) was calculated using the formula

$$C_A V_A = C_B V_B$$

(where C_A = Concentration of the gastric acid- which was unknown; V_A = Volume of Acid- 2ml; C_B = Concentration of the Base- 0.0025M NaOH; and V_B = volume of base- the volume that cause the colour change).

Making C_A the subject of the formula, the above formula becomes:

$$C_A = \frac{C_B V_B}{V_A}$$

c. Measurement of gastric Lesions

Each gastric sample was placed on a slide and then captured into a computer. The gastric damage area (mm2) was determined with "Image J" image processing software. The Ulcer Index (UI) for each rat was calculated with the following formula:

$$UI = \left(\frac{TAML\ (mm^2) \times 100)}{TMA\ (mm^2)}\right)$$

Where TMA is the total mucosal area and TAML the total area of mucosal lesion of each rat.[18]

The percentage protective ratio (PPR) of a given drug was calculated by the equation of Adinortey *et al*.[19]

$$\%UI = \left(\frac{UI\ of\ Ulcer\ control - UI\ of\ treated}{UI\ of\ ulcer\ control}\right) \times 100\%$$

d. Determination of oxidative stress markers and gastric endogenous antioxidants activity

The stomachs were harvested and devoid of fat and accessory tissues. They were then patted dry with tissue paper and weighed and placed in a plain bottle containing homogenize buffer solution (phosphate buffer 1:10 w/v). The stomach was homogenized (grind using homogenizer machine) and the content (homogenate) centrifuged at 3000 rpm for 10 minutes to obtain the supernatant and stored at minus 20°C. This was used for the determination oxidative stress. The protein concentration of the homogenate samples was determined by means of the Biuret method as described by[20]. 5.0ml of blank Biuret reagent was prepared by dissolving $CuSO_4$.5H2O crystals in 500mls of distilled water added to sample blank. These was mixed well and allowed to stand for 20 minutes at room temperature 25-27°C. Absorbance was read for test and standard against a blank at 540nm. The concentration of protein was calculated using:

$$\frac{Optical\ density\ for\ standard\ \times concentration\ of\ standard}{Optical\ density\ for\ test\ sample}$$

Malondialdehyde, MDA (an index of lipid peroxidation) was determined using the method of Buege and Aust.[21] l.0ml of the supernatant was added to 2ml of the Tricarboxylic acid-Thiobarbituric acid-Hydrochloric acid reagent. (TCA - TBA-HCL) reagent boiled at 100°C for 15 minutes and allowed to cool. Flocculent materials were removed by centrifuging at 3000rpm for 10 minutes. The supernatant was removed and the absorbance read at 532nm against a blank. MDA was then calculated using the molar extinction coefficient for MDA – TBA — Complex of 1.56 x $105M^{-1}$ CM^{-1}.

The method used for analysis of *Superoxide Dismutase* was as described by Misra and Fridovich.[22] The reaction mixture (3 ml) contained 2.95 ml 0.05 M sodium carbonate buffer pH 10.2, 0.2 ml of homogenate solution and 0.3 ml of epinephrine in 0.005M acetic acid was used to initiate the reaction. Change in absorbance was recorded at 1 min interval for five minutes.The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate (epinephrine) and 0.2 ml of water. Enzyme activity was calculated by measuring the change in absorbance at 480 nm for 5 min. The slope of the linear part of graph of absorbance against time was used to obtain specific unit of activity which was expressed as U/min/ml per mg protein.

Catalase activity was determined according to the method described by.[23] This was done by measuring the decrease in absorbance at 570nm due to the decomposition of H_2O_2 in a UV recording spectrophotometer (Jenway 6305). The reaction mixture (1.32 ml) contained 0.130 ml of tissue homogenate in phosphate buffer (50 mM, pH 7.4) and 0.530 ml of 200 mM H_2O_2 in 0.660ml phosphate buffer pH 7.0. Change in absorbance was used as extent of decomposition of H_2O_2 extrapolated from a standard curve. Rate of 34 decomposition of catalase was used to obtain activity of catalase. Specific activity of catalase was expressed as mill moles of H_2O_2 decomposed per min/ ml /mg protein.

e. Determination of gastric PGE2 concentration

Enzyme-linked immunosorbent assay kit (CUSABIO Biotech Co., Ltd, China) was utilized for measurement of gastric (stomach tissue homogenate supernatant) content of PGE2 as described by the instruction provided by the manufacturer.

f. Determination of gastric IL-8 and TNF-α

Enzyme-linked immunosorbent assay kits (CUSABIO Biotech Co., Ltd, China) were utilized for measurement of gastric (stomach tissue homogenate supernatant) content of IL - 8 and TNF-α as described by the instruction provided by the manufacturer.

h. Macroscopic (Gross) evaluation of gastric lesions

The stomachs were washed with saline water and examined for macroscopical mucosal lesions using magnifying lens. Ulcers of the gastric mucosa appear as inflammation and as elongated bands of hemorrhagic lesions parallel to the long axis of the stomach rugus.

Involvement of the NO, cGMP, K_{ATP} pathways in gastroprotection

The role of NO, cGMP, K_{ATP} in gastroprotective effects of aqueous extract of *Telfairia occidentalis* were investigated. Animals were distributed into five groups of 5 rats each. These groups of rats were subjected to intraperitoneal treatment with the following drugs:

Group A (control).

Group B was administered 8% tween 80, 10 mLs/kg.

Group C was administered L-NAME (N-nitro-Larginine methyl ester 70 mg/kg, a NO synthase inhibitor),

Group D was administerd ODQ (1H[1,2,4] oxadiazolo [4,3-a]quinoxaline-1-one 10 mg/kg, a guanylate cyclase inhibitor).[24-26]

Group E was was administered glibenclamide (K_{ATP} channel blocker, 3 mg/kg).

1 hour later groups B, C, D and E were administered *Telfairia occidentalis* extract (500mg/kg) as documented in Ortiz *et al*.[24,] After 60 mins, groups B, C, D and E were orally treated with 5mL/kg body weight 75% absolute ethanol for gastric ulcer induction. The rats were sacrificed 1 hour after ethanol administration, and the stomachs removed for laboratory analysis.

Involvement of non-protein sulfhydryl compounds (NP-SH) or calcium ion channels in gastroprotection

The role of non-protein sulfhydryl compounds (NP-SH) or calcium ion channels in gastroprotection was investigated as follows. The animals were distributed into four groups (n = 6), and subjected to intraperitoneal treatment with the following drugs:

Group A (control) was administered 8% tween 80, 10 mL/kg

Group B was administered 8% tween 80, 10 mL/kg

Group C was administered NEM (N-ethylmaleimide 5 mg/kg, a NP-SH compounds blocker)

Group D was administered verapamil (a calcium channel blocker, 5 mg/kg).

1 hour later, *Telfairia occidentalis* extract (500mg/kg; to all groups b) were administered to groups B, C, and D.

60 minutes after, groups B, C and D were orally treated with 5mL/kg body weight 75% absolute ethanol for gastric ulcer induction. The rats were sacrificed 1 hour after ethanol administration, and the stomachs removed for laboratory analysis.

Macroscopic (Gross) and Histological Presentations

Macroscopic observations of the gastric mucosa were represented in pictures and histological observation in slide viewed under the microscope.

Statistical Analysis

The Statistical Package for Social Sciences (SPSS version 20) was used for data analysis. The one-way analysis of variance (ANOVA) was employed for data analysis and where applicable LSD was determined and confidential interval of $p \leq 0.05$ considered statistically significant. Results were presented as mean ± standard error of mean using suitable tables and charts.

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Results and Discussion

Peptic ulcer diseases comprise heterogeneous disorders, which manifest as a break in the lining of the gastrointestinal mucosa bathed by acid and pepsin. It is the most predominant of the gastrointestinal diseases.[27] Several orthodox pharmaceutical drugs such as anticholinergic drugs, histamine H2-receptor antagonists, antacids, and more recently, proton-pump inhibitors have been employed in the management of peptic ulcers, but they provoke many adverse effects. In view of this challenge, there has been growing interest in alternative therapies especially from plant sources due to their perceived lower side effects, ease of accessibility and affordability.[5]

Ethanol is considered a risk factor for developing gastric ulcers. It readily penetrates the gastric mucosa due to its ability to solubilize the protective mucous and expose the mucosa to the proteolytic and hydrolytic actions of hydrochloric acid and pepsin,[28] causing damage to the membrane.[29] Moreover, alcohol stimulates acid secretion and reduces blood flow leading to microvascular injuries, through disruption of the vascular endothelium and facilitating vascular permeability; it also increases activity of xanthine oxidase. Ethanol also triggers imbalances in cellular antioxidant processes. For example, it causes the release of superoxide anion and hydroperoxy free radicals, and hence increased oxidative stress in the tissues, evidenced by increased levels of malondialdehyde, a marker of increased lipid peroxidation.[30,31]

Gastric acidity in ethanol induced ulcer

Table 1 shows the effect of aqueous leave extract of *Telfairia occidentalis* on gastric acidity after ethanol induced gastric damage. The increase in the gastric acidity observed in ethanol and the pretreatment groups were mild and insignificant. Therefore the result of this study regarding gastric acidity showed that there was no significant difference in gastric acidity across the groups. Similarly, the result of this study as expressed in figure 1, does not suggest any significant effect on gastric acidity in the different treatment groups.

Table 1: The effect of aqueous leaf extract of *Telfairia occidentalis* on gastric acidity after ethanol induced gastric damage

Groups	Gastric HCl (mEq/L)
Control	11.64 ± 3.68
Ulcer untreated	12.67 ± 1.59
500 mg/kg *T. occidentalis*	9.25 ± 21.93
500 mg/kg *T. occidentalis* + Ulcer	18.62 ± 1.63

Values are mean ± SEM; n = 5

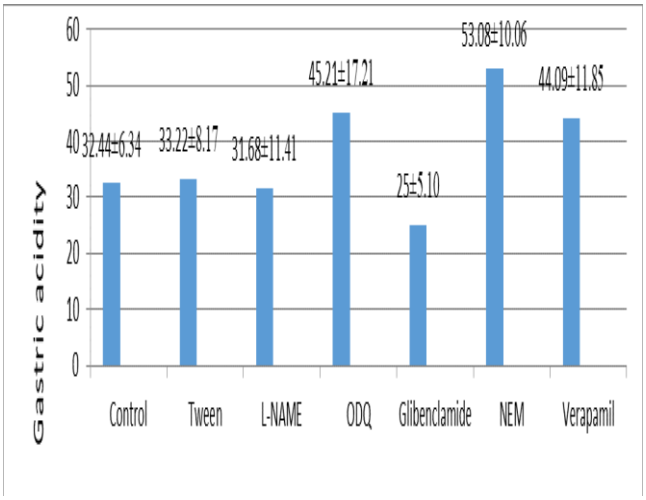

Figure 1: The effect of aqueous leaf extract of *T. occidentali* on gastric acidity after treatment with NO, cGMP and K+ATP, NP-SH, or calcium ion channel blocker.

Effect of Telfairia occidentalis on Gastric wall mucus contents in ethanol induced ulcer

Figure 2 compares the gastric wall mucus contents in the different treatment groups compared with the control. Compared to the control (250.94±3.31 µg of alcian blue/g of wet gland), there was significant lower ($p<0.05$) gastric wall mucus content in the ulcer untreated (63.40±6.15 µg of alcian blue/g of wet gland) and the ulcer group pre-treated with 500mg/kg TO (110.43±6.63 µg of alcian blue/g of wet gland) while the 500mg/kg TO treated group (282.09±6.54 µg of alcian blue/g of wet gland) presented significant higher ($p<0.05$) gastric wall mucus content compared to the control group in ethanol induced ulcer.

This study showed that 500mg/kg aqueous leave extract of *Telfairia occidentalis* increases gastric mucus production (Fig 2). It demonstrated that the 500mg/kg aqueous leave extract of *Telfairia occidentalis* may serve gastroprotective function via stimulating gastric mucus production.

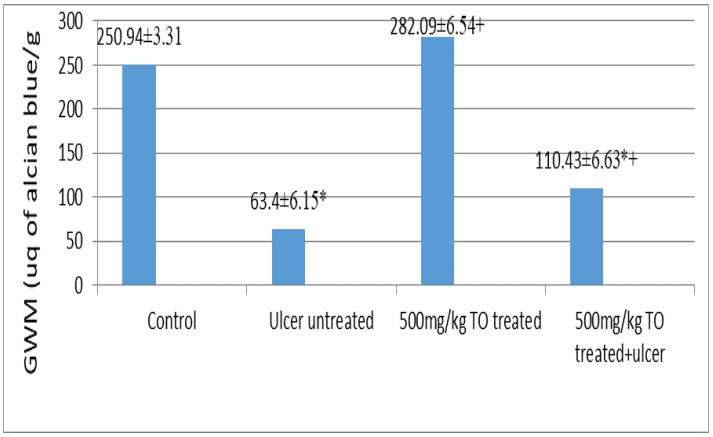

Figure 2: Gastric wall mucus content in the different treatment groups compared with control.

Effect of aqueous leaf extract of Telfairia occidentalis on ulcer parameter in ethanol induced gastric ulcers.

Table 2 shows the effect of aqueous extract of *Telfairia occidentalis* on ulcer index in ethanol induced gastric ulcer. The table showed a significant increase ($p<0.05$) in ulcer index in ulcer untreated (44.24±2.98mm^2) and pretreated (22.46±2.14mm^2) groups compared to the control (0.03±0.05mm^2), while the 500mg/kg *TO* (3.99±7.49mm^2) as well as the 500mg/kg *TO* pretreated groups (22.46±2.14mm^2) showed a significant decrease in the ulcer area compared to the ulcer untreated group (44.24±2.98mm^2). The ulcer parameter also revealed a significant decrease in the ulcer index in the group pretreated with the extract prior to ethanol induced ulceration, while the percentage protective ratios were as high as 60.64% (fig. 3) in the pretreatment group. This revelation further suggests gastroprotective capacity of aqueous leaf extract of *Telfairia occidentalis*

Table 2: The effect of aqueous leaf extract of *Telfairia occidentalis* on ulcer index in ethanol induced gastric damage

Groups	Ulcer index (mm^2)
Control	0.03 ± 0.02
Ulcer untreated	44.24 ± 2.98*
500 mg/kg *T. occidentalis*	3.99 ± 3.35$^+$
500 mg/kg *T. occidentalis* + Ulcer	22.46 ± 2.14*$^+$

Values are mean ± SEM; n = 5; * indicates significant difference at $p<0.05$ compared with control (group A); + indicates significant different at $p<0.05$ compared with group B.

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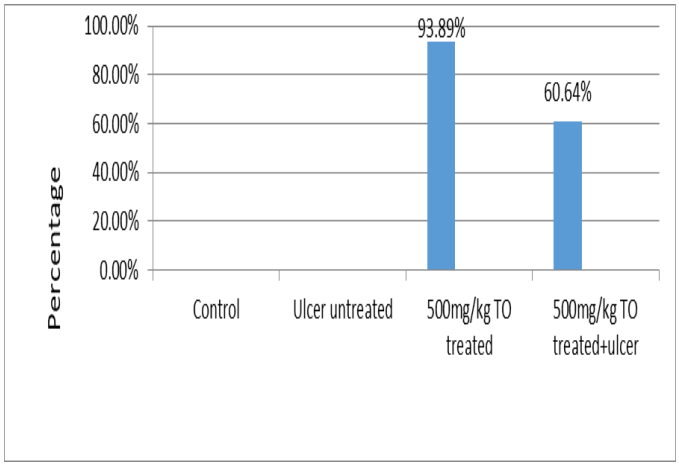

Figure 3: Percentage protective ratio of aqueous leaf extract of *T. occidentalis* on ethanol induced gastric ulcer.

Effect of Telfairia occidentalis on Gastric oxidative stress indicators

Table 3 compared the gastric protein and MDA respectively in the different treatment groups compared with the control. Gastric protein was significantly lower ($p<0.05$) in the ulcer untreated group ($1.62\pm0.02^{*}$ mg/g protein) compared to the control (2.169±0.04 mg/g protein), a significant increase in gastric protein level in the 500mg/kg TO treated group ($2.43\pm0.04^{*+}$ mg/g protein) compared to control (2.169±0.09 mg/g protein) and ulcer untreated group ($1.62\pm0.02^{*}$ mg/g protein). There was a significant decrease ($p<0.05$) in gastric protein level in 500mg/kg pretreated group ($1.85\pm0.04^{*+}$ mg/g protein), but significant increase compared to ulcer untreated group ($1.62\pm0.02^{*}$ mg/g protein). There was significant increase ($p<0.05$) in gastric MDA level in ulcer untreated group ($11.15\pm1.14^{*}$) compared to the control groups (4.90±0.17 mg/g protein), a significant increase in 500mg/kg TO treated ($5.34\pm0.28^{+}$ U/ng protein) compared to control (4.90±0.17 U/ng protein). There was a significant increase in MDA level in ulcer group pre-treated with 500mg/kg *TO* ($6.46\pm0.28^{*+}$ U/ng protein) compared to the control (4.90±0.17 U/ng protein), but significant decrease compared to ulcer untreated ($11.15\pm1.14^{*}$ U/ng protein) group. On oxidative stress markers, the extract was observed to stimulate gastric protein and to significantly stimulate gastric protein prior to gastric damage (Table 3); this means that the extract suppressed oxidative stress. It also showed a significant decrease in gastric MDA when the extract was ingested prior to induce gastric damage (Table 3). This further confirmed our earlier reports on ameliorative potentials of *Telfairia occidentalis.*[9,10,32] This finding suggests the extract contains some antioxidant potentials. To support this assertion, several studies have reported *Telfairia occidentalis* to be a rich source of antioxidants and vitamins.[5,11] Thus, the decrease in gastric MDA may be owned to its antioxidants and vitamins component. The result of this study also showed a reversal effect on gastric protein following treatment with NEM (NP-SH compounds blocker) as presented in fig 4.

Table 3: Gastric protein and MDA as Markers of Oxidative Stress in the different treatment groups compared with the control

Groups	Protein (mg/g protein)	MDA (µg/mg protein)
Control	2.17 ± 0.04	4.90 ± 0.17
Ulcer untreated	1.62 ± 0.02*	11.15 ± 1.14*
500 mg/kg *T. occidentalis*	2.43 ± 0.04*+	5.34 ± 0.28+
500 mg/kg *T. occidentalis* + Ulcer	1.85 ± 0.04*+	6.46 ± 0.28*+

*Values are mean ± SEM; n = 5; * indicates significant difference at p<0.05 compared with control (group A); + indicates significant different at p<0.05 compared with group B.*

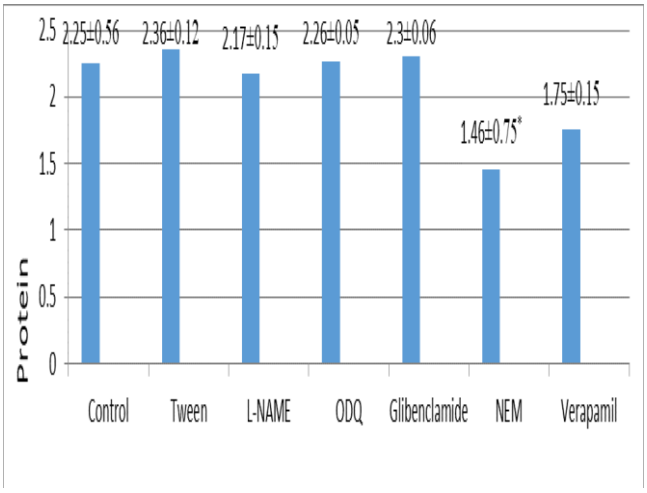

Figure 4: The effect of aqueous leaf extract of *T. occidentalis* on gastric protein after treatment with NO, cGMP and K+ATP, NP-SH or calcium ion blocker.

NP-SH, mainly GSH, is the antioxidant compound involved in the maintenance of gastric integrity.[33] It controls the cascade of inflammatory cytokines and promotes detoxification and excretion of ROS produced by aggressors such as ethanol, indomethacin, and stress.[34,35,36] Therefore, the reverted effect observed in gastric protein suggests possible involvement of NP-SH compound in mediating gastroprotective effect of *Telfairial occidentalis.*

Gastric endogenous antioxidants effect of aqueous leaf extract of Telfairia occidentalis on ethanol-induced gastric ulcers

Table 4 compared the gastric SOD and Catalase activities in the different treatment groups compared with the control. The ulcer untreated group showed a significant decrease ($p<0.05$) in SOD activity (144.25±1.80 U/mg protein) compared to control (204.80±0.69 U/mg protein). There was a significant increase in the SOD activity of 500mg/kg *TO* treated group (218.62±4.47) compared to the ulcer untreated group (144.25±4.02 U/mg protein). While the 500mg/kg of *T. occidentalis* pretreated group (152.31±4.0 U/mg protein) showed a significant decrease ($p<0.05$) in SOD activity compared to control group (204.80±0.69 U/mg protein). The ethanol untreated group showed a significant decrease ($p<0.05$) in CAT activity (205.42±6.06) compared to control (419.85±3.81 U/mg protein). The 500mg/kg (458.99±6.51) *TO* showed a significant increase ($p<0.05$) in CAT activity compared to the ulcer untreated group (205.42±6.06 U/mg protein), while the 500mg/kg *TO* pretreated group (294.72±9.97) showed a significant decrease ($p<0.05$) in CAT activity compared to control (419.85±3.81).

Table 4: Gastric endogenous antioxidants effect of aqueous leaf extract of *Telfairia occidentalis* on ethanol-induced gastric ulcers

Groups	SOD (U/mg protein)	CAT (U/mg protein)
Control	204.80 ± 0.69	419.85 ± 3.81
Ulcer untreated	144.25 ± 1.80*	205.42 ± 6.06*
500 mg/kg *T. occidentalis*	218.62 ± 4.47+	458.99 ± 6.51+
500 mg/kg *T. occidentalis* + Ulcer	152.31 ± 4.00*	294.72 ± 9.97*

*Values are mean ± SEM; n = 5; * indicates significant difference at p<0.05 compared with control (group A); + indicates significant different at p<0.05 compared with group B.*

The extract was shown to stimulate gastric antioxidants status when ingested prior to induce gastric damage. These findings suggest that the extract may boost antioxidant status and might produce gastroprotective potential on the long run. The extract was observed to stimulate gastric SOD and CAT (Table 4) activities which are known

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to protect against gastric damage. This endogenous antioxidant potential may be due to the constituent present in the extract. These stimulatory beneficial effects of the extract on endogenous antioxidant may be attributed to the presence of high amount of polyunsaturated fatty acids, including linoleic and α-linolenic acid (ALA), along with considerable amounts of tocopherols, polyphenols, flavonoids, tannins and carotenes in the extract.[37,3]

Effect of aqueous leaf extract of Telfairia occidentalis on Gastric PGE2 content in ethanol-induced gastric ulcers

Table 5 compares the gastric PGE2 contents in the different treatment groups compared with the control. Compared to the control (555.06±4.88 ng/mg protein), there was significant decreased ($p<0.05$) gastric PGE2 concentration in the ulcer untreated (501.70±9.61* ng/mg protein) and 500mg/kg *TO* pretreated group (514.67±4.31). However, the 500mg/kg *TO* treated group (576.91±6.19) showed a significant increase ($p<0.05$) in gastric PGE2 concentration compared to the ulcer untreated group (501.70±9.61) The findings on gastric PGE2 production in this study point toward gastroprotective potential of the extract against induced gastric damage (Table 5). The PGE2 stimulatory effect of the extract may be owned to the chemical components of the plant. This assertion is based on the fact that polyphenolic compounds have been reported to have a beneficial role in gastric ulcers, as it has been suggested that phenols stimulate PG formation.[39] Thus, the extract may serve gastro protective function on the long run considering the stimulatory effect of PGE2. The result of this study (figure 5) also shows the reversal of what was previously reported in table 5, following treatment with glibenclamide (K_{ATP} channel blocker). This reversal, suggests possible involvement of K_{ATP} channel in mediating the gastroprotective effect of *Telfairia occidentalis*.

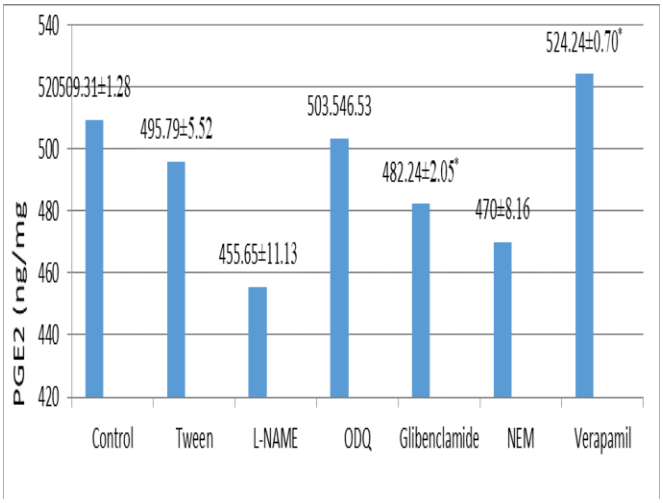

Figure 5: The effect of aqueous leaf extract of *T. occidentalis* on gasstric PG-E2 after treatment with NO, cGMP and K_{ATP}, NP-SH or Calcium ion blocker.

Table 5: Effect of aqueous leaf extract of *Telfairia occidentalis* on Gastric PGE2 contents in ethanol-induced gastric ulcers

Groups	Gastric PGE_2 (ng/mg protein)
Control	555.06 ± 4.88
Ulcer untreated	501.70 ± 9.61*
500 mg/kg *T. occidentalis*	576.91 ± 6.19^{+}
500 mg/kg *T. occidentalis* + Ulcer	514.67 ± 4.31*

*Values are mean ± SEM; n = 5; * indicates significant difference at p<0.05 compared with control (group A); + indicates significant different at p<0.05 compared with group B*

Effect of aqueous leaf extract of Telfairia occidentalis on Gastric IL-8 and TNF-α concentration in ethanol-induced gastric ulcers

Table 6 compared the gastric IL-8 and TNF-α concentration respectively in the different treatment groups compared with the control. There was a significant increase ($p<0.05$) in IL-8 and TNF-α in all the groups compared to control. Significant decrease in IL-8 in the 500mg/kg *TO* treated group (79.12±1.99 pg/g tissue) and 500mg/kg *TO* pretreated group (101.50±2.50 pg/g tissue) compared to ulcer untreated group (128.42±5.74 pg/g tissue), significant decrease in TNF-α in 500mg/kg *TO* treated group (96.57±1.57 pg/g tissue) compared to ulcer untreated group (167.68±1.64 pg/g tissue), but a significant increase in TNF-α in 500mg/kg *TO* pretreated group (181.61±1.22) compared to the ulcer untreated group (167.68±1.64 pg/g tissue). Cytokines are produced mainly by macrophages and the Th cells in response to inflammatory stimuli[40]. Macrophages produce pro-inflammatory cytokines, which include among others TNF-α and IL-6 and anti-inflammatory cytokines such as IL-10[41]. TNF-α has a central role in initiating the cascade of other cytokines and factors that make up the immune response to infection. It is involved in many forms of injury to the gastric mucosa associated with infection with H.pylori, use of NSAIDs,[42] and ethanol.[43] The inhibition of the TNF-α synthesis results in the reduction of the harmful effect of these ulcerogenic agents.[42] Brzozowska *et al*.,[44] showed that the levels of pro-inflammatory cytokines such as IL-1β and TNF-α in diabetic rats are higher, resulting in persistent inflammatory reaction that delays the healing of the ulcer. The present study has shown a decreased IL8 level (Table 6) in the extract pretreated (group D) compared to ulcer untreated (group B). IL8 and TNF-α, are members of a group of cytokines that are involved in inflammation, act synergistically and stimulate acute phase reaction.

Table 6: Effect of aqueous leaf extract of *Telfairia occidentalis* on Gastric IL-8 and TNF-α concentration in ethanol-induced gastric ulcers

Groups	Gastric IL-8 (pg/g tissue)	TNF-α (pg/g tissue)
Control	54.27 ± 1.80	66.75 ± 0.40
Ulcer untreated	128.42 ± 5.74*	167.68 ± 1.64*
500 mg/kg *T. occidentalis*	79.12 ± 1.99*$^{+}$	96.57 ± 1.57*$^{+}$
500 mg/kg *T. occidentalis* + Ulcer	101.50 ± 2.50*$^{+}$	181.61 ± 1.22*$^{+}$

*Values are mean ± SEM; n = 5; * indicates significant difference at p<0.05 compared with control (group A); + indicates significant different at p<0.05 compared with group B.*

The effect of aqueous leaf extract of Telfairia occidentalis on gastric NO after treatment with NO, cGMP and K_{ATP}, NP-SH or Calcium ion channel blocker

Figure 6 compares the gastric NO levels in the different groups, after treating the groups with various blockers. The result showed a significant decrease ($p<0.05$) in gastric NO in the group treated with verapamil (18.03±5.99) compared with control group (35.65±0.99). This shows that NO production might be mediated through the Ca^{2+} channels. NO is considered to be one of the most important defensive endogenous agents in the gastric mucosa.[44] It is synthesized by the enzyme nitric oxide synthase (NOS) from L-arginine.[35] NO, together with the prostaglandins, preserves gastric mucosa integrity.[45,46] The constitutive forms of NOS, neuronal NOS (nNOS) and endothelial NOS (eNOS), play a physiological role in the homeostasis of the gastrointestinal tract, and the inhibition of these enzymes can result in disturbance of gastrointestinal motility, blood flow, and acid secretion.[47] The result of this study (Fig. 6) showed a significant decrease in gastric NO when verapamil (Ca^{2+} channel inhibitor) was applied could only mean that Ca^{2+} channel is possibly involved in mediating gastroprotective effect of *Telfairia occidentalis.*

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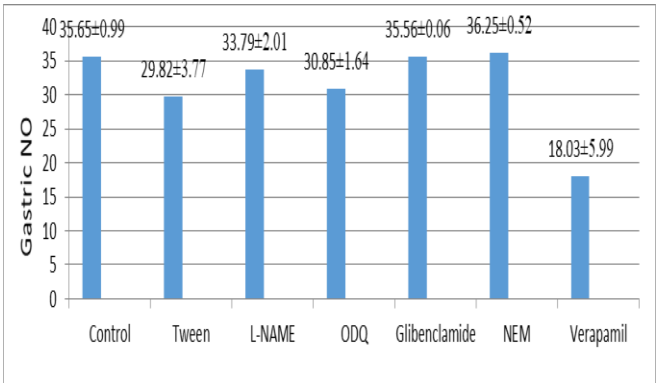


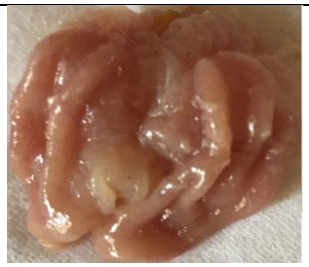
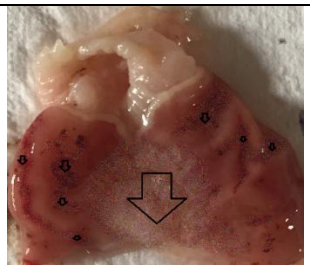
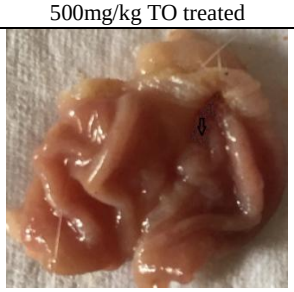
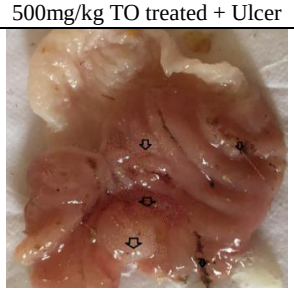
Figure 6: The effect of aqueous leaf extract of *T. occidentalis* on gastric NO after treatment with NO, cGMP and K_{ATP}, NP-SH or Calcium ion blocker.

Gastric macroscopic effect of treatments with aqueous leaf extract of Telfairia occidentalis on ethanol-induced gastric ulcers

Table 7 showed the effect of the *Telfairia occidentalis* extract on the gastric mucosa in various treatment groups and control. The macroscopic evidence revealed the ameliorative effect of the extract in the gastric mucosa of the pretreatment group compared with others as it shows less extensive inflammatory areas in the pretreatment group. This evidence further buttressed the protective potential of the plant *extract*.

Different mechanisms of gastric cytoprotection have been suggested; including increased gastric mucosal blood flow, free radical scavenging, and stimulation of cell growth and repair.[48] This study, is consistent with previous findings. Administration of 75% ethanol by orogastric cannula produced marked damage in the gastric mucosa of rats, characterized mainly by elongated macroscopic lesions with intense hemorrhaging and hyperemia, as well as loss of mucus.[49-51] Pre-treatment of rats for seven days with *TO* markedly attenuated gastric damage as evidenced in the above result. This gastroprotective effect is not without relationship with the components of the extract. This assertion is based on the fact that *Telfairia occidentalis* is rich in minerals, antioxidants, vitamins and essential oils.[5,11] Thus, the gastro protective effect with pre-treatment may be owned to its antioxidants and vitamins component.

Table 7: Gastric macroscopic effect of treatments with aqueous leaf extract of *Telfairia occidentalis* on ethanol-induced gastric ulcers

Control	Ulcer untreated	500mg/kg TO treated	500mg/kg TO treated + Ulcer
 Normal gastric mucosa	 Broad and extensive inflammatory areas	 Narrow inflammatory area	 Spread but less extentive inflammatory areas

The arrow represents the inflammatory/lesion area.

Conclusion

The results of this study, indicate that *TO* has protective effect against the ulcerative lesions induced by ethanol on gastric mucosa, its probable mechanism of action include stimulation of increased gastric wall mucus production via increased prostaglanding synthesis, increased activities of endogenous antioxidant, increased gastric protein, attenuation of free radicals and inflammatory cytokine, (IL-8). It is also possibly involving NP-SH, K_{ATP}, and Ca^{2+} channel to mediate its gastroprotective mechanism.

Conflict of interest

The authors declare no conflict of interest

Authors' Declaration

The authors her eby declare that the work presented in this article is original and that any liability for claims relating to the content of this article will be borne by them

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