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Research Article

Effect of *Telfairia occidentalis* on Markers of Oxidative Stress in Indomethacin Induced Gastric Ulcer

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ABSTRACT

Gastric ulcer is a common health challenge in terms of both morbidity and mortality. In this study, male Sprague Dawley rats of between 180-200g weights were used. The rats were divided into 4 groups of 5 rats each; group A(control) and Group B (test control) received single dose of 40mg/kg indomethacin without treatment for four hours. Group C received 500mg/kg aqueous leaf extract of *Telfairia occidentalis* for 7 days without ulcer induction, while Group D (pre-treated test group) received 40mg/kg indomethacin after pre-treated with 500mg/kg aqueous leaf extract of *Telfairia occidentalis* daily for 7 days. At the end of the experiment, the animals were sacrificed to harvest the stomachs for laboratory analysis. Comparatively, the results revealed that *Telfairia occidentalis* (group D) decreased oxidative stress in group D (pretreated group), increased gastric endogenous antioxidant level in group C (extract group), and showed apparent protective effect on gastric macroscopic architecture compared to test control group B. Judging by the findings of this study, 500mg/kg aqueous leave extract of *Telfairia occidentalis* was gastroprotective against indomethacin induced gastric ulcerations.

Keywords: *Telfairia occidentals, gastric ulceration, oxidative stress, gastric endogenous antioxidant, indomethacin*

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INTRODUCTION

Gastric ulcer is a health burden (Lanas and Chan, 2017) and one of the major causes of morbidity and mortality (Miami *et al*., 2016), with known capacity to induce gastrointestinal bleeding when not adequately treated (Lanas *et al*. 2014). There exist reports that the etiology of gastroduodenal ulcers are influenced by factors like acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (Adisa *et al*., 2019).

Studies have shown that quite a number of human diseases including peptic ulcer occur 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 (Bhattacharyya *et al*., 2014).
The accessibility of herbs over expensive pharmaceutical drugs to treat diseases amongst the people in the rural areas is fast becoming revolutionized (Airaodion *et al*., 2019). In some countries, it has been integrated into the health scheme despite advances in orthodox medicine. It is believed that the natural products if utilized in the correct form and dosage are less harmful than synthetic products, which most often elicit some side effects (Airaodion *et al*., 2019)

One of such plants that has been extensively studied and reported to have great potential is *Telfairia occidentalis*. *Telfairia occidentalis* commonly called fluted pumpkin occurs in the forest zone of West and Central Africa. The leaf is consumed in different parts of Nigeria because of the numerous nutritional and medicinal attributes ascribed to it (Oboh, 2005). The darkish green leafy vegetable of Telfairiaoccidentalis and extracts (such as aqueous and ethanol extracts) from the leaves have 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 (Kayode *et al*., 2010).

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. Hence this research to investigate further; the effect of *Telfairia occidentalis*.

MATERIALS AND METHODS

Plant material: *Telfairia occidentalis* leaves commonly called pumpkin leaves was obtained from a local market and taken to the Forest Herbarium Ibadan at the International Institute for Tropical Agriculture, for identification and authentication with batch no: FHI.111998.

Drugs and reagents: Drugs and all other chemicals 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*. (2012) with little modifications. The fresh leaves were rinsed to remove sand and other debris, sun dried 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: Two hundred and forty male rats of 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.

After two weeks (14 days) of acclimatization to the new environment, the animals were divided into 4 groups. Group A (n = 5) served as the control and received no treatment or ulcer induction. In group B animals which served as the test control 1 (n = 5), ulcer was induced with single oral administration of 40mg/kg (Akpamu *et al*., 2016) indomethacin and scarified after four hours. Group C animals served as the extract treated (n = 5). The animals in this group received 500mg/kg of the aqueous leave extract of *Telfairiaoccidentalis* for 7 days without ulcer induction. In group D animals (ulcer treated test, n = 5 rats) ulcer was induced with 40mg/kg indomethacin (Akpamu *et al*., 2016) after pre-treating the animals with 500mg/kg aqueous leaf extract of *Telfairia occidentalis* daily for 7 days. Four hours after the induction of ulcer, the animals were scarified.

Extracts were given orally according to their body weight by oro-gastric iron cannula and 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 ulcer indices, macroscopic, oxidative stress and antioxidant enzymes. The research protocols were carried out at the Ambrose Alli University, Ekpoma according to the rules in Nigeria (Helsinki Declaration, 2008) governing the use of laboratory animals as acceptable internationally.

Sample Analysis:

Measurement of gastric Lesions: Gross gastric lesions severity was measured as described by Wilhelmi and Menasse-Gdynia (1972) using the 0 to 5 scoring system. Severity factor 1 = 1 or 2 minutes, sporadic, punctuate lesion; 2 = several small lesions; 3 = one extensive lesion or multiple moderate sized lesions; 4 = several large lesions; 5 = several large lesions with stomach perforation.

The lesions score for each rat was calculated as the number of lesions in the rat multiplied by their respective severity factor. The ulcer index (UI) for each group was taken as the mean lesion score of all the rats in that group.

Ulcer index= Total ulcer score/Number of animals ulcerated

Determination of markers of oxidative stress: 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 Gornal *et al*., (1949). 5.0ml of blank Biuret reagent was

prepared by dissolving CuSO4.5H2O 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 the formular:

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

Malondialdehyde, MDA (an index of lipid peroxidation) was determined using the method of Buege and Aust (1978). l.0ml of the supernatant was added to 2ml of the Tricarboxylic acid-Thiobarbituric acid-Hydrochloric acid reagent. (TCA - TBA-HCL) reagent boiled at 100oC 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.

Determination of gastric antioxidants activity: The method used for analysis of Superoxide Dismutase was as described by Misra and Fridovich (1972). 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 (mins).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 Sinha (1979). This was done by measuring the decrease in absorbance at 570nm due to the decomposition of H2O2 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 H2O2 in 0.660ml phosphate buffer pH 7.0. Change in absorbance was used as extent of decomposition of H2O2 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 milli moles of H2O2 decomposed per min/ ml /mg protein.

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.

Macroscopic (Gross) and Histological Presentations: Macroscopic observations of the gastric mucosa were represented in pictures.

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**..**

RESULTS

Effect of aqueous leaf extract of *Telfairia occidentalis* on ulcer parameter in indomethacin induced gastric ulcer: Figure 1 shows the effect of aqueous extract of *Telfairia occidentalis* on ulcer index in indomethacin induced gastric ulcer. The ulcer index in control is zero. The ulcer untreated group (16.51±1.83mm2) shows a significant increase ($p<0.05$) in ulcer index compared with control, the 500mg/kg TO treated group (6.01±3.84mm2) shows a significant decrease ($p<0.05$) in ulcer index compared to ulcer untreated group. There is significant increase in ulcer index for 500mg/kg TO treated + Ulcer (23.60±4.50mm2) compared to the control and ulcer untreated groups.

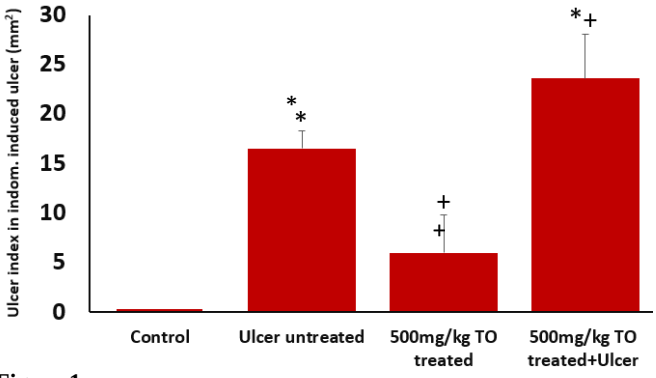


Figure 1
The effect of aqueous leaf extract of *Telfairia occidentalis* on ulcer index in indomethacin induced gastric damage
Each bar represents mean ± SEM; n = 5;
x indicates significant different at p<0.05 compared with control (group A); + indicates significant different at p<0.05 compared with group B

Protective ratio of aqueous leaf extract of *Telfairia occidentalis* on induced gastric ulcers: Figure 2 shows the percentage protective ratio of aqueous leaf extract of *Telfairia occidentalis*. The figure shows 35.45% protective capacity for 500mg/Kg TO treated (C) group, and 41.81% protective capacity for 500mg/Kg TO treated+Ulcer (D) group, in indomethacin induced ulcer.

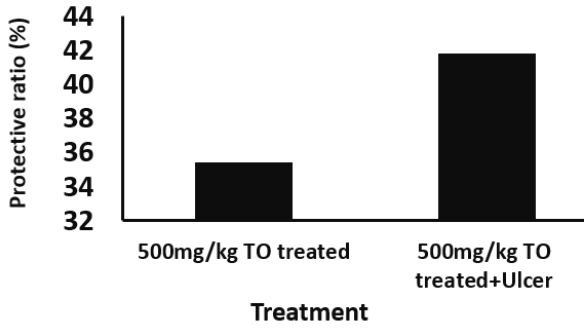


Figure 2
Protective ratio (%) aqueous leaf extract of Telfaria occidentalis on indomethacin-induced gastric ulcers

Gastric oxidative stress markers: Table 3.2 and figure 3.2 compared the gastric protein and MDA respectively in the different treatment groups compared with the control. Gastric protein was significantly higher ($p<0.05$) in the ulcer group pre-treated with 500mg/kg TO (1.77±0.06*+ mg/g protein) compared to the control (1.19±0.10 mg/g protein) or ulcer untreated group (1.39±0.07 mg/g protein). Although gastric protein level was higher in the 500mg/kg TO treated group (1.27±0.12 mg/g protein), it was not significant compared to the control. There was no significant different ($p>0.05$) in gastric MDA level across the groups, however, it was lowest in the ulcer group pre-treated with 500mg/kg TO (0.50±0.58 U/ng protein) compared to the control (0.56±0.10 U/ng protein) or ulcer untreated (0.63±0.04 U/ng protein) or 500mg/kg TO treated (0.58±0.11 U/ng protein).

Gastric endogenous antioxidants effect of aqueous leaf extract of *Telfairia occidentalis* on indomethacin-induced gastric ulcers: Table 2 compared the gastric SOD and Catalase respective in the different treatment groups compared with the control. There was significant lower ($p<0.05$) SOD activity in the ulcer untreated (92.67±14.20 U/mg protein) and the ulcer group pre-treated with 500mg/kg TO (158.22±27.60 U/mg protein) compared to the control (280.96±29.80 U/mg protein) while the 500mg/kg TO treated group (293.35±29.79 U/mg protein) has non-significant higher ($p>0.05$) SOD activity compared to the control. Gastric CAT was significantly lower ($p<0.05$) in the ulcer untreated group (384.61±34.14 U/mg protein) compared to the control (520.26±29.63 U/mg protein) but was non-significantly lower ($p>0.05$) in the 500mg/kg TO treated group (480.10±53.64 U/mg protein) and ulcer group pre-treated with 500mg/kg TO (470.03±33.74 U/mg protein).

Table 1
Gastric oxidative stress markers in the different treatment groups compared with the control

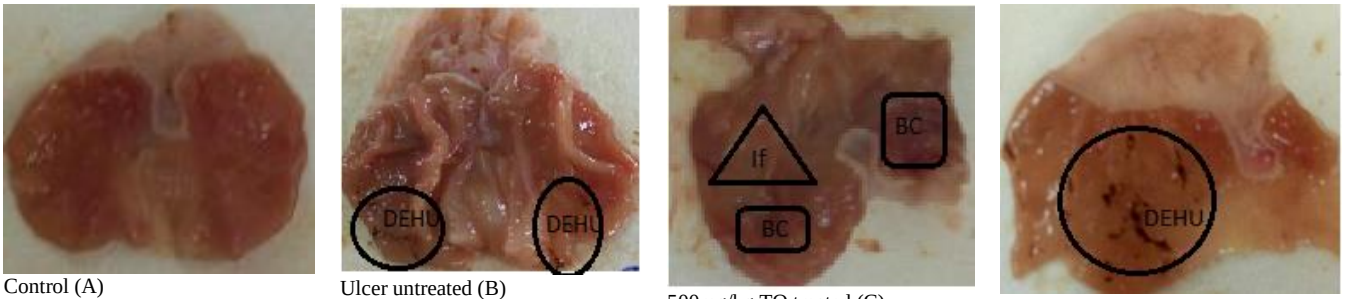
	Control (A)	Ulcer untreated (B)	500mg/kg TO treated (C)	500mg/kg TO treated+Ulcer (D)
Protein (mg/g protein.) in indomethacin induced ulcer	1.19±0.10	1.39±0.07	1.27±0.12	1.77±0.06*+
MDA (ng/ng protein.) in indomethacin induced ulcer	0.56±0.10	0.63±0.04	0.56±0.12	0.5±0.58

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

Table 2:
Gastric endogenous antioxidants effect of aqueous leaf extract of *Telfairia occidentalis* on indomethacin and ethanol-induced gastric ulcers

	Control (A)	Ulcer untreated (B)	500mg/kg TO treated (C)	500mg/kg TO treated+Ulcer (D)
SOD (U/mg protein) in indomethacin induced ulcer	280.96±29.80	92.67±14.20*	293.35±29.79^{+}	158.22±27.60*
CAT (U/mg protein) in ethanol induced ulcer	520.26±29.63	384.61±34.14*	480.10±53.64	470.03±33.74

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



Control (A)
Normal gastric mucosa

Ulcer untreated (B)
Deep and elongated hemorrhagic ulceration

500mg/kg TO treated (C)
Widely spread inflammations with mild focal blood coagulation

500mg/kg TO treated+Ulcer (D)
Severely deep and elongated hemorrhagic ulcerations

Plate 1
Gastric macroscopic effect of treatments with aqueous leaf extract of *Telfairia occidentalis* on indomethacin -induced gastric ulcer
If = inflammations, BC = blood coagulations, DEHU = deep and elongated hemorrhagic ulcerations.

Gastric macroscopy in treatments with aqueous leaf extract of *Telfairia occidentalis* on indomethacin and ethanol-induced gastric ulcers: Plate 1 shows the macroscopic observations of the protective potentials of aqueous leave extract of *Telfairia occidentalis* on indomethacin -induced gastric ulcers. Note the normal gastric mucosa in group A, deep and elongated hemorrhagic ulceration in group B, widely spread inflammations with mild focal blood coagulations in group C, and severely deep and elongated hemorrhagic ulcerations in group D.

DISCUSSION

Peptic ulcer diseases comprise heterogeneous disorders, which present as a break in the lining of the gastrointestinal mucosa bathed by acid and pepsin. It is the most common

amongst gastrointestinal diseases (Malik *et al*., 2023). Several orthodox pharmaceutical drugs have been employed in the management of peptic ulcers; however, 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 (Airaodion *et al*., 2019). Several studies have implicated reactive oxygen species (ROS) as one of the major causes of mucosal lesions and gastrointestinal damage (Bhattacharyya *et al*., 2014).

This study showed that the extract of *Telfairia occidentalis* stimulated gastric protein in indomethacin induced ulcer (Table 3.2) and decreased gastric MDA when the extract was administered prior to induce gastric damage in indomethacin induced ulcer. Gastric protein and MDA are makers of oxidative stress or ROS. This finding suggests that the extract possess the ability to scavenge ROS and therefore has antioxidant potential. This is found to be in line with the findings of Airaodion *et al*., (2019), that *Telfairia occidentalis* was able to remedy the effect of ethanol by regulating the oxidative stress biomarkers, thus possesses therapeutic effect against ethanol-induced oxidative stress and can protect the body against free radicals arising from oxidative stress. This is also consistent with the report that the leave-extract of Telfairia occidentalis has gastro-protective with minimal healing potentials mediated through reduced oxidative stress (Adisa *et al*., 2020).

The extract was also found to stimulate gastric antioxidants status when ingested prior to inducing 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 activities which are known 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 (Kaithwas and Majumdar, 2010). This assertion is supported by several studies reporting Telfairia occidentalis to be a rich source of antioxidants and vitamins (Kayode *et al*., 2010; Imosemi, 2018). Thus, the decrease in gastric MDA may be owned to its antioxidants and vitamins component.
The ulcer parameter also revealed the percentage protective ratios to be as high as 42.83% in the pretreatment group. This revelation further suggests gastroprotective capacity of aqueous leaf extract of *Telfairia occidentalis*.

Different mechanisms of gastric cytoprotection have been suggested, including increased gastric mucosal blood flow, free radical scavenging, and stimulation of cell growth and repair (Guzmán-Gómez *et al*., 2018). This study is consistent with previous findings; where administration of 40mg/kg indomethacin 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 (Almasaudi *et al*., 2016; Antonisamy *et al*., 2015). Pre-treatment of rats for seven days with TOE markedly attenuated gastric damage as evidenced in the above results. This gastroprotective effect is not without relationship with the components of the extract. This assertion is because *Telfairia occidentalis* is rich in minerals, antioxidants, vitamins and essential oils (Imosemi, 2018). Thus, the gastro protective effect with pre-treatment may be owed to its antioxidants and vitamins component.

In conclusion, the result of this study shows that TOE has protective effect against the ulcerative lesions induced by indomethacin on gastric mucosa. TO probable mechanism of action includes stimulation of increased activities of endogenous antioxidant and increased gastric protein.

Abbreviations Used

TOE, Telfairia occidentalis extract; TO, Telfairia occidentalis; IND, indomethacin

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