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Mucosal wound healing and anti peptic ulcer effect of *Gongronema latifolium* in rats

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ABSTRACT

Background: The freshly leaves and stem of *Gongronema latifolium* is eaten as vegetables in most part of tropical Africa and used in treatment of peptic ulcer disease in folklore medicine.

Objectives: The air dried methanolic leave extract was investigated for anti-peptic ulcer and mucosal wound healing effect on indomethacin-induced gastric and duodenal ulcers in rats in order to demonstrate the pharmacological activity of the plants and justify its use in healing.

Method: Fresh green leaves and stems of *Gongronema latifolium* were harvested, air dried, macerated in methanol and evaporated to dryness using rotary evaporator. Phytochemistry was carried out on the crude extract. Acute toxicity study was carried out in mice to determine the LD_{50} and guide dosing. Forty five rats of average weight of 150grams were divided into nine metal cages (A-I) of five rats per group. All animals were acclimatised for a week and fasted for 24hours prior to commencement of experiment. Group A was used as pilot study to demonstrate the effect of indomethacin and received orally 30mg/kg of indomethacin. The animals were sacrificed after 24hours and gastric and duodenal mucosal bleeding spots were used as positive indicator of ulcer induction. The stomach and duodenum were sent for histology. Group A was later taken as positive control. Group B was given orally, 2ml/kg of drinking water only to act as negative control. Groups C and D were treated like A but, were sacrificed at 2 or 4wks to act as 2 and 4wks positive control respectively. Groups E to I were given orally, 30mg/kg of indomethacin and after 24hours were treated with either *Gongronema latifolium* or cimetidine. Two dosage regimens were used for the methanolic extract while treatment period lasted for 2 or 4wks before the animals were sacrificed. Their stomach and duodenum were weighed and examined for bleeding spots before being sent for histological study. Data were presented as mean±Standard deviation of organ

weight and number of bleeding spots while comparative histological architecture was presented as micrograph. Statistical analysis was done with SPSS version 16.0 using one-way ANOVA and students t-test for comparative statistics. P≥0.05 was adjudged non significant.

Results: Phytochemistry showed the richly presence of alkaloids and flavonoids in line with earlier findings. The LD_{50} was 1,581mg/kg(IP) in mice using Lorke's 1983 method. All non treated (Groups C and D) animals died within three to four days of commencement of the experiment. There was no significant (P>0.25) change in incised organ weight of treated animals compared to negative control animals. There were multiple bleeding spots on the gastric and duodenal mucosa of the positive (group A) control. The histology also exhibited multiple gastric and duodenal damages. These bleeding spots and mucosal damages were also demonstrated in animals that were given 2wks treatment with *Gongronema latifolium* but, to a much lesser extent. The results obtained with cimetidine were similar to those of 2wks high dose treatment with *Gongronema latifolium.* At 4wks treatment duration with both dosage regimens of *Gongronema latifolium,* no bleeding spot was demonstrated and the mucosal wound had healed as compared with the negative control.

Conclusion: *Gongronema latifolium* possesses demonstrable anti-peptic ulcer and mucosal healing/repairing effects. These effects were greater than that of cimetidine, a standard anti-peptic ulcer agent. The study may justify the use of *Gongronema latifolium* in treatment of peptic ulcer disease by traditional medicine healers. Clinical trial in human is recommended.

Key words: *Gongronema latifolium*, anti-peptic ulcer, gastric and duodenal mucosal wound healing

1. INTRODUCTION

Gasrtic ulcer is a major health hazard in terms of both morbidity and mortality (Abdallah *et al.*, 2011). The aetiology of gastroduodenal ulcers is influenced by various aggressive and defensive factors such as acid-pepsin secretion, parietal cell, mucosal barrier, mucus secretion, blood flow, cellular regeneration and endogenous protective agents (prostaglandins and epidermic growth factors) (Abdallah *et al.*, 2011).

According to Kim (2008), Ahn *et al*., (2014) some other factors, such as inadequate dietary habits, cigarette smoking, stress, hereditary predisposition, excessive ingestion of non-steroidal anti-inflammatory agents, and infection by *Helicobacter pylori*, may be responsible for the development of peptic ulcer.

Untreated gastric ulcer, a resultant effect of NSAIDS therapy is capable of inducing upper gastrointestinal bleeding with its fatal consequence (Gargallo *et al*., 2014).

Reflux oesophageal complication could arise from post surgical treatment of peptic ulcer (Veligotskiĭ *et al*., 2014).

Patients with gastric ulcers have significantly higher risk of gastric cancer, especially within first 2 years after diagnosis (Lee *et al*., 2014).

Ulcer therapy has progressed from vagotomy to anticholinergic drugs, histamine H_2 receptor antagonists, antacids and to proton pump inhibitors. Although the implementation of guidelines into clinical practice takes time, several studies have shown a recent and profound decrease in hospitalizations due to upper gastrointestinal complications, which has been linked to widespread use of proton pump inhibitors, better NSAID prescription, and decreased prevalence of *Helicobacter pylori* infection (Gargallo *et al*., 2014).

Several pharmaceutical products have been employed for the treatment of gastroduodenal ulcer and peptic diseases, resulting in decreasing mortality and morbidity rate but, they are not completely effective and produce many adverse effects (Abdallah *et al.*, 2011).

For example a widely used drug but, associated with rare idiosyncratic hepatotoxicity is the histamine H_2 receptor antagonist, ranitidine (Abdallah *et al.*, 2011).

An analysis of reporting and statistical data showed the considerable changes in clinical-epidemiological indices of gastric and duodenum ulcer at the period from1998 to 2012 as the prevalence of the disease and the number of primary patients decreased in 2-3 times (Larichev *et al*., 2014).

There was reduction of the rate of perforations and ulcerous bleeding but, a tendency of frequency of occurrence increased and efficacy indices of currently available antiulcer drugs reduced in the last recent years (Larichev *et al*., 2014).

Medicinal plants are readily available in most part of the world especially, the tropics and medicinal plants could be as efficacious as their orthodox drugs counterpart but, they generally possess less toxic adverse effects once used appropriately (Azikiwe *et al*., 2007; Azikiwe *et al*., 2009).

Gongronema latifolium is a tropical rain forest plant found throughout Nigeria and other tropical countries such as Guinea-Bissau, Western Cameroon and Sierra Leone. It has been used in the traditional system of medicine for various gastrointestinal disorders such as diarrhoea, ulcers and dyspepsia and in the management of diabetes mellitus (Akah *et al.*, 2011).

In southern Nigeria the Igbo people call the plant 'utazi' and the Yoruba people 'arokeke'. They are sharp-bitter with tint-sweet and widely used as a leafy vegetable and as a spice for sauces, soups and salads and the leaves are used to spice locally brewed beer (Eleyinmi, *et al*., 2008).

An infusion of the aerial parts is taken to treat cough, intestinal worms, dysentery, dyspepsia and malaria and also taken as a tonic to treat loss of appetite (Dike, 2010).

Azikiwe et al.
Mucosal wound healing and anti peptic ulcer effect of *Gongronema latifolium* in rats,
Discovery, 2015, 10(25), 103-112,

In Sierra Leone an infusion or decoction of the stems with lime juice is taken as a purge to treat colic and stomach-ache and in Senegal and Ghana the leaves are rubbed on the joints of small children to help them walk while the boiled fruits in soup are eaten as a laxative (Antai *et al.,* 2010).

In Nigeria a leafy stem infusion is taken as a cleansing purge by Muslims during Ramadan and decoction of leaves or leafy stems is commonly taken to treat diabetes and high blood pressure (Akudor *et al.,* 2010).

In Sierra Leone the pliable stems are used as chew sticks. The bark contains much latex and has been tested for exploitation. The latex is applied to teeth affected by caries and also taken for controlling weight gain in lactating women and overall health management of pregnant cum lactating women (Antai *et al.,* 2010).

Asthma patients chew fresh leaves to relieve wheezing and cold maceration of the roots is also taken as a remedy for asthma (Akudor *et al.,* 2010).

A decoction of the roots, combined with other plant species, is taken to treat sickle cell anaemia and a maceration of the leaves in alcohol is taken to treat bilharzia, viral hepatitis and as a general antimicrobial agent (Okeke *et al.,* 2008).

The leaves contain several 17β-marsdenin derivatives (pregnane glycosides) as well as β-sitosterol, lupenyl cinnamate, lupenyl acetate, lupeol, essential oils and saponins. The essential oil from the leaves contains linalool (19.5%), (E)-phytol (15.3%) and aromadendrene hydrate (9.8%) (Eleyinmi, 2007).

The aqueous extract of *G. latifolium* leaves possesses hypoglycaemic as well as anti-lipid peroxidative properties thus an antioxidant (Nwanjo et al., 2006; Akah *et al*., 2011). Hypoglycaemic effect has been documented in man unlike most other experimental works (Okolie *et al*., 2008). The hypoglycaemic effect was highest in diabetic mice when the plant was combined with *Vernonia amygdalina* and *Viscum album* (Itelima *et al*., 2014)

No serious adverse effects have been recorded with normal consumption of *Gongronema latifolium* but, chronic toxicity study carried in rats showed histological hepatotoxicity and elongation of intestinal mucosa (Emeka and Obioa, 2009).

2. MATERIAL AND METHOD

Rats and mice of close weight range were purchased from Chris farm, Awka. Cimetidine and indomethacin were bought from Anambra State University Teaching Hospital Pharmacy, Awka. Methanol was bought from a chemical shop at Onitsha, Anambra State.

Plant collection, extraction and Phytochemistry

The freshly leaves along with some part of the stem were harvested in June, 2013 and from Nimo, Anambra State, South-East Nigeria.

The leaves were air dried for 7days and ground into fine powder. 300 grams of the fine powder was macerated in 500ml of methanol and stood at room temperature for 48hours. Sieving and subsequent filtration was carried out using Whatman No 1 filter paper. The filtrate was evaporated to dryness using rotary evaporator and the scraped out dry yield refrigerated until required (Azikiwe *et al*., 2014).

Phytochemistry was carried out using the methods described by Trease and Evans, 1996. Phytochemistry qualitatively tested for alkaloids, terpenoids, Steroids, flavonoids, saponins, tannins, proteins, carbohydrates, glycosides, resins, fats/oils, acidic and reducing substances.

Acute toxicity study

Acute toxicity study was done using Lorke's 1983 method and as used by Akah *et al*., 2003; Arkila *et al*., 2007; Azikiwe *et al*., 2010. Mice of both sexes were used for LD_{50} to determine the safety margin and guide dosing. The study was divided into two phases and involving 13 mice. At the first phase, 9 mice of average weight of 28grams were divided into 3 metal cages (A-C) of 3 each and had access to water and feeds al dilatium. Group A was given 10mg/kg of the extract intraperitoneally, group B received 100mg/kg and group C received 1000mg/kg. All animals were observed for 24 hours for any clinical signs of toxicity and or death. At the second phase since no death occurred at the first phase, 4 mice of same average weight were divided into 4 cages of a mouse each and were dosed intraperitoneally 2500mg/kg, 5000mg/kg, 7500mg/kg and 10,000mg/kg. They were again observed for 24hours for any clinical signs of toxicity and or death.

Anti-peptic ulcer and mucosal healing study

Forty five rats of average weight of 150grams were divided into nine metal cages (A-I) of five rats per group. All animals were acclimatised for a week and fasted for 24hours prior to commencement of experiment.

Group A was used as pilot study to demonstrate the effect of indomethacin and received orally 30mg/kg of indomethacin. The animals were sacrificed after 24hours and gastric and duodenal mucosal bleeding spots were used as positive indicator of ulcer induction. The stomach and duodenum were sent for histology. Group A was later taken as positive control.

Group B was given orally, 2ml/kg of drinking water only to act as negative control.

Groups C and D were treated like A but, were sacrificed at 2 or 4wks to act as 2 and 4wks positive control respectively.

Groups E to I were given orally, 30mg/kg of indomethacin and after 24hours were treated with either *Gongronema latifolium* or cimetidine. Two dosage regimens were used for the methanolic extract while treatment period lasted for 2 or 4wks before the animals were sacrificed under chloroform anaesthesia (Amazu *et al,* 2014)

Their stomach and duodenum were weighed and examined for bleeding spots before being sent for histological study. Tissues were processed for histology using automatic tissue processing unit and stained with haematoxylin and eosin method then microscopically examined (Nwanjo and Alumanah, 2006; Nwanjo *et al*,. 2009).

Data presentation and Statistics

Data were presented as mean±Standard deviation of organ weight and number of bleeding spots while comparative histological architecture was presented as micrograph. Statistical analysis was done with SPSS version 16.0 using one-way ANOVA and students t-test for comparative statistics. P≥0.05 was adjudged non significant.

3. RESULTS

The phytochemical composition of the methanolic extract of *Gongronema latifolium* showed a richly presence of alkaloids, flavenoids and saponins (Table 1). Moderately present were terpenoids, steroids, resins, glycosides, proteins and carbohydrates while reducing and acidic substances and tannins were absent (Table 1).

Table 1

Phytochemical Composition of methanolic Extract of *Gongronema latifolium.*

S/N	Parameter Tested	Result
1	Alkaloids	+++
2	Flavenoids	+++
3	Terpenoids	++
4	Steroids	++
5	Resins	++
6	Glycosides	++
7	Saponins	+++
8	Proteins	++
9	Carbohydrates	++
10	Reducing substances	Neg
11	Acidic substances	Neg
12	Tannins	Neg
13	Fats/oils	Neg

Key: *Neg means tested substance was absent. + means tested substance was present in small amount. ++ means tested substance was present in moderate quantity and +++ means tested substance was abundantly present:*

Result on Acute toxicity (LD_{50}) study

Lorke's method employs two phases of testing.

All animals died in the night of the second phase of the experiment thus LD_{50} was calculated out as the geometry mean of 2500 and 1000 being the least lethal dose and the highest non-lethal dose respectively. The LD_{50} was therefore deduced as 1581.1mg/kg (IP) in mice based on Lorke.s 1983 method.

Anti-peptic ulcer and mucosal healing study

The results of anti peptic ulcer and mucosal healing effect are presented as histological micrographs. In Fig 1, the histology of non-treated animals (Negative control) showed normal histological architecture whereas the histology of positive control (Fig 2) showed extensive erosion of the mucosal epithelium with marked oedema and degenerative appearance of some of the muscularis mucosa. The submucosa, muscularis externa and serosa showed shrinkages. In Fig 3, the result post four weeks treatment with cimetidine showed moderate mucosal epithelial oedema and increase in cellular density. But unlike in the two weeks treatment with cimetidine (Fig 4) that showed shrinkages of the various layers, that of four weeks showed a normal appearance of the submucosa, muscularis externa and serosa.

On the other hand, extract of *Gongronema latifolium* exhibited protection of the mucosal layers in dose dependent fashion (Fig 5-8). At low dose treatment for two and four weeks, the histological architecture was similar (Fig7&8).

Whereas at four weeks of high dose treatment (Fig 5); the gastric and duodenal mucosa were completely restored as compared with negative control. The high dose treatment showed partial restoration of mucosal epithelium (Fig 6).

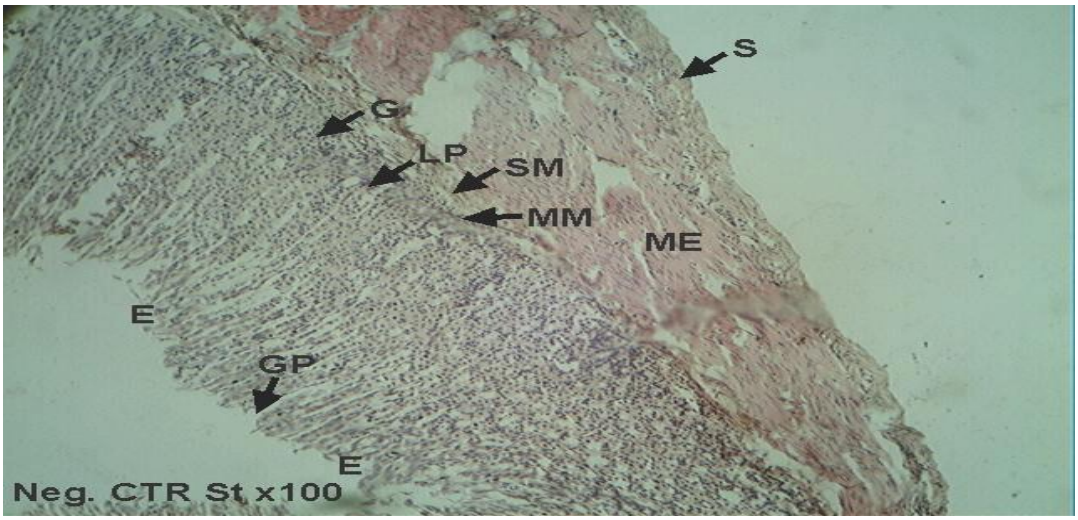

Figure 1

Mcrograph of gastric mucosa of non-treated rats (Negative control): The histological section showed four layers: the mucosa (M), submucosa (SM), muscularis externa (ME) and the serosa (S). The mucosa was subdivided into three layers; the epithelium (E), lamina propria (LP) and the muscularis mucosa (MM). The mucosal epithelium was made up of simple columnar cells that continued as the epithelium of the gastric pits to end in the gastric glands (G). The gastric glands consisted of two cell types; the parietal and the zygmogenic or chief cells. The lamina propria consisted of loose connective tissues, while muscularis mucosa was made up of the inner circular and outer longitudinal muscles. The submucosa has loose connective tissue containing arterioles (A) and venules (V). The muscularis externa was made up of three muscle layers; innermost oblique (O),innercircular (C) and outer longitudinal (L) layers. (Magnification: 100X)

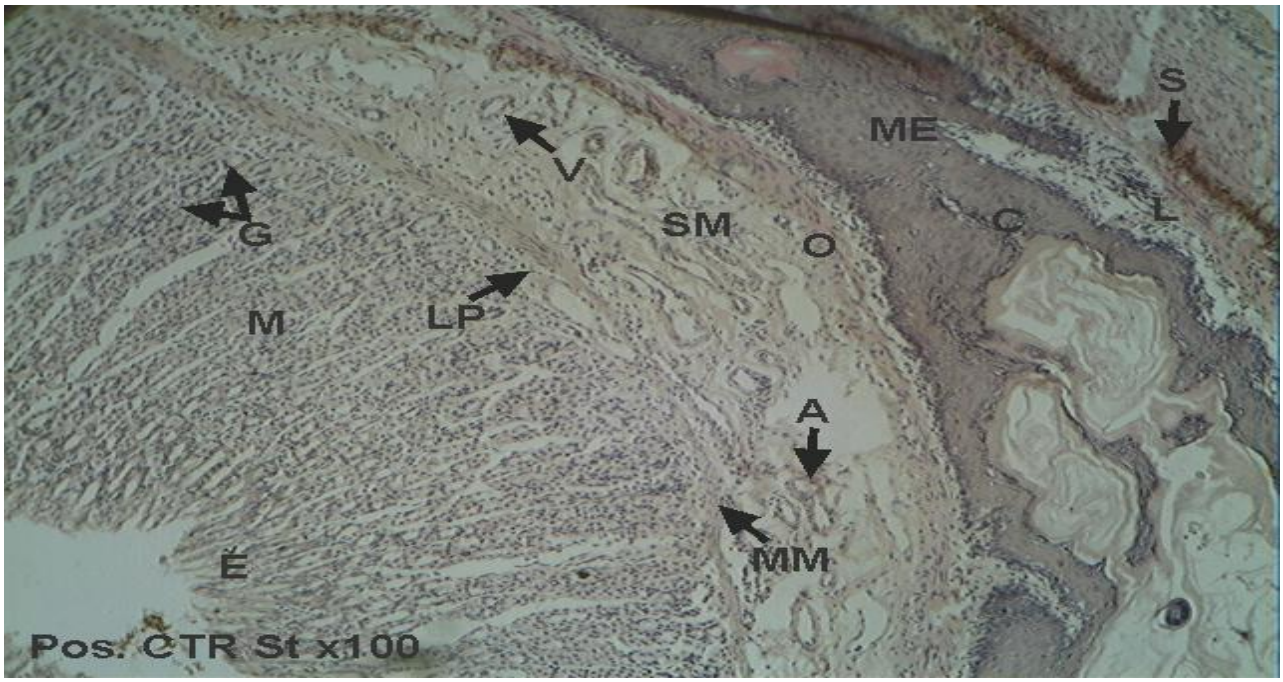

Figure 2

Micrograph of rat's gastric mucosa post indomethacin-induced peptic ulcer. (Positive control- Group A). The histological section showed extensive erosion (C) of the mucosal epithelium and marked oedema (O) with degenerative (D) appearance of some of the muscularis mucosa (MM). The submucosa (SM) muscularis externa (ME) and serosa (S) had shrinkages unlike the negative control group. (Magnification: 100X)

Azikiwe et al.
Mucosal wound healing and anti peptic ulcer effect of *Gongronema latifolium* in rats,
Discovery, 2015, 10(25), 103-112,

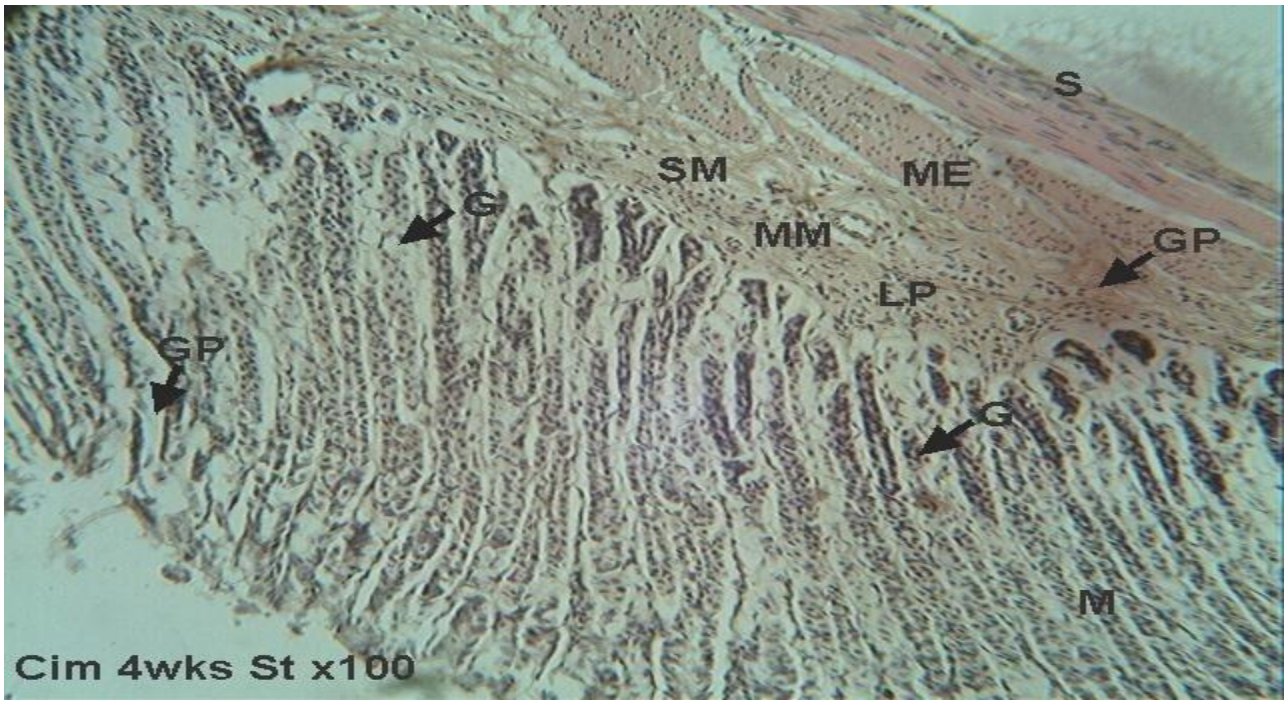

Figure 3
Micrograph of rat's gastric mucosa post 4 weeks treatment with daily 100mg/kg of cimetidine. The histological section showed moderate mucosal epithelial oedema (O), and increase cellular density. The submucosa (SM), muscularis externa (ME) and serosa (S) appeared unaffected compared with the negative control group. (Magnification: x100).

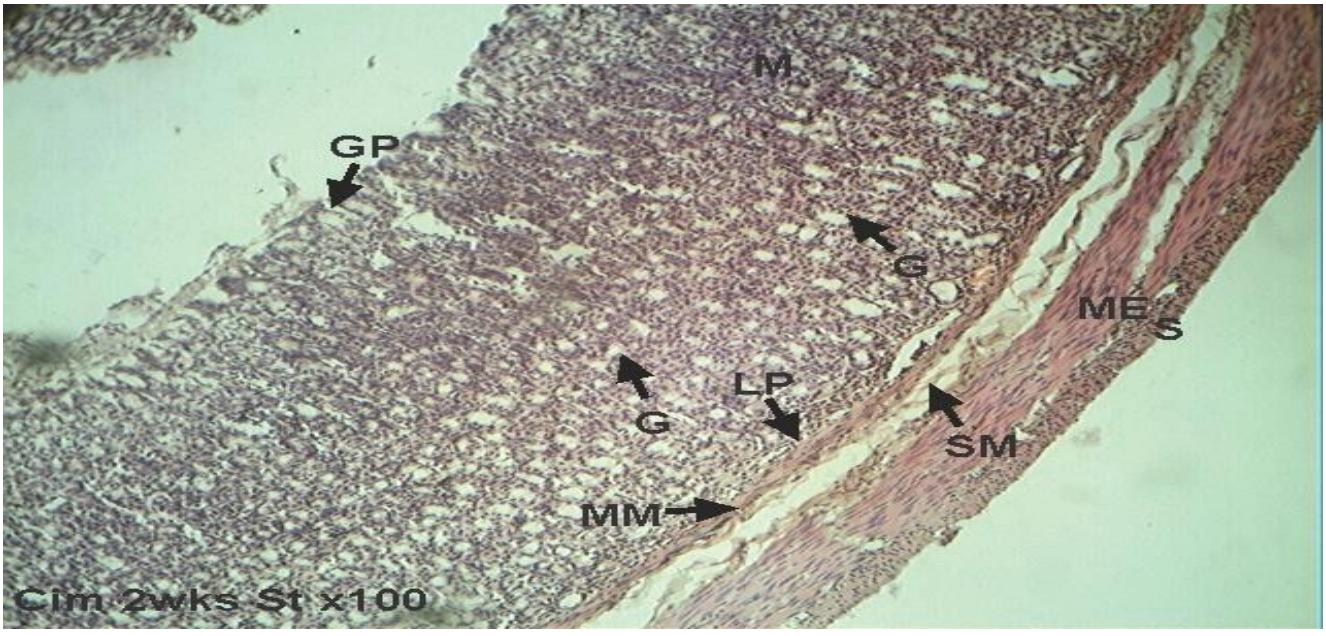

Figure 4
Micrograph of rat's gastric mucosa post 2 weeks treatment with daily 100mg/kg of cimetidine. The histological section showed moderate mucosal epithelial oedema (O), and increase cellular density. The submucosa (SM) and the muscularis externa (ME) showed shrinkages, while the serosa (S) appeared unaffected compared with the negative control group. (Magnification: 100X).

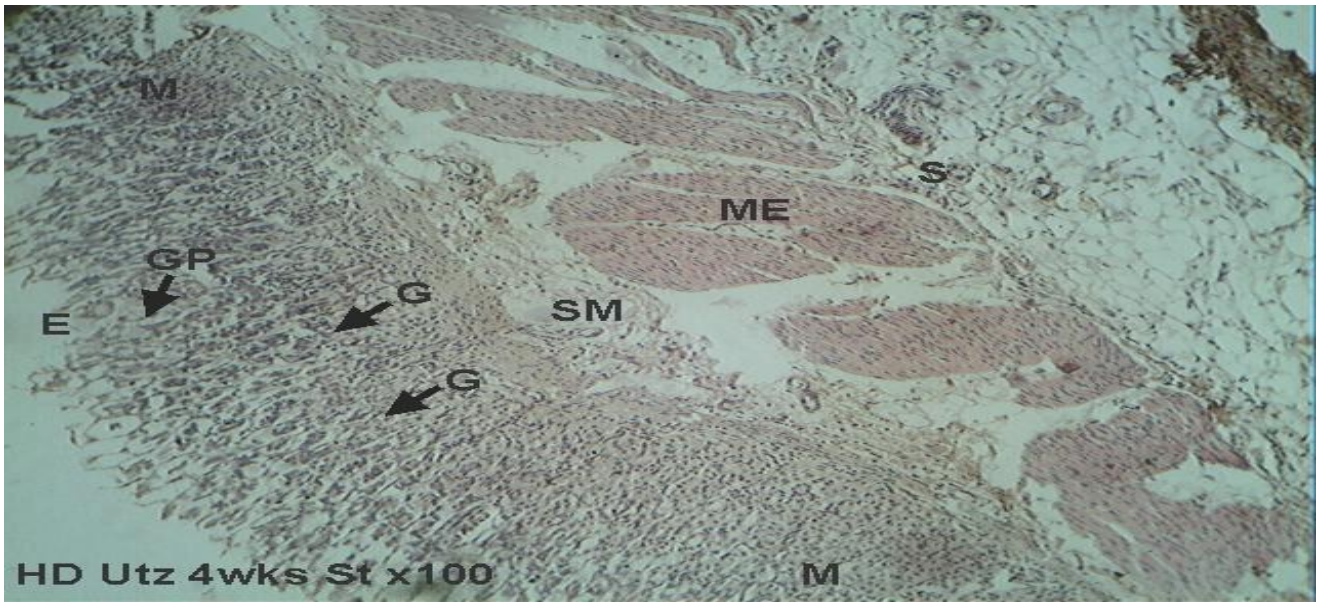

Figure 5

Micrograph of rat's gastric mucosa post 4 weeks treatment with daily oral high dose of extract (300mg/kg) of *Gongronema latifolium*. The histological showed a complete restoration of mucosal epithelium (E) and muscularis mucosa (M). The gastric cells (G) appeared more prominent. The submucosa (SM), the muscularis externa (ME) and serosa (S) appeared unaffected compared with the negative control group. (Magnification:100X).

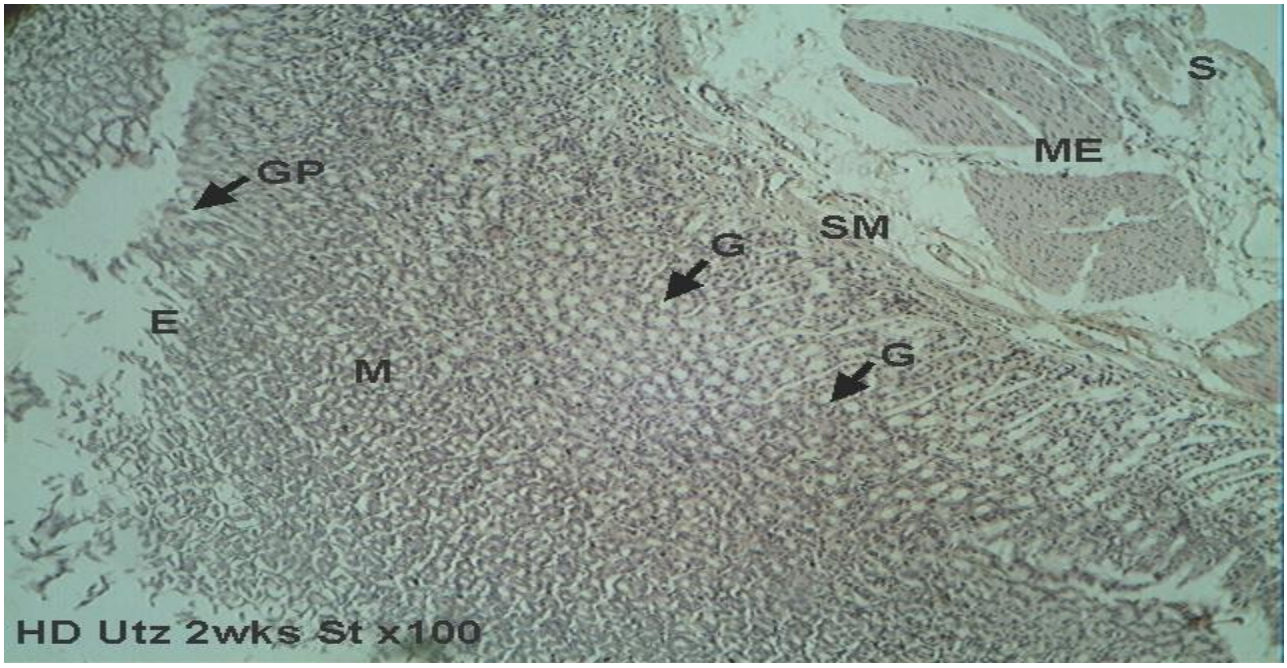

Figure 6

Micrograph of rat's gastric mucosa post 2 weeks treatment with daily oral high dose of extract (300mg/kg) of *Gongronema latifolium*. The histological section showed a partial restoration of the mucosal epithelium (E) and muscularis mucosa (M) but, increase in cellular population density with the gastric cells (G) appearing more prominent. The submucosa (SM), the muscularis externa (ME) and serosa (S) appeared unaffected compared with the negative control group. (Magnification: 100X).

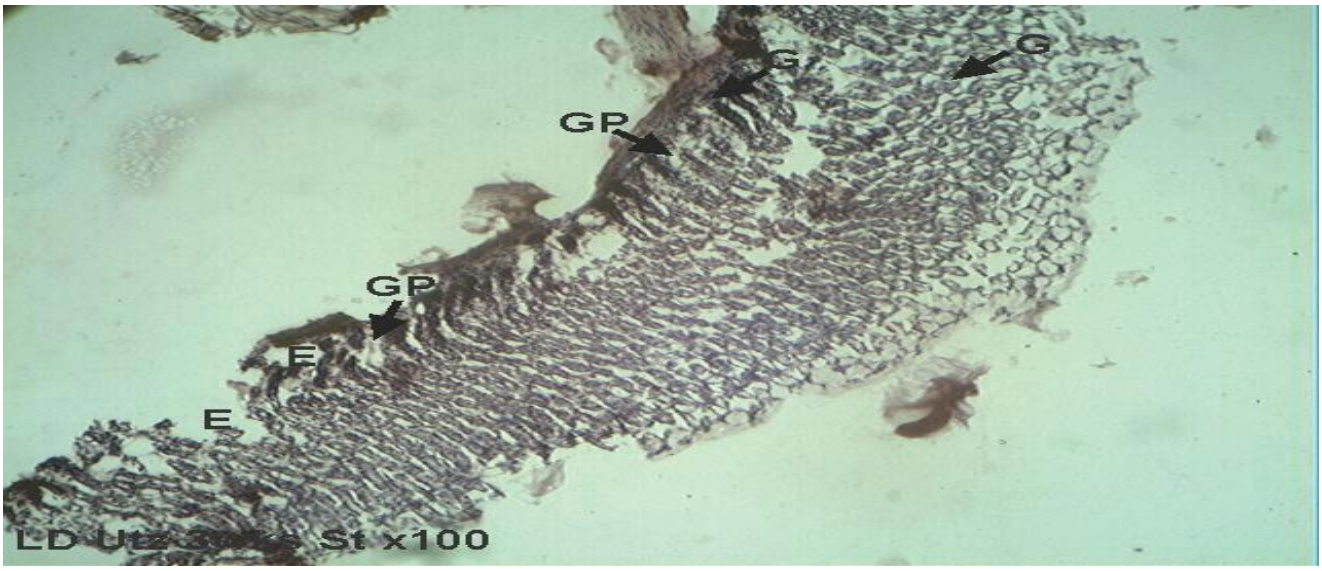

Figure 7

Micrograph of rat's gastric mucosa post 2 weeks treatment with daily oral low dose (150mg/kg) of extract of *Gongronema latifolium*. The histological section showed mucosal epithelial erosion (E), oedematous (O) and degenerative (D) mucosal cells (M) with increase cellular population density. The gastric cells (G) appeared more prominent and the submucosa (SM) appeared slightly reduced in size. The muscularis externa (ME) and serosa (S) appeared unaffected compared with the negative control group. (Magnification: x100).

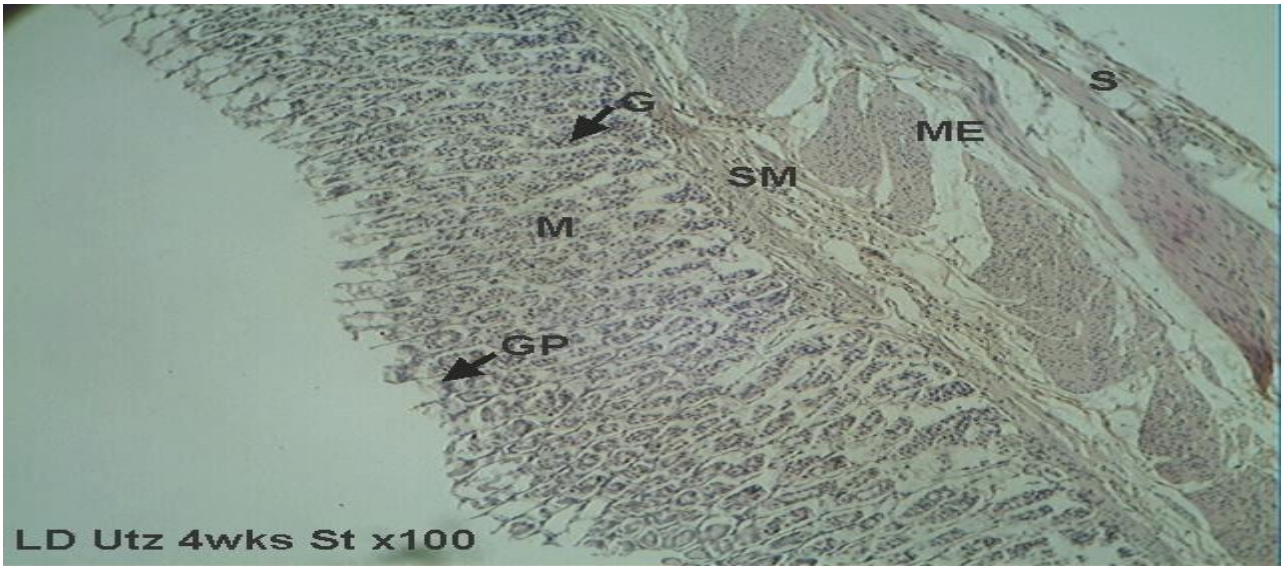

Figure 8

Micrograph of rat's gastric mucosa post 4 weeks treatment with daily oral low dose of extract of Gongronema latifolium (150mg/kg). The histological section showed mucosal epithelial erosion (E), oedematous (O) and degenerative (D) mucosal cells (M) with increase cellular population density. The gastric cells (G) appeared more prominent and the submucosa (SM) appeared slightly reduced in size. The muscularis externa (ME) and serosa (S) appeared unaffected compared with the negative control group. (Magnification: x100).

4. DISCUSSION

Acute toxicity studies aid us in proper choice of dosage regimen while chronic toxicity studies aid in guidance on possible adverse/unwanted effects as well as histological studies are usually more sensitive than biochemical parameters (Azikiwe *et al* 2014). For example more than 50% of the kidney would be damaged before creatinine and urea levels could be significantly elevated in the blood (Crook, 2007). Our work found out that the acute toxicity of *Gongronema latifolium* was 1581.1mg/kg (IP) in mice. Effiong *et al* (2012) got LD_{50} of greater than 5000mg/kg(oral) but, 1500mg/kg (IP) in mice and in a 60day sub-chronic toxicity study demonstrated no toxic effect compared to control.

Our present finding is therefore in concordance with previous literature and goes on to demonstrate the safety margin of the plant. Again noting that the freshly leaves and stems of the plant are edible vegetables and spices in most tropical African diet adds further justification to these scientific findings.

The phytochemical composition of extract of *Gongronema latifolium* showed a richly presence of alkaloids, flavenoids and saponins. These substances possess antioxidant activity (Oloyede *et al*., 2010; Faggion *et al*., 2011) thus could possibly be a means of *Gongronema latifolium* pharmacological property.

Ulcer is defined as a break in the continuation of epithelial lining and accompanied by inflammation while peptic ulcer is defined as ulcer occurring on the gastrointestinal tract or a circumcised wound of an area on the gastrointestinal tract bathed by acid and pepsin.

Indomethacin and other non steroidal anti-inflammatory drugs (NSAIDS) are commonly used to induce peptic ulcer in experimental animals (Jainu and Davi, 2006; de Andrade *et al*., 2007; Olaleye *et al*., 2008). Stress and hunger can also be used to induce peptic ulceration (Zhang *et al*., 2014). In our present study, peptic ulceration was achieved by administration of indomethacin on 24hours fasted rats. The histology of non-treated animals (Negative control) showed normal histological architecture whereas the histology of positive control showed extensive erosion of the mucosal epithelium with marked oedema and degenerative appearance of some of the muscularis mucosa.

In cimetidine treated rats the submucosa, muscularis externa and serosa showed shrinkages, moderate mucosal epithelial oedema and increase in cellular density like the positive control whereas the extract of *Gongronema latifolium* showed anti ulcer and wound healing effects in dose and time dependent fashion. At four weeks of daily 300mg/kg oral dose, the rats gastric mucosal epithelium had almost returned to normal as compared with non treated animals, negative control. Efficacy of *Gongronema latifolium* may therefore be adjudged to be superior to cimetidine. Alkaloids and flavenoids were richly demonstrated in the plant and they have been shown to possess antioxidant effect. Alkaloids and flavenoids are not present in cimetidine.

Alkaloids of *Mahonia beali* have been demonstrated to possess anti proton pump and anti gastrin secretion thus antipeptic ulcer activity (Zhang *et al*., 2014). Hydroalcoholic extract of *Achyrocline satureoides* displayed antiulcer activity, as demonstrated by the significant inhibition of the formation of induced ulcers but, this activity appears not be related to the antisecretor mechanisms (Santin *et al*., 2010).

The extract of green *propolis* at 250 and 500 mg/kg displayed an anti-secretory activity of the ligated pylorus model which led to a reduction in the gastric juice volume, total acidity and pH (de Barros *et al*., 2007). *Gongronema latifolium* antiulcerative activity is due to its prevention of chemically-induced stomach injury (Owu *et al*., 2012). Antioxidation effect could inhibit inflammation, a defined mechanical process of gastro-ulceration.

5. CONCLUSION

Gongronema latifolium possesses demonstrable anti-peptic ulcer and mucosal healing/repairing effects. These effects were greater than that of cimetidine, a standard anti-peptic ulcer agent. The study may justify the use of Gongronema latifolium in treatment of peptic ulcer disease by traditional medicine healers. Clinical trial in human is recommended.

REFERENCES

1. Ahn DG, Kim BJ, Kim JW, Kim JG (2014): [Clinical impact of dual antiplatelet therapy on peptic ulcer disease]. *Korean J Gastroenterol.*;64(2):81-6.
2. Akah P,A; Ezike A,C; Nwafor S,V; Okoli C,O; Enwerem N,M; {2003}: Evaluation of antiasthmatic properties of Asystasis angetica leaf extract. *Journal of Ethnopharmacology* 74{203}pg.25-30.
3. Akah PA, Uzodinma and Okolo CE (2011): Antidiabetic activity of aqueous and methanol extract and fractions of Gongronema latifolium (Asclepidaceae) leaves in Alloxan Diabetic Rats. Journal of Applied Pharmaceutical Science 01(09): 99-102
4. Akhila JS, Deepa S and Alwar MC(2007): Acute toxicity studies and determination of
5. median lethal dose. *Current Science*, 93, (7) 917-920.
6. Akuodor, G.C., Idris-Usman, M.S., Mbah, C.C., Megwas, U.A., Akpan, J.L., Ugwu, T.C., Okoroafor, D.O. and Osunkwo, U.A (2010): Studies on anti-ulcer, analgesic and antipyretic properties of the ethanolic leaf extract of Gongronema latifolium in rodents. African Journal of Biotechnology 9(15): 2316–2321.
7. Amazu LU, Omorogie P, Ajugwo AO, Ifezulike CC, Azikiwe CCA (2014): Anticonvulsant potency of *Chromoleana odorata* in rats. *Unique research journal of medicine and medical sciences.* 1(6); 064-069.
8. Antai AB, Ofem OE, Ikpi DE, Ukafiasi, Agiang EA (2011): Phytochemistry and some haematological changes following oral administration of ethanol root extract of *Gongronema Latifolium* in rat: *Assian Journal of science*:4: 540-547
9. Azikiwe CCA, Ifezulike CC, Amazu LU, Nwabali MO (2014): Haemostatic activities of *Jatropha curcas* (Linn) in rats. *Drug Discovery*, 8(19), 16-20
10. Azikiwe CCA, Siminialayi IM, Brambaifa N, Amazu LU, Enye JC and Ezeani MC(2012): Anticonvulsant activity of the fractionated extract of Crinum jagus bulbs in experimental animals. Asian Pacific Journal of Tropical Disease S446-S452
11. AZIKIWE, C.C.A; Amazu, L.U; Unekwe, P.C; Luechai,S; Nwosu, P.J.C; Ezeani, M.C;& Ajugwo, A.O (2009): Antitussive activity of Triclisia dictyophylla, Family of Menispermisae. *Asian Pacific Journal of Tropical Medicine,* 2(4) 33-40.
12. AZIKIWE, C.C.A; Amazu, L.U; Unekwe, P.C; Chilaka, K.C; Afonne, O & Nwankwo, M.J (2007): Anticoagulation and Antithrombotic effect of

Azikiwe et al.
Mucosal wound healing and anti peptic ulcer effect of *Gongronema latifolium* in rats,
Discovery, 2015, 10(25), 103-112,

Triclisia dityophylla, Moonseed family. *Nigerian Journal of natural product and medicine* (11) 29-31.
13. Crook AM (2006): Clinical chemistry in diagnosis and treatment. 6th edition. BookViews-Edward Arnold Publishers Ltd, UK. Pg- 112-456.
14. deAndrade SF, Lemos M, Comunello E, Noldin VF, Filho VC and Niero R (2007): Evaluation of the antiulcerogenic activity of Maytenus robusta (Celastraceae) in different experimental ulcer models. J Ehnopharmacolo 113(2): 252-257 (Pubmed).
15. deBarros MP, Sousa JP, Bastos JK and de Andrade SF (2007): Effect of Brazilian green propolis on experimental gastric ulcers in rats. J Ethnopharmacol: 110(3): 567-571(Pubmed)
16. Dike, M.C (2010): Proximate, phytochemical and nutrient compositions of some fruits, seeds and leaves of some plant species at Umudike, Nigeria. ARPN Journal of Agricultural and BiologicalScience. 5(1):7–16.
17. Effiong, G. S, Udoh, I.E, Mbagwu, H. O. C, Ekpe, I. P, Asuquo, E. N, Atangwho, I. J. And Ebong, P. E (2012): Acute and chronic toxicity studies of the ethanol leaf extract of *Gongronema latifolium*: *International Research Journal of Biochemistry and Bioinformatics,* 2(7): 155-161.
18. Eleyinmi, A.F (2007): Chemical composition and antibacterial activity of *Gongronema latifolium. Journal of Zhejiang University, Science* B 8(5): 352–358.
19. Eleyinmi, A.F. Sporns, P. and Bressler, D.C (2008). Nutritional composition of Gongronema latifolium and Vernonia amygdalina. Nutrition & Food Science 38(2): 99–109.
20. Emeka, E.J.I. & Obioa, O.(2009): Effect of a long term consumption of a diet supplemented with leaves of Gongronema latifolium Benth. on some biochemical and histological parameters in male albino rats. Journal of Biological Sciences 9(8): 859–865.
21. Faggion SA, Cunha AO, Fachim HA, Gavin AS, dos Santos WF, Pereira AM and Beleboni .(2011): Anticonvulsant profile of the alkaloids (+)-erythravine and (+)-11-α-hydroxy-erythravine isolated from the flowers of Erythrina mulungu Mart ex Benth (Leguminosae-Papilionaceae). *Epilepsy Behav.* ;20(3):441-446.
22. Gargallo CJ, Sostres C, Lanas A (2014): Prevention and Treatment of NSAID Gastropathy. Curr Treat Options Gastroenterol. Pubmed Sep 11. [Epub ahead of print].
23. Itelima, J. U. Nyam, M.A. Ogbonna, A. I. Onwuliri, E.A. Maiangwa, D. T.(2014): Comparative hypoglycaemic effect of three Nigerian medicinal plants, Gongronema latifolium, Vernonia amygdalina and Viscum album on alloxan induced diabetic mice. *Journal of Pharmacy and Biological Sciences;* 9(2): 27-33.
24. Jainu M and Devi CS (2006): Antiulcerogenic and ulcer healing effects of Solanum nigrum (L) on experimental ulcer models: possible mechanism for the inhibition of acid formation. J Ethnopharmacol 8(1-2):156-163. (Pubmed).
25. Kim, J. S., Ju, T. B., Choi, C. W. and Kim, B. C. (2008). Hypoglycemic and anti hypoglycemic effects of four Korean Medicinal Plants in alloxan induced Diabetic rats. *A.M.J. Biochem. Biotech*. 2(4) 154-160
26. Larichev AB, Maïorov MI, Favstov SV, Shalop'ev AG (2014):.Clinical-epidemiological aspects of gastric and duodenum ulcer: *Vestn Khir Im I I Grek*.; 173(2): 100-104
27. Lee TY, Wang CB, Chen TT, Kuo KN, Wu MS, Lin JT, Wu CY (2014): A Tool to Predict Risk for Gastric Cancer in Patients With Peptic Ulcer Disease on the Basis of a Nationwide Cohort by Taiwan Gastrointestinal Disease and Helicobacter Consortium. *Clinical Gastroenterol Hepatol:* S1542-3565(14)01088-X. doi:10.1016/j.cgh.2014.07.043. [Epub ahead of print]
28. Lorke, D (1983). Determination of median lethal dose. *Arch. Toxicol*., 53, 275–289.
29. Nwanjo, H.U. & Alumanah, E.O(2006): Effect of aqueous extract of Gongronema latifolium on some indices of liver function in rats. Global Journal of Medical Sciences 5(1): 17–20.
30. Nwanjo H. U, Okafor M. C and Oze G. O. (2006): Anti-lipid peroxidative activity of *gongronema latifolium* in streptozotocin-induced diabetic rats. Nigerian Journal of Physiological Sciences 21 (1-2): 61-65.
31. Okolie, U.V., Okeke, C.E., Oli, J.M. and Ehiemere, I.O(2008): Hypoglycaemic indices of Vernonia amygdalina on postprandial blood glucose concentration of healthy humans. African Journal of Biotechnology 7(24): 4581–4585.
32. Olaleye SB, Owoyele VB and Odukanmi AO (2008): Antiulcer and antisecretory effects of Landolphia owariensis extracts in rats. Niger J Physiol Sci. 23(1-2): 23-26.
33. Okeke, E.C., Eneobong, H.N., Uzuegbunam, A.O., Ozioko, A.O. & Kuhnlein, H., 2008. Igbo traditional food system: documentation, uses and research needs. Pakistan Journal of Nutrition 7(2): 365–376.
34. Oloyede KG, Oke MJ, Raji J and Olugbade T(2010): Antioxidant and anticonvulsant alkaloids in Crinum ornatum bulb extract. *World journal of chemistry* 5(1) 26-31. (Pubmed).
35. Owu DU; Nwokocha CR; Obembe AO; Essien AD; Ikpi DE; Osim EE (2012):West Indian med. j. vol.61 no.9 Mona Dec. 2012
36. Santin JR, Lemos M, Klein LC, Niero R and de Andrade SF (2010): Antiulcer effects of Achyrocline satureoides (Lam) DC (Asteraceae) (Marcela), a folk medicine plant, in different experimental models. J Ethnopharmacol 130(2): 334-339 (Pubmed).
37. Trease G.E and Evans W.C (1983): Textbook of Pharmacognosy. 12th ed. Bailliere, Tindall, London Pg: 102-111.
38. Veligotskiĭ NN, Komarchuk VV, Trushin AS, Gorbulich AV, Komarchuk EV (2014): [Postoperative reflux in treatment of complicated forms of duodenal ulcer disease]. *Klin Khir*; (4):5-7.
39. Zhang SL, Li H, He X, Zhang RQ, Sun YH, Zhang CF, Wang CZ and Yuan CS (2014): Alkaloids from Mahonia bealei posses anti-H(+)-ATPase and anti-gastrin effects on pyloric ligation-induced gastric ulcer in rats. Phytomedicine 21(11): 1356-1363 (Pubmed).