THE PROTECTIVE EFFECT OF *Gongronema latifolium* ON WISTAR ALBINO RATS INDUCED WITH INDOMETHACIN

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Abstract

This study was to determine the protective effects of *Gongronema latifolium* on male wistar albino rats induced with indomethacin. Twenty five (25) male wistar albino rats weighing 150 g were used in this study. The rats were randomly selected and divided into five groups of five rats. Groups A, B and C were blank, negative and positive controls while Groups D and E were low (100 mg/kg) and high (400 mg/kg) dose treated groups of the extracts of *Gongronema latifolium* respectively. The rats were housed in metallic cage and allowed to acclimatize for fourteen days. The ulcerative index increased significantly in group B at ($p<0.05$) (68.00 ± 2.83) but the low dose treated groups reduced on oral administration (100 mg/kg) of extracts of *Gongronema latifolium* at ($p<0.05$) (12.00 ±4.24). The percentage ulcer inhibition increased significantly at ($P<0.05$) (77.68 ± 4.61) when treated with a named ulcer drug, Omeprazole (Group C) while the percentage of ulceration reduced significantly at ($p<0.05$) (38.26 ± 6.09) to a reasonable amount when treated with low dose (100 mg/kg) (Group D) of *Gongronema latifolium.* The pH value was at the lowest level at the negative control group (Group B) at ($p<0.05$) (2.92 ± 0.63) but was significantly increased ($P<0.05$) (4.60 ± 0.18) when treated with high dose of extracts of *Gongronema latifolium.* Indomethacin induced ulcer increased the mucosa acidity of wistar albino rats (Group A) with the highest value ($p< 0.05$) (5.29±0.12), but when treated with low dose (Group D) of *Gongronema latifolium*, it significantly reduced ($p<0.05$) (2.75±1.061). This study shows *Gongronema latifolium* is potent, effective and efficacious due to its phytochemical properties such as antioxidants and anti-ulcer agents.

Key Words: *Gongronema latifolium,* Indomethacin, Ulcer, Omeprazole.

1.1 Background of Study

Open sores or lesions on the skin or mucous membrane known as ulcers are brought on by the breakdown of surface tissue[1, 2]. Peptic ulcer is one of the most common Gastro- Intestinal (GI) diseases creating a lot of pain and discomfort[3]. Ulcer has been attributed to effect of acid/ethanol[4], Non-Steroidal Anti-Inflammatory Drugs (NSAIDs), such as aspirin and indomethacin, used to inhibit pains, arthritis and inflammation which can be complicated by *Helicobacter pylori* infection[5]. Oxidative disturbances in the digestive system have also been

implicated in ulcers especially, that of the activities of Reactive Oxygen Species (ROS) [6]. Mucosal protection has been attributed to endogenous prostaglandin synthesis that stimulates the secretion of mucosa and bicarbonate layer along the GIT. Almost half of the world's population is colonized by *H. pylori*, which remains one of the most common causes of peptic ulcer disease[7].

The prevalence of *H. pylori* is higher in developing countries, especially in Africa, Central America, Central Asia, and Eastern Europe[8]. The organism is usually acquired in childhood in an environment of unsanitary conditions and crowding, mostly in countries with lower socioeconomic status. *H. pylori* cause epithelial cell degeneration and injury, which is usually more severe in the atrium, by the inflammatory response with neutrophils, lymphocytes, plasma cells, and macrophages. The mechanism by which *H. pylori* induces the development of different types of lesions in the gastro-duodenal mucosa is not fully explained. *H. pylori* infection can result in either hypochlorhydria or hyperchlorhydria, thus determining the type of peptic ulcer. The main mediators of *H. pylori* infection are cytokines that inhibit parietal cell secretion, but *H. pylori* can directly affect the H+/K+ ATPase α-subunit, activate Calcitonin Gene-Related Peptide (CGRP), a sensory neurons linked to somatostatin, or inhibit the production of gastrin[9]. Although the formation of gastric ulcers is associated with hyposecretion, 10–15% of patients with *H. pylori* infection have increased gastric secretion caused by hypergastrinemia and reduced antral somatostatin content.

Peptic ulcer is a hetergenous disease with worldwide distribution but the disease affects people from all countries and different races. Its average prevalence is between 5 - 10 percent of the general population over a lifetime[10]. This represents approximately 10 - 20 percent of people infected with *Helicobacter pylori* globally, with wide variations between different races and countries of the world having been confirmed, as its prevalence is inversely related to the economic level of the population, degree of development and level of hygienic social environmental.

The prevalence of both sexes is fairly close. The adjusted incidence in relation to age, some experts established the average for onset of gastric and duodenal ulcer. The peak incidence of gastric ulcer is between 55 - 65 years, while the average age for duodenal ulcer onset is decade earlier, at around 45 years[11]. Treatment of peptic ulcer disease should include eradication

of *H. pylori* in patients with this infection. The recommended duration of therapy for eradication is 10 to 14 days; however, shorter treatment courses (regimens of one, five, and seven days) are being assessed[12]. Potential benefits of shorter regimens include better compliance, fewer adverse effects, and lower costs. Antiulcer agents and medications for acid peptic disease are commonly used drugs that rarely cause liver injury. Most agents act by inhibition of gastric acid production, neutralization of acid or protection of the gastrointestinal mucosa from acid injury. These agents are used for both prevention and therapy of duodenal and gastric ulcer disease as well as to alleviate acid reflux, esophagitis and minor upper intestinal discomforts.

The most commonly used antiulcer agents are antacids such as aluminum or magnesium hydroxide (Maalox, Mylanta and many others) and calcium carbonate (Tums, Rolaids and others). Antacids are minimally absorbed and have no known adverse effects on the liver. Antacid use may cause a minor rise in urinary pH and rarely the calcium salts cause hypercalcemia. The major, most potent and effective antiulcer medications are the selective histamine type 2 receptor blockers (H2 blockers) and the Proton Pump Inhibitors (PPIs). Both classes of antiulcer medications block the pathways of acid production or secretion, decreasing gastric acidity, improving symptoms and aiding in healing of acid-peptic diseases. These are some of the most commonly used drugs in medicine and are generally well tolerated and rarely result in serious adverse events.

Nevertheless, both of these classes of agents have been linked to rare instances of acute liver injury. Pharmacological intervention utilizing histamine H2 blockers, antacids and anticholinergics have not succeeded to confer immunity from recurrence of disease or total restoration due to a number of limitations such as, the drugs being expensive and most of them can be injurious to the body and some have side effects[3]. Hence the need to study the efficacy of *Gongronema latifolium* on rats' systems in order to foster information regarding its medical application in ulcer infection.

Gongronema latifolium belongs to the family Asclepiadaceae. It is an edible medicinal plant mostly found in the rain forest zones in Nigeria and other tropical African countries such as Guinea-Bissau, western Cameroon and Sierra Leone. It is indicated as one of the aromatic plants of medicinal importance in Nigeria[13] (fig. 1)

Fig. 1: The plant of *Gongronema latifolium*
A: Leaves of *G. latifolium* plant

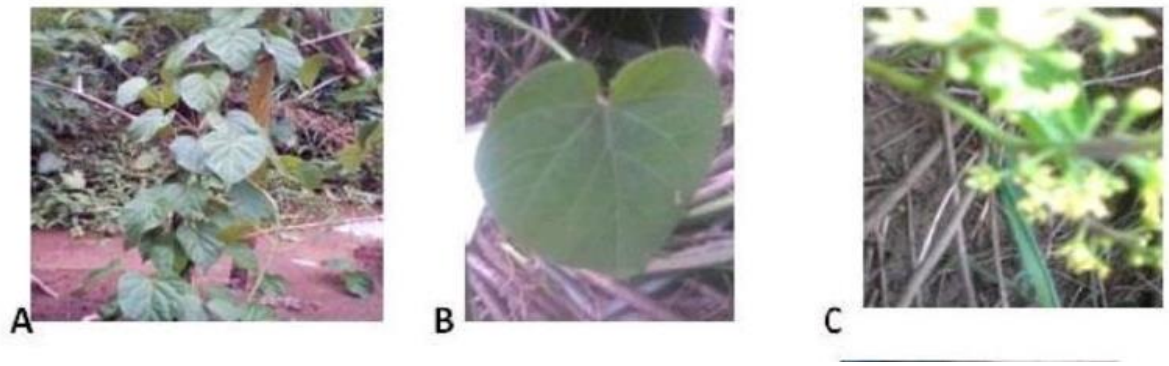
B: A single leaf of *G. latifolium*

C: The inflorescence

Source: Osuagwu *et al*.[14]

1.2 Justification of the Study

Despite the fact that scientists have discovered several anti-ulcer medications, these



medications have not been able to completely eradicate the condition. The majority of these medications are fairly pricey to purchase and some of them have adverse effects. The goal of the current study was to examine how ulcers are treated internally by utilizing a *Gongronema latifolium* extract, which is widely available, affordable, and used in economically underdeveloped African nations like Nigeria.

1.3 Main Objective of the Study

The main objective of the study was to determine the effect of the oral administration of *Gongronema latifolium* leave extract on wistar albino rat induced with indomethacin.

Materials and Methods

3.1 Animal Model and Experimental Procedure

Twenty- five (25) male wistar albino rats was used for the study and was divided into five groups. Each group with five rats. The blank control group was designated as Group A. Rats

were not either treated or induced in this instance. Group B served as the adverse control. In this case, indomethacin 40 mg/kg was used to create ulcers in the rats. The positive control was designated as Group C. In this case, indomethacin was used to generate the rats' ulcers, and the medicine omeprazole (20 mg/kg) was used to cure them. Low dose extract was given to Group D. In this case, indomethacin was used to stimulate the rats, and a modest dose of 100 mg/kg of *Gongronema latifolium* was administered orally once per day. High dose was given to Group E. The rats in the latter group were Induced with indomethacin and treated with high dose of 400 mg/kg of *Gongronema latifolium* preparation orally once daily.

3.2 Procurement of Animal

Twenty five (25) male albino rats weighing between (150 g) were selected for this study. They were purchased from the Department of Veterinary Medicine at the University of Nigeria Nsukka, Enugu State, Nigeria. All were housed in metallic cages at room temperature (28-30C) under controlled light cycles (12-hr day/night cycles). They were given water *ad libitum* and acclimatized for fourteen days before the study in the Animal House of Brian Phosphorelationship Scientific Solution Services, Enugu.

3.3 Induction of Animal

The rats were put to death after being given a chloroform inhalation anesthesia at the conclusion of the studies. The kidneys and the colon of the rats were removed and promptly preserved in 10% neutral buffered formalin for histological research. Blood samples from each of the rats were taken by heart puncture into well-labeled dry plain tubes for biochemical analyses. The intestines were processed right away after being fixed for 24 hours by dehydrating the tissue in escalating alcohol concentrations, clearing in xylene, and infiltrating with paraffin wax before embedding. Hematoxylin and eosin staining was applied after sections were cut, mounted, and stained.

3.3.1 Effect of crude ethanol extract on Indomethacin-induced ulcer

The method of Urishidani *et al*.[15] was utilized. Gastric ulceration was induced in 4 groups of five rats each using oral administration of 40 mg/kg of indomethacin, 30 min after each group had received oral administration of its respective extract, standard drug or saline. After 7 h, the animals were sacrificed in ether chamber and stomachs excised, dissected, washed and fixed in formal saline and mounted on slab. Ulcer craters or wounds were counted, rated from 1-3 and used to compute the ulcer scores. The ulcer indices of a group are summation of ulcer scores (number of ulcer spots × their rating and divided by the magnification). The percent ulcer inhibition was calculated.

3.3.2 Effect of crude ethanol extract and its fractions on indomethacin-induced ulcer

The effect of the crude ethanol fractions (400 mg/kg) and crude were tested at a higher level of induction using indomethacin (40 mg/kg). 5 groups of five rats each were administered their respective extracts and the same procedures as above were followed to determine ulcer indices.

3.4 Collection of Plant Materials

Fresh leaves of *Gongronema latifolium* were purchased at Ogbette Main Market, Enugu State in a large quantity and washed thoroughly to remove contaminants. After washing, they were air-dried in room temperature for about one week. Thereafter, they were grinded to fine powdered form using a manual blender.

3.4.1 Plant Extraction

The pulverized leaves (1.0 kg) were macerated in 1.0 ml/kg of 97% ethanol using a soxhlet extractor, a beaker, a flat bottom flask, a heater and a sieve to get the plant extract according to Wei *et al*.[16]. The fresh leaves of *Gongronema latifolium* were carefully selected and dried at room temperature for about two weeks. Two hundred and fifty grams of grinded shades of *Gongronema latifolium* phytochemicals where extracted using soxhlet apparatus, heating mantle and ethanol. Then ten grams of the concentrated crude extract was reconstituted with 100ml of distilled water to get extract concentration of 100 mg/ml ready for use.

3.5 Formulation and Administration of Indomethacin

Indomethacin was purchased from a pharmacy dealer in Enugu, the weight of the indomethacin was taken and recorded by the use of a beam balance in mg/kg. 40 mg/kg of indomethacin was diluted in 100 ml of water to indomethacin treated solution. The indomethacin was administered orally.

3.6 Induction of Ulcer

Gastric ulceration was induced according to the procedure described by Sayanti *et al.*[17] Briefly; rats were administered with a single oral dose of indomethacin (40 mg/kg body weight) they were deprived of food but had free access to water 24 hours prior to ulcer induction.

3.7 Standard Drug

The standard drug Omeprazole was administered at a dosage of 20 mg/kg.

3.8 Statistical analysis

All statistical analysis was processed using Statistical Program of Social Science (SPSS) software for window version 18. The values of the measured parameters were expressed as mean ± SEM. A one-way Analysis of Variance (ANOVA) was used to determine the effects of indomethacin at different doses on wistar albino rats infected with ulcer and the test for significance was recorded as $p< 0.05$ using Duncan New Multiple Range Test (DNMRT).

RESULTS

4. 1 Ulceration index

The result in table 1, showed that the negative control group (Group B) which was induced with indomethacin and not treated had the highest ulceration index value, this inferred that indomethacin induced ulcer significantly at ($p<0.05$) (68.00 ± 2.38). The ulceration index of the wistar albino rats on administration of high dose of *Gongronema latifolium* extracts was significantly reduced ($p<0.05$) (12.00 ± 4.24).

Table 1: Ulceration index

Groups	Ulceration Index
A(blank control)	0^{a}
B(negative control)	68.00 ± 2.38^{d}
C(positive control)	21.50 ± 3.54^{b}
D(low dose)	64.50 ± 3.54^{b}
E(high dose)	12.00 ± 4.24^{c}

In a column, mean values with different letter as superscript are significantly different ($p<0.05$)

4.2 Percentage ulcer inhibition

The result from table 2 showed that there was no percentage ulcer inhibition in the blank and the negative control group (0, 0) respectively. But, there was a significant increase ($p<0.05$) (77.68 ± 4.61) in positive control group (Group C), where ulcer was induced and treated with 20mg/kg of a standard drug, Omeprazole. But significantly reduced ($p<0.05$)(38.26 ± 6.08) when treated with low dose (100 mg/kg) of extracts of *Gongronema latifolium.* This result inferred that Omeprazole increases the percentage ulcer inhibition of the wistar albino rats but on oral administration of extract of *Gongronema latifolium* the percentage ulcer inhibition was reduced.

Table 2: Percentage ulcer inhibition

Groups	**% ulcer inhibition**
A(blank control)	0^{a}
B(negative control)	0^{a}
C(positive control)	77.68 ± 4.61^{c}
D(low dose)	38.26 ± 6.08^{b}
E(high dose)	57.09 ± 2.70^{d}

In a column, mean values with different letter as superscript are significantly different ($p<0.05$)

4.3 Gastric pH

The result from table 3 showed that the negative control group (Group B) had the lowest pH value, (2.92 ± 0.63) but was significantly increased ($p<0.05$) (4.60 ± 0.18) when treated with high dose of extracts of *Gongronema latifolium*, this inferred that indomethacin induced ulcer makes the intestine of the wistar albino rat highly acidic and the high dose extract of *G. latifolium* served as a buffering agent to normalise the pH of the wistar albino rats.

Table 3: Gastric pH

Groups	Gastric pH
A(blank control)	5.95 ± 0.46^{b}
B(negative control)	2.93 ± 0.63^{c}
C(positive control)	5.14 ± 0.23^{b}
D(low dose)	3.02 ± 0.21^{a}
E(high dose)	4.60 ± 0.18^{d}

In a column, mean values with different letter as superscript are significantly different ($p<0.05$)

4.4 Mucosa acidity

The result from table 4, showed that indomethacin induced ulcer increases the mucosa acidity of wistar albino rats the blank control (Group A) had the highest value (5.29 ± 0.12), but when treated with low dose (Group D) of *Gongronema latifolium*, it significantly reduced ($P<0.05$) (2.75 ± 1.061), the reduction showed that the extracts of *Gongronema latifolium* reduces mucosa acidity in the induced ulcerated rats.

Table 4: Mucosa acidity

Groups	Mucosa acidity
A(blank control)	5.29 ± 0.18^{a}
B(negative control)	2.60 ± 0.28^{c}

C (positive control)	4.70 ± 2.40^{b}
D (low dose)	2.75 ± 1.06^{c}
E (high dose)	3.60 ± 0.85^{d}

In a single column, mean values with different letter as superscript are significantly different ($p < 0.05$)

DISCUSSION AND CONCLUSION

5.1 Discussion

This result on the presence of the ulceration index showed that indomethacin induced ulcer elevates the ulceration index of the experimental wistar albino rats and the oral administration of low dose of extracts of *Gongronema latifolium* brought down the ulceration index. This is consistent with Morebise *et al.*[18] who found out that alcohol induced ulcer reduces ulceration index. The work also agreed with the work of Mosango[19] who found out that the ulceration index increased in the same fashion of aspirin induced ulcer. This work is also consistent with the work of Ojo *et al.*[20] who said that *Gongronema latifolium* reduces ulceration index. This result on the mucosa acidity showed that indomethacin induced ulcer increases the mucosa acidity of wistar albino rats but on oral administration of high dose of extracts of *Gongronema latifolium* reduced the mucosa acidity. This result is consistent with the work of Edim *et al*.[21] who from his research work concluded that *Gongronema latifolium* reduces the mucosa acidity of wistar albino rats on oral administration. From the result, it can be seen that indomethacin induced ulcer treated with a standard drug, omeprazole increases the Gastric pH of the wistar albino rats making the intestine of the experimental rat highly acidic but on administration of extracts of *Gongronema latifolium* it normalised the gastric pH, here the extracts of *Gongronema latifolium* acts as a buffering agent. This result is in consistent with Ezekwe *et al*.[22] who said that *Gongronema latifolium* normalises the gastric pH of wistar albino rats in alcohol induced ulcer. The result of the presence of the percentage ulcer inhibition showed that indomethacin induced ulcer increases the percentage ulcer inhibition of wistar albino rats but when treated with extracts of *Gongronema latifolium*, the percentage ulcer

inhibition reduced, the reduction showed that the extracts of *Gongronema latifolium* reduces percentage ulcer inhibition in the induced ulcerated rats. This result agreed with the work of Morebise *et al.*[18] who confirmed from his work that extracts of *Gongronema latifolium reduces* the percentage ulcer inhibition of the experimental wistar albino rats in indomethacin induced ulcer. Mosango *et al.*[19] also concluded from his work that the extracts of *Gongronema latifolium* reduces the percentage ulcer inhibition of wistar albino rats in alcohol induced ulcer.

5.2 Conclusion

This study revealed that the oral administration of *G. latifolium* extract in the treatment of indomethacin induced ulcer have significant effect in moderating the incidence of ulcer as seen from the results got. The result also showed that the extracts of *Gongronema latifolium* has an effective, potent antioxidant agents and has the ability to inhibit, reverse and scavenge the reactive oxygen species (ROS) generated by the ulcer infection before reaching the intestine.

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