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GASTROPROTECTIVE EFFECT OF *CUCURBITA MAXIMA* POLYPHENOLIC EXTRACT IN DICLOFENAC INDUED-ULCER IN MALE ALBINO RATS

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ABSTRACT

Peptic ulcer disease has remained one of the most common health problems impacting a huge number of people. *Cucurbita maxima* is a tropical fruit bearing vegetable with analgesic, anti-inflammatory, antiviral, urinary problems, antidiabetic, and antioxidant effects. In folkloric medicine, it can also be used to cure ulcers. In this work, diclofenac-induced ulceration in male albino rats was used to assess the anti-ulcer activity of *Cucurbita maxima* polyphenolic extract. For the investigation, thirty (30) male albino rats were randomly divided into six (6) groups, each having five (5) rats. Group 1 was the normal control group. Group 3 received 30 mg/kg b.w lansoprazole; group 4 received 100 mg/kg b.w *Cucurbita maxima* polyphenolic extract; group 5 received 200 mg/kg b.w *Cucurbita maxima* polyphenolic extract while group 6 received 400 mg/kg b.w *Cucurbita maxima* polyphenolic extract. The study showed that *Cucurbita maxima* polyphenolic extract exerted a significant gastroprotective activity against diclofenac induced gastric mucosal damage in rats.

KEYWORDS: Peptic ulcer, *Cucurbita maxima*; Polyphenols, antioxidants.

INTRODUCTION

Peptic ulcers are lesions found in the gastrointestinal mucosa that extend throughout the muscularis mucosae normally characterized by stages of necrosis, neutrophil infiltration, blood flow reduction, increased oxidative stress and inflammation.[1,2] Basically, gastric ulcer results from an imbalance between some endogenous and exogenous aggressive factors such as increased release of pepsin, hydrochloric acid, refluxed bile, leukotrienes, reactive oxygen species (ROS) as well as incidence of prolonged consumption of non-steroidal anti-inflammatory drugs and alcohol.[3, 4] Helicobacter pylori infection is another cause of peptic ulcer. *Helicobacter pylori* Gastroprotective activity of plants or plant-derived product could be attributed to the phytoconstituents of the plant which possibly increase antioxidant enzyme and reducing oxidative stress.[5] It could also be due to the ability of plant extract to modulate the activities of H^+/K^+ATPase thereby normalizing the pH of the gastric juice.[2]

Diclofenac, a non-steroidal anti-inflammatory medicine, is one of the peptic ulcers most aggressive contributors. It has a catastrophic effect on the stomach mucosa, making it an effective drug for inducing gastric ulcers in animal models.[6] NSAIDs and other ulcer-causing agents, such as ethanol, have been shown to compromise the integrity of the gastric mucosa by causing acid reflux into the mucosa's subluminal layer and submucosa, as well as affecting hormone release and nerve function regulation.[7] Basically, NSAIDs cause ulcers by inhibiting prostaglandin synthetase in the cyclooxygenase pathway.[8] Prostaglandins are present in many tissues such as the stomach, where they play a protective role by stimulating the secretion of bicarbonate and mucus, maintaining mucosal blood flow, and regulating mucosal cell turnover and repair.[8] Hence, the suppression of prostaglandin synthesis by NSAIDs exposes the gastric mucosal to injury and ulceration.

Due to the increasing side effect of conventional drugs used in the treatment of peptic ulcer, there is a need for development of alternative drugs from natural product such as plant, which would indicate better efficacy and minimal or no side effect. One of the plants that have been useful in the management of peptic ulcer in traditional medicine is *Cucurbita maxima*. *Cucurbita maxima* is a member of the family, Cucurbitaceae which contains about 118 genera and 825 species.[9, 10] The plant is a fruit bearing vegetable that is widely grown in the tropics, and used in many countries for treating various diseases. Some of the important pharmacological properties of the plant include analgesic, anti-inflammatory, anti-ulcer,

antiviral, urinary disorders, antidiabetic, and antioxidant activities.[11] Polyphenolic compounds are important phytochemicals which have demonstrated significant antioxidant activities. A number of medicinal properties have been attributed to polyphenolic compounds, thus there is a need to evaluate the possible gastroprotective roles of polyphenolic compounds.

MATERIALS AND METHODS

Collection of plant material*Cucurbita maxima* leaf sample was collected from Nsukka, Enugu State, Nigeria on 4th August 2020. The plant was authenticated by the Taxonomy Section, Bioresources Development and Conservation Programme (BDCP), Nsukka, Enugu State, Nigeria.

Extraction of *Cucurbita maxima* leaf

Cucurbita maxima leaf air-dried and pulverized into fine powder. The powder (1000 g) macerated in 80% methanol (8:2 v/v methanol/water) (6 litres) for 48 hrs. The mixture was filtered using muslin cloth and Whatman No. 1 filter paper respectively. The filtrate was then partitioned using n hexane to remove fat soluble components of the extract. The concentrated under pressure using a rotary evaporator to obtain a semi solid extract.

Phytochemical Evaluation

The quantitative determination of polyphenolic compounds such as total phenolics, total flavonoids and tannins were carried out using the standard spectrophotometric methods as described by Ondo *et al*.[12] and Rohman *et al*.[13] respectively.

Animals

Male Wistar albino rats of weight range (130g-170g), used for this study were obtained from the Department of Zoology and Environmental Biology, University of Nigeria, Nsukka.

Experimental design

After acclimatization, the animals were randomly distributed into six (6) groups having four (4) animals each. The polyphenolic extract and standard drug were administered for one week. The route of administration of the extract and standard drug was oral using oral intubation tube. The rats were handled according to the guidelines of the National Institute of Health on the case and use of laboratory animals (NIH, 1985).

Group 1: Normal Control (Distilled water)

Group 2: Positive control

Group 3: 30 mg/kg b. w. lansoprazole

Group 4: 100 mg/kg b. w. *Cucurbita maxima* leaf polyphenolic extract

Group 5: 200 mg/kg b. w. *Cucurbita maxima* leaf polyphenolic extract

Group 6: 400 mg/kg b. w. *Cucurbita maxima* leaf polyphenolic extract

Induction of ulcer using Diclofenac model

Diclofenac was used as ulcerogenic agent at a dose of 150 mg/kg. After 24 hours of fasting, the graded doses of the polyphenolic extract of *Cucurbita maxima* leaf (100, 200 and 400 mg/kg b.w.) and 30 mg/kg b.w. lansoprazole were administered. One h after the last administration of the polyphenolic extract animals in groups 2-6 received diclofenac 150 mg/kg by the same oral route. Rats were sacrificed 6 h after NSAID administration as described by Sánchez *et al.*[14] while the stomachs removed and opened along the greater curvature to remove gastric content.

Estimation of gastric ulcerative index changes

The lesion index was determined by measuring each lesion in mm along its greater length. The stomach tissues opened along greater curvature were washed with normal saline, pinned flat on a cork board, and were examined for gastric ulcers with the aid of magnifying lens (×10) and each given a severity rating according to the method of Main and Whittle[15] as follows: <1mm = 1; > 1mm ≤ 2mm = 2 and >2mm ≤ 3mm = 3. The overall total score divided by 10 was designated as the ulcer index (UI). The percentage ulcer inhibition (percentage ulcer protection) was calculated for each of the groups according to method described by Vinothapooshan and Sundar.[16] The ulcer preventive/ inhibition index was calculated using the formula prescribed by Onwukwe *et al.*[17]

$$\text{Ulcer protective index (\%)} = \frac{\text{Ulcer index of untreated} - \text{Ulcer index of treated}}{\text{Ulcer index of untreated}} \times 100$$

Determination of gastric parameters

Gastric juice volume was determined according to the method of Kiranmai *et al.*[18] The collected gastric content was centrifuged at 3000 rpm for 10 min, then separated and the volume measured using a graduated cylinder.

Histological studies

The stomachs of the scarified rats were excised and immersed in 10 % formalin solution. The fixed specimens were trimmed, washed, and dehydrated in ascending grades of alcohol. The Specimens were further cleared in xylol, embedded in paraffin, sectioned at 4-6 microns thickness, and stained with heamtoxylin and eosin for examination as described by Drury *et al*.[19]

Statistical analysis

The results obtained from the study were statistically analyzed using One-way ANOVA, and the Duncan multiple test range was used to compare means. The results were expressed as mean ± SD. The analysis was carried out using the IBM SPSS statistical package version 20.

RESULTS

Phytochemical evaluation of *Cucurbita maxima* leaves polyphenolic extract

The profiling of polyphenolic compounds in the extract showed the presence phenolics, flavonoids and tannins as presented in table 1.

Table 1: Polyphenolic contents of *Cucurbita maxima* extract.

S/N	Phytoconstituent	Amount
1	Total phenolics	32.08±2.62 mg GAE/g extract
2	Flavonoids	23.16±4.01 mg/g
3	Tannins	14.28±2.08 mg/g TAC eq.

Values are presented as mean±SD. n=3.

Effect of *Cucurbita maxima* leaves polyphenolic extract on ulcer parameters

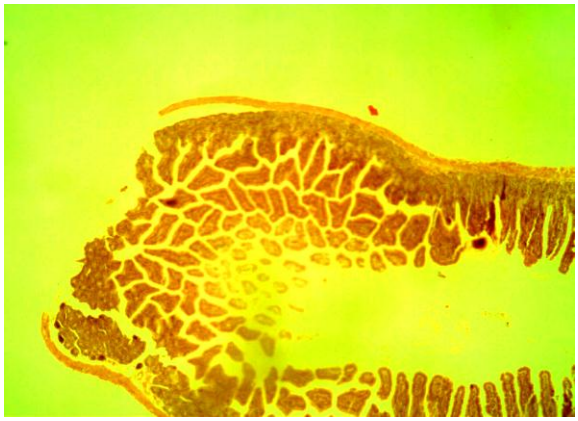
The results of the effect of *Cucurbita maxima* leaves polyphenolic extract on ulcer parameters **(**pH, gastric juice volume, ulcer index and ulcer protective index) as presented in table 2 revealed that the administration of diclofenac to experimental rats after overnight fast resulted to a significant increase ($p < 0.05$) in gastric juice volume as observed in the group that received diclofenac only. On the other hand, significant ($p < 0.05$) decreases in gastric juice volume were observed in the treatment groups that received graded doses of the polyphenolic extract. More so, the pH values of the gastric juice of experimental rats that received graded doses of the polyphenolic extract showed a reduced acidity level while that of the positive control had an increased acidity level. Ulcer protective index of the experimental rats that received graded doses of the polyphenolic extract increased significantly compared to the positive control rats which had a reduced ulcer protective index as well as an increased ulcer index.

Table 2: Effect of *Cucurbita maxima* leaf polyphenolic extract on Gastric Parameters.

S/N	Groups	Gastric Juice Volume (ml)	pH	Ulcer index	Ulcer protective index (%)
1	Normal Control	1.02 ± 0.09^{a}	7.64 ± 2.03^{f}	-	-
2	Positive Control	2.69 ± 0.41^{f}	3.46 ± 0.86^{a}	3.06 ± 0.22^{e}	-
3	30 mg/kg b. w. lansoprazole	1.28 ± 0.68^{b}	7.44 ± 1.31^{e}	1.02 ± 0.42^{a}	66.67
4	100 mg/kg b. w. CMLPE	1.84 ± 0.06^{e}	6.41 ± 1.12^{b}	2.01 ± 0.26^{d}	34.3
5	200 mg/kg b. w. CMLPE	1.80 ± 0.21^{d}	7.27 ± 1.48^{d}	1.72 ± 0.22^{c}	43.80
6	400 mg/kg b. w. CMLPE	1.62 ± 0.38^{c}	7.12 ± 1.08^{c}	1.31 ± 0.36^{b}	57.19

Values are expressed as mean±SD. Values in the same column with different superscript differ significantly ($p<0.05$). CMLPE: *Cucurbita maxima* leaf polyphenolic extract

HISTOLOGICAL STUDIES



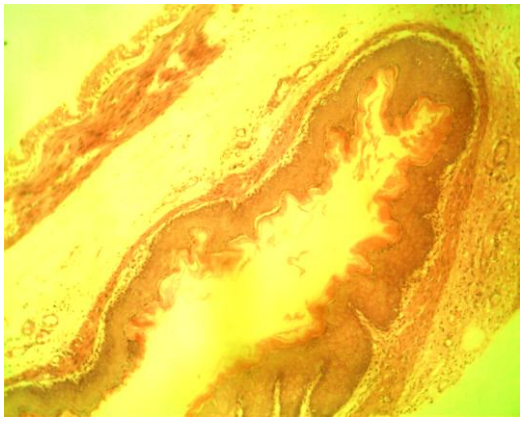
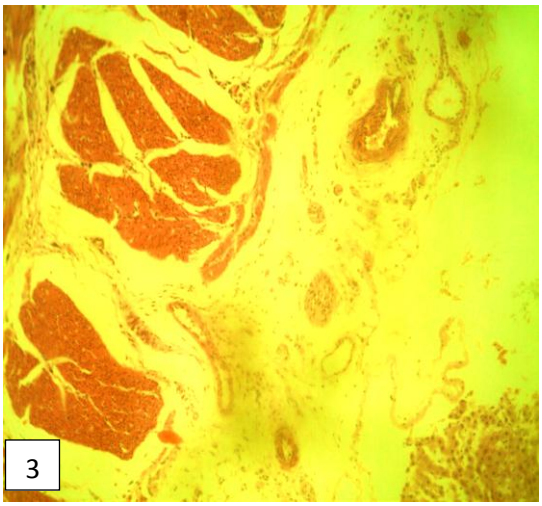


Figure 1: shows the photomicrograph of normal control group (H&E ×100) characterized by an intact muscularis mucosa. Figure 2 shows the photomicrograph of positive control group. The photomicrograph shows a severe mucosal damage with loss of epithelium (H&E ×100).



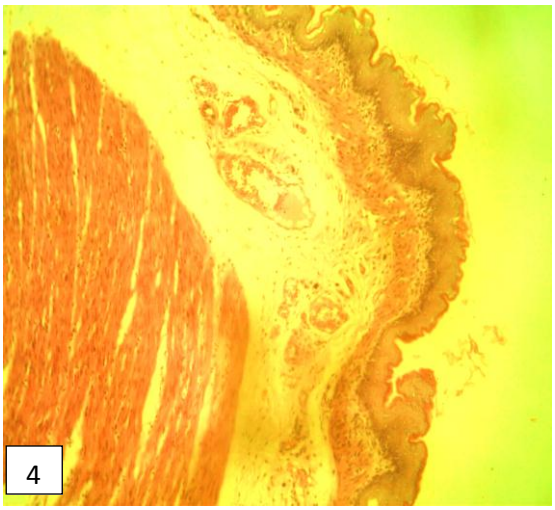


Figure 3: shows the photomicrograph of rat that received with lanosprazole (standard drug) (H&E ×100). The photomicrograph showed moderate mucosal lesions. Figure 4 is

the photomicrograph of rat that 100 mg/kg b.w of polyphenolic extract. It shows gastric lesions characterised by inflammatory infilterate (H&E ×100).

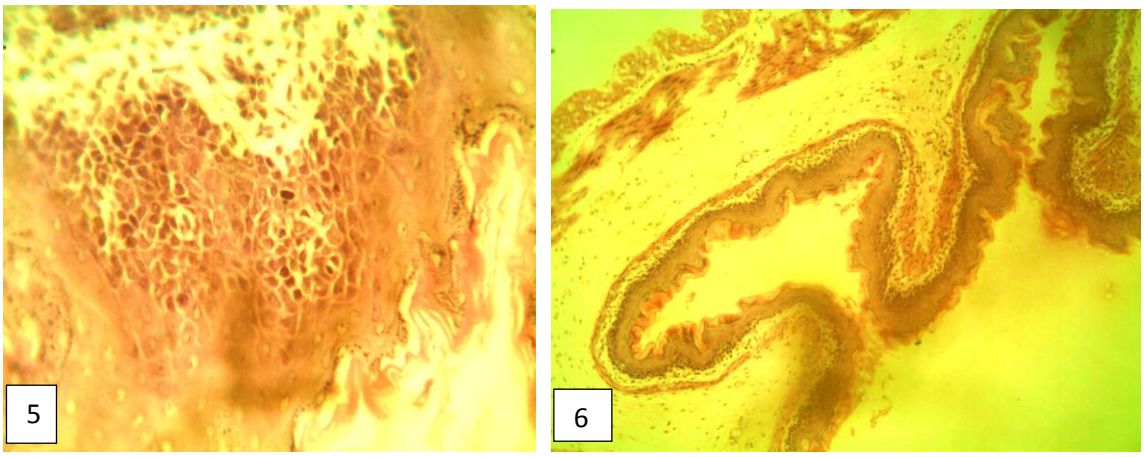


Figure 5: shows the photomicrograph of rat that received 200 mg/kg polyphenolic extract (H&E ×100). The photomicrograph showed moderate mucosal lesions. Figure 6 shows the photomicrograph of rat that 400 mg/kg b.w of polyphenolic extract. It shows gastric tissue with mild inflammations. (H&E ×100).

DISCUSSION

The medicinal properties of many plants are attributed mainly to the nature of the phytochemicals they contain. The phytochemical screening of the extract revealed the presence of flavonoid, tannin and phenolics table 1. Polyphenolic compounds have been classified as flavonoids such as isoflavonols, flavanones, flavones, anthocyanins, catechins, and non-flavonoids such as phenolic acids and hydroxycinnamic acids based on their chemical composition.[20] A number of studies have shown that polyphenolics such as flavonoids and tannins possess gastroprotective effects. Flavonoids exert gastroprotective properties through various mechanisms such as improved mucus secretion, antisecretory effect, antioxidant activities as well as free radical scavenging effect and inhibition of the *Helicobacter pylori* growth.[20] Also, tannins exert gastroprotective effect by protein precipitation and vasoconstricting effect.[20] The astringent property of tannins enables the precipitation of microproteins on the ulcer site, thus forming an impervious layer over the lining, which prevents ulceration.[21]

In this present study, a significant ulceration was observed in the positive control group as shown by the increased ulcer index and histological micrograph of the tissue which revealed ulcerations with deep hemorrhagic lesions, severe vascular congestions as well as

hypergranulation of the stomach tissue. These observations could be due to diclofenac treatment which has been reported to cause peptic ulceration in animal models.

A significant ($p<0.05$) decrease in the gastric volume of normal control and the groups that received graded doses of the polyphenolic extract compared to the positive control group was observed. In addition, there was a restoration of pH of the intestinal content of the groups that received the graded doses of the polyphenolic extract compared to the positive control group as presented in Table 2. On the other hand, a significant ($p<0.05$) decrease in ulcer index was observed in the groups that received graded doses of the polyphenolic extract when compared to the positive control group. In addition, a significant ($p<0.05$) increase in the ulcer protective index of normal control and groups that received the graded doses of the polyphenolic extract and lansoprazole were observed when compared to the positive control group. The gastroprotective effect observed in the groups that received *Cucurbita maxima* polyphenolic extract is similar to the reports of Onoja[22] who observed a significant gastroprotective effect when graded doses of polyphenolic extract of *Anarcadium occidentale* leaf was administered to ethanol-and indomethacin induced ulcer in rats. The lesions which are described by the ulcer index are caused by loss of mucous layer and increase of acid secretion, hence an increase in gastric volume leads to ulcer occurrence.[23,6] However, the gastro-protective effect of the extract could be partly attributed to the antioxidant effects of the polyphenolic compounds, as well as the specific effects of some of the phytoconstituents such as flavonoids, which have anti-secretory effect, inhibiting secretion of HCl by the parietal cells of the gastric mucosa, as a result, reduce the volume of gastric content and increasing the pH of the gastric content.[3,6]

There was no mucosal damage in the photomicrograph of normal control as shown in Figure 1. The histology revealed an intact gastric wall (muscularis mucosa) submucosa and serosa with no lesions to the gastric mucosa observed. The positive control group showed a severe and extensive mucosal damage as presented in Figure 2, while there was a gradual gastro-protective effect in the groups that received 100 and 200 mg/kg b.w extract and 400 *Cucurbita maxima* polyphenolic extract respectively as presented in figures 4-6. This is similar to the result of Onoja.[22] who reported gastroprotective effect in rats that received higher doses of *A. occidentale* polyphenolic extract. During ulcer healing, epithelial cells in the mucosa of the ulcer margin proliferate and integrate onto the granulation tissue to re-epithelialize the ulcer. Epidermal growth factor, basic fibroblast growth factor, trefoil

peptides, platelet derived growth factor, and other cytokines generated locally by regenerating cells regulate re-epithelialization and glandular structure rebuilding.[24] The standard drug, lansoprazole is a proton-pump inhibitor and acts by inhibiting gastric acid secretion by blocking the H^+/K^+-adenosine triphosphate enzyme system (the proton pump) of the gastric parietal cells.[25] Though, proton pump inhibitor such as lansoprazole has been effective in alleviating ulcer, \ number of adverse effects such as distortion of the immune structure and function as well as placental toxicity have been reported.[26]

CONCLUSION

The results of the present study show that *Cucurbit maxima* leaf polyphenolic extract possesses gastro-protective effect as indicated by its dose dependent ameliorative effect against diclofenac induced gastric ulceration.

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