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Evaluation of the anti-inflammatory, anti-ulcer and gastric histopathology activities of ethanol leaf extracts of *Gongronema latifolium* in diclofenac induced albino rat models

Daniel Ikechukwu Oraekei *, Chukwuka Benjamin Uzodinma, Uchechukwu Harrison Orji, Onyeka Chinwuba Obidiegwu, Marcellus Ejike Nnamani, Nnamdi Markben Adione and Cynthia Chioma Ibeh

Department of Pharmacology and Toxicology, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka, Anambra State, Nigeria.

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Abstract

The stomach is the roomiest portion of gastrointestinal tract. It serves as a reservoir for ingested food, secrets enzymes and hydrochloric acid for digestion of foods. This study was aimed at evaluating the anti-ulcer activity of ethanol leaf extracts of *Gongronema latifolium* in diclofenac induced albino rat models. *G. latifolium* leaves powder (1600 g) was weighed and extracted by cold maceration for 72 hours in 15 L of ethanol. Qualitative phytochemical analysis and acute toxicity study of the extract were done. Anti-inflammatory activities of the extract were tested on both heat and hypo tonicity induced hemolysis. Ulcer index, percentage ulcer protection and percentage mucus production were estimated for various concentrations of the extract. There was also histopathology examination of the gastric epithelium. The extractive percentage yield of *G. latifolium* was 24.69%. The phytocomponents were small concentrations of alkaloids, tannins, flavonoids, steroids and saponins. Only glycosides were present in moderately high concentrations. The median lethal dose (LD50) was > 5.000 mg/kg body weight. The extract exhibited dose dependent percentage inhibition of inflammation; and at the dose of 1,000 µg/ml, attained percentage inhibitions of 86.75 and 85.87% for heat and hypo tonicity induced inflammations respectively. At the dose of 800 mg/kg body weight, *Gongronema latifolium* leaf extract recorded percentage ulcer inhibition of 57.18%, which was comparable with the percentage inhibition of omeprazole (62.71%). In conclusion, the good anti-ulcer activity of ethanol leaf extracts of *Gongronema latifolium* can be attributable to its anti-inflammatory as well as enhancement of gastric mucus production.

Keywords: Anti-inflammatory; Anti-ulcer; Diclofenac; *Gongronema latifolium*; Histopathology

1. Introduction

The gastrointestinal (GI) system comprises the GI tract and accessory organs. The GI tract consists of the oral cavity, pharynx, esophagus, stomach, small intestine, large intestine, and anal canal. The accessory organs include the teeth, tongue, and glandular organs such as salivary glands, liver, gallbladder, and pancreas. The main functions of the GI system include ingestion and digestion of food, nutrient absorption, secretion of water and enzymes, and excretion of waste products [1]. The various roles performed by the stomach depend largely on the quality of the gastric mucosa. The pyloric gland is responsible for gastric secretion as well as the quality of mucus [2]. The mucus ensures that the lining of the stomach is not damaged by excess acidity which may be activated by food buffer that causes high luminal pH in the stomach [3]. The Stomach is a notable organ, because it provides water, electrolytes and nutrients to the body [4]. Stomach has also been found to provide protection from numerous aggressive factors that may be ingested with food by increasing defensive factors such as gastric mucus, bicarbonate ions, and prostaglandins among others. However, an interruption in balance of these hostile and protective factors results in the formation of gastric diseases

* Corresponding author: Daniel Ikechukwu Oraekei; Email: di.oraekei@unizik.edu.ng

like peptic ulcer [5]. There are various type of ulcer that affect human stomach. Some of these include: duodenal, gastric and esophageal ulcers. Peptic ulcer is an unwrapped sores that develop in the mucosa epithelial coating of the stomach, and duodenum At times it occurs in small and large bowels or in areas of ectopic-gastric tissue as in Zollinger-Ellison syndrome [6]. Some of the intrinsic hostile factors produced in the stomach are refluxed bile, leukotrienes, abnormal gastric motility, reactive Oxygen species, Helicobacter pylori infection, Non-steroidal anti-inflammatory drug and environmental substances such as alcohol, stress, smoking and lifestyle constitute what is known as exogenous aggressive factors. The defensive factors involve the regulation of gastric blood flow, endogenous Prostaglandins, mucus secretion, Bicarbonate, Nitric Oxide, metalloproteins, melatonin, protein rich diets and recently discovered peptides that control food intake such as ghrelin, orein-A and leptin [7]. Once the innate defending factors of the gastric epithelial cells are overwhelmed via aggressive factors, ulcer is formed. These factors constantly challenge the gastric mucosa and if they are not countered, it may lead to development of ulcers. There has been an increasing rise in cases of stomach ulcer in recent times and most cases are drug induced. A lot of pharmacological therapies had being studied for the prevention and management of peptic and gastric ulcers. Despite years of research work, controversy still surrounds the standardization of prophylactic therapy [8]. Hence effective and less toxic antiulcer agents are needed. The use of herbs have shown promising results in prophylaxis as well as treatment of ulcers [9]. This study is therefore aimed at evaluating the anti-ulcer activity of ethanol leaf extracts of *Gongronema latifolium* in diclofenac induced albino rat models. *Gongronema latifolium* commonly known as utazi, from family Apocynaceae is a leafy green plant characterized by its large, glossy, dark green leaves. They are used for their potential anti-inflammatory and digestive properties.

2. Material and methods

2.1. Plant Materials

Gongronema latifolium leaves were collected from Ngwo in Enugu State Nigeria in the month of December, 2023. The plant material was authenticated by a trained taxonomist, Mr Felix Nwafor of Department of Pharmacognosy and Environmental Medicine, University of Nigeria Nsukka, Enugu State, Nigeria. Voucher specimen was deposited at the herbarium of the Department of Pharmacognosy and Traditional Medicine, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University, Awka for future reference. The plant material was subsequently air-dried under room temperature for three weeks and pulverized with a mechanical grinding machine (GX160 Delmar 5.5HP).

2.2. Chemicals

Chemicals and experimental reagents used include: Methanol, (JHD, China). Tween-80, Fehlings solution (A&B, Guangdong Guanghua Chemical Factory Co., Ltd, China), Ammonia solution, Millions reagent, ferric chloride (Griffin & George, England), $AlCl_3$ (JHD, China), Folin-Ciocalteu's reagent (Loba Chemie, India), HCL, Potassium dichromate, Potassium ferricyanide (Hopkin and Williams Ltd, England). All solvent/reagent purchased were of analytical grade. All laboratory reagents were freshly prepared and freshly distilled water was used when required.

2.3. Extraction and percentage yield (%) calculation

G. latifolium leaves powder 1600 g was weighed using a weighing balance (Camry EK5350 Model, China) and extracted by cold maceration for 72 hours in 15 L of ethanol. The resulting solution was filtered using Whatman filter paper and the filtrate concentrated to dryness *in vacuo* using rotary evaporator (RE300 Model, United Kingdom) at 40 °C. The extract was stored in refrigerator between 0 - 4 °C. Percentage yield was calculated by the following formular:

$$\% \text{ yield} = (\text{actual yield} / \text{quantity of extract}) \times 100$$

2.4. Phytochemical Analysis

The qualitative phytochemical analysis of the extract was carried out using standard methods described by Odoh *et al*., (2019) [10].

2.5. Test for Alkaloids

The plant extract was dissolved in methanol and the resulting solution was used for the following test:

- Dragendorff's Test: A 5 ml of the sample was placed in labeled test tubes and 1 ml of Dragendorff's reagent was added. Formation of orange or red precipitates indicates the presence of alkaloids.
- Hager's Test: A 5 ml of the sample was placed in labeled test tubes and a few drops of Hager's reagent (saturated picric acid solution) were added. Formation of yellow precipitate confirms the presence of alkaloids.

- Wagners Test: A 5 ml of the sample was placed in labeled test tubes and a few drops of wagners reagent (solution of iodine and potassium iodide) were added. A reddish brown precipitate indicates the presence of alkaloids.
- Mayers Test: A 5 ml of the sample was placed in labeled test tubes and a few drops of the Mayers reagent (potassium mercuric iodide solution) were added. Formation of cream color precipitate indicates the presence of alkaloids.

2.6. Test for Saponins

The plant extract was dissolved in methanol individually and the resulting solutions was used for the following test:

- Frothing Test: A 10 ml of the samples was placed in labeled test tubes; 5 ml of distilled water was added and the mixture shaken vigorously. The test tube was observed for the presence of stable persistent froth.
- Emulsion Test: To the frothing solution, three drops of olive oil was added and the content shaken vigorously and observed for the formation of emulsion.

2.7. Test for Tannins

The plant extract was dissolved in methanol individually and the resulting solutions were used for the test. To 3 ml of each of the samples a few drops of 0.1% Ferric chloride was added and observed for brownish green or a blue-black coloration.

2.8. Test for Flavonoids

The plant extract was dissolved in methanol individually and resulting solutions were used for the following test:

- Ammonium Test: A 5 ml of dilute ammonia solution was added to a portion of each of the sample followed by addition of concentrated sulphuric acid (H2SO4) to the inclined test tube containing the mixture. Yellow coloration which disappears on standing indicates the presence of flavonoid.
- 1%Aluminium Chloride Test: Few drops of 1% aluminum solution was added to a portion of each of the samples and observed for yellow coloration.

2.9. Test for Steroids and Terpenoids

- **Liebermann-Burchard Test:** A 2 ml of acetic anhydride was added to 0.5 g of each of the ethanol extract. A 2 ml of H_2SO_4 was carefully added to the resulting mixture and observed for color change from violet to blue or green.
- **Salkowski Test:** The plant extract was dissolved in methanol individually and the resulting solutions were used for the test. A 5 ml of the sample was mixed in 2 ml of chloroform and concentrated H_2SO_4 was carefully added to form a layer. A reddish brown coloration at the interface indicates the presence of terpenoids.

2.10. Test for Cardiac Glycosides

- Keller-Killani Test: A 0.2 g of the ethanol extract was dissolved in 5 ml of methanol individually and was treated with 2 ml glacial acetic acid containing one drop of ferric chloride solution. A 3 ml concentrated H2SO4 was carefully poured to the inclined test tube containing the mixture. A brown ring at the interface indicates a deoxysugar characteristic of cardenolides.

2.11. Animals

Swiss Albino rats (140 – 170 g) of both sex were used for this study. All the animals were obtained from the Animal House of the Department of Pharmacology, Enugu State University of Science and Technology, Enugu State, Nigeria. Animals were allowed to acclimatize for one week prior to the commencement of the study. Food and water were provided *ad libitum*. All animal experiments were conducted in compliance with NIH guide for care and use of laboratory animals (National Institute of health (NIH) (2011) Pub No: 85-23).

2.12. Acute Toxicity Studies

Acute toxicity analysis of the extract was performed using Lorke's method (1983) [11]. This method has two phases (Phase 1 and Phase 2).

- PHASE 1: Nine adult albino mice were weighed, marked and randomized into three groups of three mice each. Each group of animals were administered different doses (10, 100 and 1000 mg/kg) of the extract. The mice were observed for 24 hours for signs of toxicity as well as mortality. In the absence of any mortality, the assay was extended to phase 2.
- PHASE 2: Four mice were weighed, marked and randomized into four groups of one mouse each. They received 2000, 3000, 4000 and 5000 mg/kg of the extract. Observation for 24 hours for obvious signs of toxicity and death was recorded accordingly. The LD50 was calculated using the formula:

$$LD_{50} = \sqrt{(D_0 \times D_{100})}$$

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

2.13. *In vitro* Anti-inflammatory study

2.13.1. Heat-induced hemolysis

Portion of 5 ml of the isotonic buffer containing 62.5, 125, 250, 500 and 1000 µg/ml of the extract was put into two duplicate sets of centrifuge tubes. The same amount of vehicles was added up in another tube as control. A 50 µl of RBC suspension was added to each tube and gently mixed by inverting the test tube. One pair of tubes were incubated at 54 °C temperature for 20 minutes in water bath. The other pair was preserved at temperature 0 - 5 °C in ice bath. Centrifugation of the mixture was done at 1000 rpm for 5 minutes and the absorbance was taken at 540 nm by using a spectrophotometer. Indomethacin was used as reference standard.

The percent inhibition of hemolysis was calculated according to the equation:

$$\text{Inhibition of hemolysis} = 100\ x\ \left(1 - \frac{OD2-OD1}{OD3-OD1}\right)$$ (Gandhidasan *et al*., 1991) [12].

Where, OD1 = Test Sample Unheated; OD2 = Test Sample Heated and OD3 = Control Sample Heated.

2.13.2. Hypotonicity-induced hemolysis

The isotonic solution was made by composing 154 mM NaCl in 10 mM sodium phosphate solution and the buffer of this solution was 7.4 pH. Stock RBC suspension 50 µl was mixed with 5 ml of the hypotonic solution (distilled water) containing the extract at concentrations of 62.5, 125, 250, 500 and 1000 µg/ml, while the control sample was mixed with drug-free solution. After incubating for 30 minutes at room temperature the whole mixture was centrifuged 1000 rpm for 5 minutes and the absorbance was taken at 540 nm. Dexamethasone was used as a reference standard. The percent inhibition of hemolysis was calculated according to the equation:

$$\text{Inhibition of hemolysis} = 100\ x\ \left(1 - \frac{OD2-OD1}{OD3-OD1}\right)$$ (Choi *et al*., 1997) [13].

Where, OD1 = Test sample in isotonic solution; OD2 = Test sample hypotonic solution and OD3 = Control sample in hypotonic solution.

2.14. Antiulcer Screening

2.14.1. Animal grouping and treatment

Albino rats were used for the study. They were randomly divided into 8 groups of 5 animals each Group 1: Naïve group was not subjected to ulcer induction; Group 2: was subjected to ulcer induction and treated with 50 mg/kg of the extract; Group 3: was subjected to ulcer induction and treated with 100 mg/kg of the extract; Group 4: was subjected to ulcer induction and treated with 200 mg/kg of the extract; Group 5: was subjected to ulcer induction and treated with 400 mg/kg of the extract; Group 6: was subjected to ulcer induction and treated with 800 mg/kg of the extract; Group 7: was subjected to ulcer induction and treated with 40 mg/kg of omeprazole (reference standard); Group 8: was subjected to ulcer induction and given 10 ml/kg distilled water as a vehicle control group. Daily administration of Diclofenac sodium 80 mg/kg for 10 days was used to induce ulcer 2 hours after prophylactic treatment with the extract. Prophylactic extract administration was done orally for 10 days and experiments performed at fixed times of the day between 9 a.m. and 10 a.m. to remove any potential confounding factors that may be related to the diurnal rhythms of gastric acid

secretion. All animal groups were fasted for 12 hours prior to each administration. Twenty-four hours after the last treatment, pyloric ligation was done for 4 hours to collect the gastric juice. The animals were anesthetized, the abdomen was opened by making a small midline incision, and the pyloric stomach was ligated with a tread by avoiding damage to its blood supply. The abdomen was closed by interrupted sutures.

2.14.2. Macroscopic Evaluation

The stomach was opened along the greater curvature and rinsed with distilled water to remove gastric contents and examined by a magnifying lens to access for induction of ulcers. Ulcer was scored using the scoring index described by Dashputre and Naikwade, 2011 [14]. Normal colored stomach (0); Red coloration (0.5); Spot ulcer (1); Hemorrhagic streak (1.5); Deep ulcers (2); and Perforation (3). While ulcer severity was quantify based on the following scoring: No damage (0); Blood at the lumen (1); Pin-point erosions (2); One to five erosions < 2 mm (3); More than five small erosions < 2 mm (4); One to 3 large erosions > 2 mm (5); More than 3 large erosions > 2mm (6).

2.14.3. Determination of Ulcer index and ulcer protective effect

The ulcer index was calculated as:

$UI = (Un + Us) + Up \times 10^{-1}$ (Abebaw *et al*., 2017) [15].

Where UI = ulcer index, Un = average number of ulcer (score), Us = average number of severity of ulcer, Up = percentage of animals with ulcer

Ulcer protective effect of treatment was calculated using the following formula:

$$100 - \frac{UI\ of\ Treatment}{UI\ of\ control}\ x\ 100$$

2.14.4. Gastric Wall Mucus Determination

The glandular segments of the stomach were removed, weighed, and assessed to determine gastric wall mucus in rats using the method of Corne *et al*., 1974 [16] as described by Ezenyi *et al*., 2019 [17]. Each segment was transferred immediately to a 1% Alcian blue solution (in sucrose solution, buffered with sodium acetate at pH 5), and the excess dye was removed by rinsing with sucrose solution. The dye complexes with the gastric wall mucus were extracted with magnesium chloride solution. A 4 mL aliquot of blue extract was then shaken with an equal volume of diethyl ether. The resulting emulsion was centrifuged and the absorbance of the aqueous layer was recorded at 580 nm. The quantity of Alcian blue extracted per gram of glandular tissue (net) was then calculated and used to calculate the percentage mucus production in the ulcer treated groups relative to naïve un-induced control.

2.15. Histopathological examination

The stomach of representative animal from the groups was transferred to a 10% Formalin solution. The tissues were processed through a series of ethyl alcohol of ascending strength (70, 80 and 95 %) for a period of 1 hour; twice in absolute alcohol (for 1 hour); and twice in xylene (for 1 hour) in order to render the tissue elements transparent. The tissues was then infiltrated with molten paraplast at 58 ^{0}C. This was done twice (for 1 hour). The transparent tissues, after clearing all elements from it, was embedded in a solid mass of paraplast. The blocks were labelled, allowed to cool and the metal blocks removed. The solid mass was then trimmed free of excess paraplast, leaving some free margins around the embedded tissues. Three microns thick longitudinal sections were cut with a rotary microtone. The sections were mounted on thoroughly cleaned gelatinized slides and the slides were placed on hot plates at 37 ^{0}C for 24 hours for proper fixation. The slides were stained by H & E stain according to the prescribed staining method (Bancroft and Stevens, 1990) [18]. The stain was prepared by dissolving hematoxylin in absolute alcohol. The mixture was boiled rapidly and mercuric oxide then added. The stain was cooled rapidly in a cold water bath; glacial acetic acid was then added and the stain ready for immediate use. The stained slides, after drying and labelling, were examined microscopically under oil immersion for comparative morphological and pathological changes in the stomach of the animals.

3. Results

Table 1 Result of % yield of ethanol extract of *G. latifolium* leaves

Extract	Yield (g)	% Yield (%w/w)
Ethanol extract	395	24.69

%Yield calculated from 1600 g of powdered leaves

Table 2 Results of phytochemical constituents of methanol extract of *G. latifolium*

Phytochemical	Methanol Extract
Alkaloids	+
Tannins	+
Flavonoids	+
Steroids	+
Glycosides	++
Saponins	+

Key: ++++ = Abundantly present; +++ = Present in high concentration; ++ = Present in moderately high concentration; + = Present in small concentration; – = Not present

3.1. Results of acute toxicity

Oral single dose administration of the extract did not produce any obvious signs of toxicity in phase 1 of the toxicity study. Weakness and reduced physical activities were however observed at higher doses of the phase 2 study with normal physical activities restored 4 hours post extract administration. No mortality was recorded at all the tested doses. The LD50 was estimated to be above 5000 mg/kg body weight.

3.2. Results of anti-inflammation assay

Table 3 Results of heat induced hemolysis

Treatments: Dexamethasone and extract (µg/ml)	Dexamethasone %inhibition	Extracts %inhibition
62.5	41.01	4.08
125	49.71	20.49
250	85.28	50.00
500	87.50	73.47
1000	93.22	86.78

Table 4 Results of hypotonicity induced RBC hemolysis

Treatments: Dexamethasone and extract (µg/ml)	Dexamethasone %inhibition	Extracts %inhibition
62.5	43.82	12.21
125	64.32	23.65
250	82.27	47.50
500	93.75	65.46
1000	94.62	85.87

3.3. Results of antiulcer studies

Table 5 Results of acidified ethanol induced ulcer

Groups	Treatments	%Ulcer protection
1	Naïve un-induced	99.45
2	50 mg/kg body weight extract	12.16
3	100 mg/kg body weight extract	29.56
4	200 mg/kg body weight extract	35.91
5	400 mg/kg body weight extract	48.90
6	800 mg/kg body weight extract	57.18
7	20 ml/kg body weight distilled water	-
8	40 mg/kg body weight omeprazole	62.71

The ulcer index was calculated to be 12.9

Table 6 Results of gastric wall mucus determination

Groups	Treatments	%Mucus production
1	Naïve un-induced	-
2	50 mg/kg body weight extract	23.48
3	100 mg/kg body weight extract	36.70
4	200 mg/kg body weight extract	45.30
5	400 mg/kg body weight extract	56.23
6	800 mg/kg body weight extract	71.59
7	20 ml/kg body weight distilled water	16.42
8	40 mg/kg body weight omeprazole	77.72

3.4. Results of histopathology assay

Representative photomicrographs of the stomach from the experimental groups showed moderate infiltration of neutrophilic inflammatory cells and edema in the groups treated with 200 mg/kg and 400 mg/kg extract but mild infiltration of neutrophilic inflammatory cells and edema occurred in groups treate with 400 mg/kg) and 800 mg/kg extract. There were apparently normal histological features of the stomach with intact submucosal layer (SM) in the group treated with Omeprazole 40 mg/kg and Naïve uninduced control. Photomicrographs of the stomach from the experimental groups (Negative control) showed infiltration of neutrophilic inflammatory cells and edema with exfoliation of the gastric epithelial cells.

4. Discussion

The extractive percentage yield of *G. latifolium* was 24.69%. This might be due to the ratio of water to raw material. Also, the polarity of the extracting solvent play a key role in increasing the recovery of phenolic and flavonoid compounds [19]. The extract percentage yield can also be affected by the extraction method. This was buttressed in another study in which extract yield, phytochemical constituents such as total phenol and withanolide content of water and water-alcohol extracts prepared using two most commonly used extraction techniques, also known as "Green Extraction" techniques, ultrasound and microwave assisted solvent extraction were compared with the conventional extraction method. The researchers concluded that extract yield, chemical composition of the extracts (total phenol and withanolide content) and antioxidant activity of the extracts varied with the extraction process as well as solvent composition [20]. According to the results of the phytochemical analysis, alkaloids, tannins, flavonoids, steroids and saponins were present in small concentrations. Only glycosides were present in moderately high concentrations. A certain study reiterated that glycosides were renowned for their anti-inflammatory activities when

the chemical components and biological activity of *Camellia* mistletoe, *Korthalsella japonica* (Loranthaceae) were investigated for their phytochemical properties and biological activity. The major plant components were chromatographically isolated using high-performance liquid chromatography and their structures elucidated using tandem mass spectrometry and nuclear magnetic resonance analysis. Furthermore, the anti-inflammatory activity of the 70% ethanol extract of *K. japonica* (KJ) and its isolated components were evaluated using a nitric oxide (NO) assay and western blot analysis for inducible NO synthase (iNOS) and cyclooxygenase (COX)-2. Three flavone di-*C*-glycosides, lucenin-2, vicenin-2, and stellarin-2 were identified as major components of KJ, for the first time. KJ significantly inhibited NO production and reduced iNOS and COX-2 expression in lipopolysaccharide-stimulated RAW 264.7 cells at 100 μg/mL while similar activity were observed with isolated flavone C-glycosides. The study concluded that KJ had a simple secondary metabolite profiles including flavone di-*C*-glycosides as major components [21]. From the acute toxicity results, it was obvious that *Gongronema latifolium* leaf extract had good safety profile since oral single dose administration of the extract did not produce any obvious signs of toxicity; and the median lethal dose (LD50) was > 5.000 mg/kg body weight. In an earlier research, although there was limited scientific evidence to establish the safety and efficacy of most herbal products, the effect of Western medicine treatment had not been satisfactory and problems of the adverse drug reaction were also very prominent. Consequently, the complementary and alternative treatment, especially the herbal medicine, has gained more attention and has also become popular [22].

In the inflammation study, the effects of *Gongronema latifolium* on heat induced and hypo tonicity induced inflammations were compared with that of a standard anti-inflammatory drug – dexamethasone. At any given equal concentration, dexamethasone showed superiority over the extracts in inhibiting inflammation. Despite this fact, the leaf extract of *G. latifolium* exhibited remarkable percentage inhibition of inflammation which was dose dependent; and at the dose of 1,000 μg/ml, attained percentage inhibitions of 86.75 and 85.87% for heat induced and hypo tonicity induced inflammations respectively. Inflammation has been reported to be involved in the initiation or progression of many health conditions. A certain study examined the anti-inflammatory potential of aqueous extracts of twelve medicinal plants used in Nigeria. The antioxidant activity was estimated using the total radical-trapping antioxidant parameter (TRAP) and ferric reducing ability (FRAP) assays. The abilities to inhibit nuclear factor kappa light chain enhancer of activated B cells (NfkB), a key regulator of the inflammatory response, and to activate nuclear factorE2 related factor 2 (Nrf2), a transcription factor that regulates cellular antioxidant defense systems, were determined using in vitro cell based assays. Extracts that showed the greatest inhibition of NfkB were S. esculentus (bark), 91.8%; E. senegalensis (leaves), 81.4%; S. birrea (stem bark), 77.5%; and S. setigera (stem bark), 75.5%. B. dalzielli (leaves) and Xylopia aethiopica (leaves) gave 7.4 and 7.7 fold activation of Nrf2, respectively. These were comparable to activation by sulphorophane [23]. Sulphorophane is the most potent naturally occurring inducers of cytoprotective enzymes yet discovered [24].

Furthermore, the results of acidified ethanol induced ulcer and gastric wall mucus determination assays followed similar trend. When compared with the naïve uninduced group 1, which had a percentage ulcer protection of 99.45%, the extracts showed a dose dependent increases in the percentage ulcer protection that were smaller than that of 40 mg/kg body weight of omeprazole(a standard anti-ulcer drug). At the dose of 800 mg/kg body weight, *Gongronema latifolium* leaf extract recorded percentage ulcer inhibition of 57.18%, which is comparable with the percentage inhibition of omeprazole (62.71 mg/kg body weight). Numerous natural products have been evaluated as therapeutics for the treatment of peptic ulcer. A review was conducted on different medicinal plant and alkaloids with antiulcer activity using the experimental parameters for antiulcer activity such as cold restraint stress-induced ulcer model, diclofenac-induced ulcer model in rats, (HCl–ethanol)-induced ulcer in mice and water immersion stress-induced ulcer in rats. From the study, it was clear that various herbal plants and plants extracts have significant antiulcer activity in animal models ranging from muco-protective to gastric anti-secretary activities when compared with that of reference drugs [25]. With respect to the gastric wall mucus determination, the 800 mg/kg body weight of the extract elicited a percentage mucus production of 74.59% which is not significantly different from the percentage mucus production by the standard anti-ulcer drug omeprazole which recorded 77.72%. Mucus is a protective substance that is secreted from multiple areas in the body such as the mouth, sinuses, throat, lungs, and stomach. The mucus shields the stomach wall from the digestive enzyme pepsin's self-digestion as well as the acidic quality of the gastric juice. Thus, gastric wall mucus is very essential in ulcer protection. In another study, the mucus layer was referred to as the first line of defense against infiltration of microorganisms, digestive enzymes and acids, digested food particles, microbial by-products, and food-associated toxins. This layer coats the interior surface of the GI tract, lubricates luminal contents and acts as a physical barrier to bacteria and other antigenic substances present in the lumen. The moist, nutrient-rich mucus layer adjacent to the epithelial barrier of the GI tract was also noted to be essential in the maintenance of intestinal homeostasis and contains a thriving biofilm including beneficial and pathogenic microbial populations [26]. In a study that investigated the gastro protective activity and the underlying mechanism of the 95% ethanol extract of *Z. officinale*, the extract was evaluated against gastric ulceration induced either by hydrochloric acid (HCl) or water immersion restraint stress (WIR) or aspirin (ASP). Pretreatment with the extract (0.1, 0.25, 0.5 or 1.0 g/kg) for 30 minutes before

inducing an ulcer by HCl, WIR and ASP decreased gastric lesions with maximal inhibitions of 81.7, 44.1 and 68.2%, respectively. Moreover, the involvement of gastric secretion on this antigastric ulcer activity was determined in a model of histamine-induced secretion in gastric fistulae of rats. The extract significantly increased visible gastric mucus secretion and had a tendency to increase the secretory rate of soluble gastric mucus. The researchers concluded that the *Z. officinale* extract exerts moderate gastric ulcer protection by increasing gastric mucus secretion [27].

5. Conclusion

In conclusion, ethanol leaf extracts of *Gongronema latifolium* showed good anti-ulcer activity which can be attributable to its anti-inflammatory property as well as enhancement of gastric mucus production. These conferred to the herb gastro protective effects as was evident in the gastric histopathology study outcome.

Compliance with ethical standards

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Disclosure of conflict of interest

- Daniel Ikechukwu Oraekei declared no conflict of interest
- Chukwuka Benjamin Uzodinma declared no conflict of interest
- Uchechukwu Harrison Orji declared no conflict of interest
- Onyeka Chinwuba Obidiegwu declared no conflict of interest
- Marcellus Ejike Nnamani declared no conflict of interest
- Nnamdi Markben Adione declared no conflict of interest
- Cynthia Chioma Ibeh declared no conflict of interest

Statement of ethical approval

Maintenance and care of all animals were carried out in accordance with EU Directive 2010/63/EU for animal experiments. Guide for the care and use of Laboratory Animals, DHHS Publ. # (NIH 86-123) were strictly adhered to. Ethical approval was obtained from the Animal Ethical Committee of the Enugu State University of Science and Technology. There was additional approval by the Nnamdi Azikiwe University's Ethical Committee for the use of Laboratory Animals for Research Purposes.

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