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Research Paper

Healing, Cytoprotective and Anti-*Helicobacter pylori* activities of stem bark extracts and butanol fraction of *Nauclea latifolia*

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ABSTRACT

In this study, we investigated the healing, cyto-protective and anti *H. pylori* activities of stem bark extract and butanol fraction (BNL) of *Nauclea latifolia*. They were evaluated for their ability to inhibit gastric lesions in histamine and aspirin induced ulcer models in rats. Their influence on pH, wall mucus, acidity and gastric acid output were recorded. The extract (50mg/kg and 100mg/kg) and BNL (50mg/kg) were administered daily for seven days for gastroprotective and healing studies. Cimetidine, omeprazole and misoprotol (20mg/kg respectively) were used as reference drugs. The *in vitro* anti-*Helicobacter pylori* activities of the extract and BNL were also investigated. They significantly increase ulcer tolerant rate and healing rate compared to controls. Increase in pH with groups treated with the extract and BNL was also observed.The results showed that the test extract and BNL have more cyto protective than healing properties. Results of anti-*Helicobacter pylori* studies showed the minimum inhibitory concentration (MIC) for crude extract to be 25mg/ml while the minimum bactericidal concentration (MBC) is 100mg/ml. This study demonstrates that *Nauclea latifolia* possesses significant cyto-protective activities on aspirin and histamine induced gastric lesion and promising anti-*Helicobacter-pylori* effect thus justifying its use in folkloric medicine as antiulcer.

Keywords: *Helicobacter Pylori*; Cytoprotective; Ulcerogenic; *Nauclea latifolia*

INTRODUCTION

Gastrointestinal ulcer (GIU) is one silent but a leading tropical disease associated with discomforting and crucial pain in the stomach and intestinal region of the body. Over the years, the cause of GIU has been attributed to common use of NSAIDS, necrotizing agents and other ulcerogenic agents. Recently, the major cause has been attributed to infections arising from *Helicobacter pylori*, a micro organism which inhabits in the gastro-intestinal region of the body. Since its discovery in 1983, the microorganism has been related with the pathogenesis of several diseases of the digestive system, such as gastritis, peptic ulcer disease and gastric cancer (Kusters, et

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al., 2006). Conventional therapy for eradication of this disease is based mainly on the use of multiple drugs, such as clarithromycin, amoxicillin, furazolidone, tetracycline, metronidazole with bismuth or a proton pump inhibitor (Megraud, et al., 2007). Many and more of these synthetic drugs have been used to treat GIU disease but the major challenge remains the resistance of these drugs over a long period of usage hence continuous exploration of new therapeutic agents.

Quite a number of medicinal plants have been shown to possess gastro-protective activities. Carbenoxolone, the first systemically effective antiulcer with a cytoprotective property was isolated from a plant , *Glycyrrhiza glabra* (Brown,et al., 1959). Gefarnate, another anti-ulcer compound isolated from juice of wild cabbage has been known to demonstrate a gastric defence mechanism by increasing the mucus synthesis in the mucosa (Adami, et al., 1964). Other plants known to have antiulcer activities include *Ociumum basilium* leaves (Akhtar, et al., 1989) *Panax ginseng* (Xio-Bo,et al.,1992), *Styrax camporum*, *Caesalpinia ferrea* (Bacchi,et al.,1995). Nevertheless, research in plants, a natural product remains a field of dynamic study in providing templates for novel drugs discovery due to its munificent endowment of active components of medicinal values.

The plant under investigation is *Nauclea latifolia* Sm.(*Rubiaceae*). The leaves, stembark and roots of *N.latifolia* are used in Nigeria for treatment of various ailments such as diabetes mellitus, fever, hypertension, antihelmintic, antiplasmodial, skin diseases and microbial infections. The plant has been reported to contain terpenes, alkaloids, saponins and glycosides. Their main compounds are the indole alkaloids and saponines. (Erdelmeier,et al.,1991; Hideyuki,et al.,2003).

However, The indigenous people of Abia State in South-East, Nigeria use it extensively for management and treatment of gastrointestinal ulcers. To the best of our knowledge, no study has been systematically conducted to evaluate the cytoprotective and healing activities of hydro-methanolic extract of *Nauclea latifolia*, to support traditional uses of this plant in folklore medicine. Hence, the present study was undertaken to evaluate the cytoprotective and healing activities of *N. latifolia* in healthy Sprague-dawley rats.

MATERIALS AND METHODS

Plant materials: The stem bark of *Nauclea latifolia* was collected in the hinterland of Ikwuano, Umuahia, L.G.A, Abia State of South East Nigeria in June, 2012. The plant was identified by Mr Odewo of the department of Botany, University of Lagos and a voucher specimen LH 4016 deposited at same department.

Preparation of fractions: Air-dried *N. latifolia* stem bark was pulverized, 500g powder was extracted with 50% MeOH using hot maceration method. The extraction was done successively twice, evaporated under reduced pressure. The dried extract was stored in airtight container and kept in freezer prior to use. Extract of *N. latifolia* stembark (20g) was dissolved in 200ml distilled water and then extracted 3 times with 200ml of hexane, $CHCl_3$, EtOAc and butanol in successive order to yield HNL, CNL, ENL and BNL fractions. Each fractions of the extract was evaporated to dryness, weighed and stored for further use. In this study, only crude extract and butanol (BNL) fraction were further investigated for anti-ulcer activities after subjecting the four fractions to preliminary anti-ulcer screenings.

Animals and handling: Healthy Sprague-dawley rats of either sexes weighing between 100-200g and obtained from animal house University of Lagos, Idi-Araba Lagos were used. The rats were housed in a well-ventilated animal house under a controlled light, temperature and humidity conditions where they had free access to

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standard feed and water. The rats were fasted for 24hrs prior to GIU assay. Animals were grouped and placed in separate cages to facilitate experimental work and for accurate evaluations. Handling of animals was done in accordance with international acceptable guidelines of CRC research number letter number M2012/02 dated 31st Jan, 2013. The rats were divided into eight groups of ten animals each. Group I served as control received distilled water, Group II received aspirin; 200mg/kg while Group III, IV and V received omeprazole, cimetidine, misoprostol 20mg/kg respectively. Group VI and VII received crude extract, 50 and 100mg/kg respectively while Group VIII received BNL, 50mg/kg. The animals were subjected to 24h of fasting prior to experimental use.

Anti-ulcerogenic evaluation assay in rats: Gastric ulcers were induced in all the different groups of rats except Group I. The sequence of trials and the treatments were randomized as follows:

Aspirin-induced gastric ulceration**:** Acetyl salicylic acid (200mg/kg) solution was administered orally in one hour interval to different groups of rats until ulceration was observed by writhing movement in the rats and further observed by using ulcer scores achieved by sacrificing two out of the rats in group II. The stomach was removed and opened along the greater curvature. The number of ulcer spots in the glandular portion of the stomach was counted in both control and drug treated animals and the ulcer index was calculated (Singh and Mujumdar, 1999).

Histamine-induced gastric mucosa damage: The same grouping as aspirin was used. Histamine hydrogen chloride solution (5mg/kg) in sterile normal saline was injected intra-peritoneally (i.p.) after one hour interval to different groups of rats until ulceration was also observed.

Treatment procedures

Cyto-protective assay-using aspirin induced model: The extract (50 and 100mg/kg) and reference control, misoprostol (20mg/kg) were administered on body weight basis. A one week treatment was used. Induction of ulcer using aspirin model, (200mg/kg, p.o.) commenced on the fifth day after pre-treating with extract, BNL and misoprostol separately and treatment continued till 7th day. The treated animals were then sacrificed for further investigations and the rate at which ulcer was tolerated ***(*UTR,%)** in the stomach of the rats were evaluated.

Cyto-protective assay-using histamine induced model: The extract (50 and 100mg/kg) and two reference controls (cimetidine, omeprazole, 20mg/kg) were also administered on body weight basis. Induction of ulcer using histamine (5mg/kg i.p.) induced model, also started on the fifth day of pre-treatment with test crude extracts and reference controls separately. Treatment and ulceration with histamine continued concurrently till 7th day. The treated animals were also sacrificed for further investigations.

Cyto-healing assay-using aspirin induced model: The extract (50 and 100mg/kg) and various reference controls (cimetidine, misoprostol, omeprazole, 20mg/kg) were also administered on body weight basis. Only aspirin induced model was used in this healing assay. The various treatment groups were administered with the test drugs except Group I and Group II after four days of ulceration with aspirin (200mg/kg). The animals were then sacrificed on the 7th day for further investigations and the rate of healing of the ulcerated areas ***(*UHR,%)** in the stomach of the rats were also evaluated.

Procedures of antiulcer evaluation studies

Determination of gastric volumes, pH, and acid – outputs**:** The rat stomachs were excised out under ether anesthesia exactly after four hours of pylorus ligation and the gastric contents were collected. The stomachs were washed with luke warm sterile

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water. Both the washings and gastric contents were centrifuged together at 4000rpm for 10min. The supernatants were collected separately. All the stomachs were incised along the greater curvatures and preserved in 5% formalin solution separately in air – tight bottles at 4°C. The volume of all the supernatants of gastric contents was measured using microsyringes. The acid output was determined by titration to pH 7 with 0.1 N (NaOH) solutions and calculated by the following equation:

Acid output (µEq) = Acidity (mEq / l) X Volume of gastric juice (ml)

$$\textbf{Where; Acidity} = \frac{\text{Volume of NaOH} \times \text{Normality of NaOH} \times 100}{0.1} \textbf{ (mEq/ l)}$$

***Calculation of gastric ulcer index*:** The stomachs were opened along the great curvature, rinsed with saline to remove gastric contents and blood clots and examined under a simple magnifier. The gastric damage in the glandular regions were located in the gastric mucosa as elongated black-red lines parallel to the long axis of stomach. The numbers of ulcers were counted and the ulcer scores were read using the Magistretti scale (0 to signify no lesion and 6 to signify more than thickened lesion). Mean ulcer score for each animal was expressed as ulcer index.

$$\textbf{Ulcer index } (U_I) = U_N + U_S + U_P \times 10^{-1}$$

- U_I =Ulcer index; U_N =Number of animals with ulcers; U_S =Average number of severity score; U_P =Percentage animals with ulcers

$$\textbf{Ulcer Tolerant / Healing Rate (UTR, UHR \%)} = \frac{\textbf{UI of the treatment group (s)}}{\textbf{UI of untreated control group}} \times 100$$

***Determination of Drug effects on Gastric Mucus Contents*:** The assay was carried out according to modified method of Corne,et al.,1974. The glandular portions of stomachs from all the animals of normal untreated control, untreated control and treated groups were collected and weighed. The segments were immediately transferred to the 1% alcian blue solution in 10% sucrose. Thus, glandular mucus was allowed to complex with alcian blue for 10min. The excess dye of each segment was removed by rinsing with sucrose solution and complexed dye was extracted for 15min in 5ml of 5% magnesium chloride solution, which was then shaken with equal volume of diethyl ether. The resulting emulsions were centrifuged at 4000rpm for 15min and the absorbencies of the aqueous layers were measured at 580nm using UV-Spectrophotometer. Different concentrations i.e. 100, 200, 300, 400, 500, 600, 700, 800, 900 and 1000µg of alcian blue in 10% sucrose solution were also prepared and their absorbance were recorded at 580nm.

Histology assay

***Tissue Processing*:** Processes involved in preparing tissues for micrometry which include grossing, cutting up, tissue processing, embedding, fixation and wax impregnation were caaried out. The tissues scrapped from the stomach were cut into thin slices with the aid of scalpel blade, placed in the embedding cassettes and passed through 10% formal saline, 70% alcohol, 90% absolute alcohol and xylene in separate glass chambers by the use of automatic tissue processor for 18h. This was done to ensure tissue consistency suitable for microtomy.

***Microtomy*:** Rotary microtomy was used in this assay. It involves obtaining sections from tissues. The tissues were clamped and trimmed to required thickness 3-5µm and placed on a glass slide containing 20% alcohol and then gently lowering the strip of each section from the slide on the surface of water at 5-10°C below the melting point of wax. The sections were floated, expanded slightly and flattened then isolated on the hot plate for the surrounding wax to melt off the tissue.

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Haemotoxylin and eosin staining technique: The staining technique demonstrates general tissue structures. This was done by de-waxing, staining the tissues with haemotoxylin for 1min and differentiated with 1% acid alcohol. The sample was then stained with 1% eosin for 3min, and dehydrated with alcohol and cleared with xylene. It was finally examined under microscope. Blue coloration indicates nucleus while pink shows cytoplasm.

Anti-helicobacter pylori assay

***Isolation of micro-organism*:** *H. pylori* bacteria strain isolated from a human gastric biopsy was obtained from the University of Lagos, Teaching hospital, Idiara-araba, Lagos, Nigeria and was identified by Dr Smith, National Institute of Medical Research by morphological and chemical methods. The *H.pylori* isolate was stored at freezing mode – 80°C prior to experimental use.

Preparation of inoculums: Freshly prepared Gab camp (GC) agar plates containing appropriate antibiotic [vancomycin (4mg/ml), polymyxin B (0.35mg/L), Trimethoprim (6mg/ml), amphotericin B (3ug/ml), cyclohexamide (20mg/L), ferric pyrophosphate (25g/L) and L-cystein (40g/L)] was autoclaved and allowed to cool in a water bath at 56°C for 60min. Defribrinated horse serum (8%; 40ml) was added with 5ml of vitamin mix and 1 vial of Vitox supplement. The mixture was poured into the plates and covered with white cloth to prevent light degradation of the antibiotics while cooling. The bacterial strain isolated and previously stored was sub cultured under micro-aerobic conditions of 5% O_2, 15% CO_2 and 80% N_2 at 37°C on the freshly prepared agar supplement until the desired amount of growth was obtained (Day 3- 5).

Preparation samples and agar-plates for agar dilution assay: Agar dilution method was used to determine the anti- *Helicobacter pylori* activities of the crude extract of *N. latifolia*. Guideline given by the NCCLS for methods for dilution antimicrobial susceptibility tests was used. Extracts were diluted from the highest concentration to the final concentration. The test extracts of *N. latifolia* and reference standard; amoxicillin were diluted 2 fold from the highest concentration 100mg/ml to 3.125mg/ml. For the incorporation of the test drugs into the agar, a 1: 10 dilution in agar was used which is 2ml of each test drug into 18ml of the molten agar at 45°C-40°C. Each was carefully mixed and poured into sterile Petri dishes. The contents were allowed to set. Each Petri dish contains 100mg/ml, 50mg/ml, 25mg/ml, 12.5mg/ml, 6.25mg/ml and 3.125mg/ml. All the plates were kept in the incubator at 37°C with the plates turned upside down for 18-24h. After overnight incubation, all the plates were checked for visible growth. No growth signifies that the plates are pure for use.

MIC determination by Agar Dilution method: 1-2µl of standard inoculums (10^4 CFU) of the organism were inoculated on the agar surface of each plate containing the test drugs and allowed to dry upside down before been incubated under micro-aerophilic conditions at 37°C for 48h using micro-aerophilic system envelopes. 1-2µl of the bacterial suspension was also inoculated on agar free antimicrobial plate and incubated alongside for control check. The minimum inhibitory concentration (MIC) was taken as the least concentration or highest dilution of the extracts that inhibit visible growth of a micro organism.

Determination of MBC: Minimum bactericidal concentration (MBC) was also taken as the least concentration at which there is no visible growth of the organism indicating 99.5% killing of the original inoculums. The MBCs were determined by sub-culturing the bacteria from the least concentration (25mg/ml) that inhibited the growth of the organism into sterile agar plates and further incubated for 48h.

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Statistical analysis: Data for each experiment were expressed as the MEAN± S.E.M (standard error of mean) value for triplicates. Data analysed by one way ANOVA. Statistical significance was placed at $P \leq 0.05$ and highly significant at $P \leq 0.01$.

RESULTS

Anti-ulcer results: Aspirin and histamine methods of ulceration are commonly used in the study of the activities of natural products in ulcerated animal model. Aspirin, a NSAID is an agent that inhibits the enzyme cyclooxygenase 1 (COX-1). They are directly responsible for the synthesis of prostaglandins (PGD) which protects the gastric mucosa by inhibiting acid secretion. Histamine, on the other hand is a local hormone which also stimulates the release of gastric acid by interacting with a specific type of histamine receptors known as the H_2 receptors (Graham, 2005). These two ulcer induction methods resulted in depleting the cytoprotective morphology of the mucosal cell walls and increased the gastric secretions (Figures 3-5, 10.

Acute and sub-acute toxicity studies carried out on the aqueous extract of *N. latifolia* in animal model using the highest dose (180mg/kg) by James,et al, 2014 and also supported by a previous toxicity report on *N. latifolia*. (Gamaniel, 2005) gave rise to the doses of test samples used for this study.

Cytoprotective assay: In order to determine the cytoprotective activities of the extracts of *N. latifolia*, the rats were pretreated with the test samples and reference drugs before being induced with ulcer agents. The results in table 1 for aspirin model showed a significant reduction ($P \leq 0.05$) in the ulcer score from 6.0 for untreated control group to 0.50 for the group that received the lower dose of crude extract (50mg/kg) and BNL (50mg/kg). The rate at which ulceration of gastric mucosa cell walls was tolerated (UTR,%) was observed at lower dose, 50mg/kg (crude extract (4.4%) and BNL(28.1%),) comparative to the higher dose (100mg/kg, 94.9%) and reference drug, misoprostol, (20mg/kg, 95.0%). For histamine model, same reduction was observed in ulcer score for lower dose compared to the negative control group (Table 3).

***Healing assay*:** To determine the healing activities, the rats were ulcerated first with aspirin and then treated with the test samples and reference drugs. The results showed reduction in ulcer score for both the higher (100mg/kg, 4.17) and lower dose (50mg/kg, 2.83) comparable to the negative control group (6.0). In this assay, the reference drug, omeprazole showed more reduction in ulcer score than the test drugs (Table-2).

The results of gastric secretions (Gastric volume, pH, acid output) and mucus secretions in both assay (cytoprotective and healing) were also recorded (Table 1-3).

Histopathological analysis: The rat stomach examined, further confirmed the antiulcer activities of crude extract and BNL fraction of *N. latifolia* by reducing the haemorrhage in gastric mucosa (Figures 3-9; 11-13).

Anti-helicobacter pylori activity: Initial anti-bacterial screening using agar disk diffusion method of crude extract of *N. latifolia*, highest concentration 400mg/ml; zone of inhibition (ZOI); 32mm was suggestive of the concentrations used in agar dilution method.The MIC and MBC results of crude extract of *N. latifolia* showed anti-*helicobacter pylori* activity but not as significant as amoxicilin used as the reference drug (Table 4).

DISCUSSION

Drug discovery and development from natural remains a field of dynamic research as far as disease conditions continue to be a global challenge. Medicinal plants are gaining more and more recognition as nutraceuticals, bioactives, food supplements

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and pharmaceuticals day by day, especially with the support of the World Health Organisation (WHO). Gastrointestinal protection including protection of mucosal cells lining is an inevitable necessity, most especially with the predominace rise of gastric ulcer due to prompting factors in form of carcinogenic chemicals present in most food spices, abusive use of NSAIDs , corticosteriods, age factors, environmental and climatic changes. The persistence of ulcers after treatment with anti-ulcer agents have also been attributed to the presence of the micro-orgnism-*Helicobacter pylori* . Orthodox drugs currently used in the treatment of gastrointestinal ulcer (GIU) have shown increased rate of resistance, relapses, drug interactions and unpalatable adverse effects. These challenges have supported the continous search for novel molecules that can provide better healing and cyto-protective activties for GIU.

Ulcer is generally believed to be due to imbalance between aggressive factors and the mucosal defence mechanism (Mukherjee,et al., 2010). The cyto-protective results in this study showed that the extract and its butanol fraction demonstrated significant antiulcer activity against aspirin and histamine induced ulcers in rats. The ulcer tolerant rate (UTR) which is the rate at which ulcer was allowed when pretreated with extract (50mg/kg, 4.4%) and BNL (50mg/kg, 28.1%) was appreciative and significantly **(***P*≤0.01**)** comparable to other treatment groups including reference control misoprostol (Table 1, Fig 6, 9). It is known that PGE2 and PGI2 (prostaglandin) of gastric and duodenal mucosa are responsible for mucous production and maintaining cellular integrity of the gastric mucosa (Graham, 2005).However, the results of histological slides suggested that the extract and BNL (50mg/kg) in pretreatment method (Figure 6, 9) may have stimulated the release of prostagladin by competiting with aspirin at the COX-1 binding sites. The increased covering of the goblet cells with mucin is an indication of increased mucus secretion (Fig 6). The ulcer healing rate which is the rate at which the ulcerated areas were healed (post treatment) in aspirin induced model was significant increase in the test group (50 mg/kg and 100 mg/kg; 58.23% and 60.35% respectively) compare to the untreated control group (*P*≤ 0.01) and reference control (*P*≤ 0.05) cimetidine (32.6 %) but less than that of omeprazole (94.1 %).

Aspirin is known to induce gastric damage by suppression of synthesis of endogenous prostaglandins which play protective role in the stomach by stimulating secretion of bicarbonate and mucus sustaining mucosal blood flow and moderation of cell turn over and repair (DeAndrade, et al., 2007). Omeprazole is known as proton pump inhibitors which act irreversibly by blocking the H+/K+adenosine triphosphatase enzymes (the H^+/K^+ ATPase) system of the gastric parietal cells, simply called the gastric proton pump (Graham, 2005). This pump is the terminal stage in gastric acid secretion, being directly responsible for secreting H^+ ions into the gastric lumen, making it an ideal target for inhibiting acid secretion.

Histamine is also known to induce gastric ulcers through stimulation of H2 receptor leading to enhanced gastric acid secretion and vasodilation (Amagase & Okabe, 2003). In pretreatment method, the extract and BNL (50mg/kg**)** showed highly significant (*P*≤0.01) UTR activity 44.4 and 62.80% respectively compared to the untreated positive control (only histamine). The higher dose (100mg/kg**)** produced better cytoprotective activity (83.02%). This result suggests that the extract and BNL antiulcerogenic properties may be mediated through anti-histamine activities. Furthermore, gastric acid secretions have been observed to be stimulated and regulated by activities of NSAIDs, proton pump or H^+ / k^+ ATP ase (Merkelbach,et al., 2002; Kwiecien, et al., 2001). Results of pretreatment group in aspirin induced ulceration showed a significant (*P*≤0.05) decrease in gastric secretion (volume and

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acid output) extract (50mg/kg and 100mg/kg) and BNL .44ml; 0.56ml, 3.60µEq/hr and 0.55ml, 2.75µEq/hr respectively (Table 1). Increase in gastric secretion pH was also observed with the extract. Reduction in gastric secretion (volume and acid output) was also observed in histamine induced models (Table 3). Mucus secretion production in aspirin and histamine models was appreciated with groups pretreated and post treated with extract (50mg/kg, 100mg/kg) and BNL (50mg/kg) as shown in their absorbance values (Tables 1-3). The results revealed better mucus secretory activities with test groups than with reference control groups. The extract and its fraction showed decreased ulcer index, increased curative ratio, decreased number of gastric ulcers and decreased total acidity thus they have potent anti-ulcerogenic effects against histamine- and aspirin- induced gastric ulcer.

Anti-*Helicobacter pylori* assay was also carried out to investigate the activities of extract on the main organism-*H. pylori* known to cause inflammation on the stomach wall. Metronidazole, amoxicilin,furazolidone and clarithromycin, among others have long use in the treatment of GIU in different combinations therapy regimens (Sener-Muratoglu, et al., 2001; Choudhary, et al., 2001, Fakheri, et al., 2001). In spite of this, *H. pylori* still developed resistance to these antibiotics and its infection has been considered a major cause of gastric (stomach) cancer, associated with an increased risk of gastric mucosa-associated lymphoid tissue (MALT) lymphoma. It develops ways of interfering with local immune responses, making them ineffective in eliminating the bacteria (Atherton, 2006; Kusters, et al., 2006). This is an awaken call for continous search for antibiotics that will be potent against the micro-organism. Agar dilution result showed that extract demonstrated noticeable anti-*H. pylori* activities which was significantly less than amoxicillin (Table 4).

N. latifolia stem bark was reported to contain phenolic compounds like flavonoids and tannins (Egbun, et al., 2013) which are special metabolites with significant antioxidant activity that participate significantly in the gastroprotection and anti-*H. pylori* activities (Zayachkivska, 2005). Presence of these secondary metabolites may be responsible for observed activities.

CONCLUSION

Butanol fraction of *N. latifolia* stem bark has promising cytoprotective and healing activities in different ulcer induced models as well as anti-*H. pylori* activities.

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REFERENCES

Adami, E., Marazzi –Uberti, E. Turba, C., (1964): Pharmacological research on gefarnate a new synthetic isoprenoid with antiulcer action. *Pubmed*., 147:113-45.

Akhtar, N.S., Munir, M., (1989): Evaluation of the gastric anti-ulcerogenic effects of *Solanum nigram*, *Brassica oleracea* and *Ocimum basilicum* in rats. *J.Ethnopharmacol.*, 27: 163-175.

Amagase, K., Okabe, S., (2003): On the mechanisms underlying histamine induction of gastric mucosal lesions in rats with partial gastric vascular occlusion. *J Pharmacol Sci.* 92 (2):124-36.

Atherton, J.C., (2006): The pathogenesis of *Helicobacter pylori*-induced gastro-duodenal diseases. *Annual Review of Pathology*. *PubMed* Abstract., 1:63-96.

Bacchi, E.M., Sertie, J.A.A., Villa, N., Katz, H., (1995): Anti –ulcer action and toxicity of *Styrax camporunm* and *Caesalpinia ferra*. *Planta Medica*; 61: 204 - 207

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Brown, H.M., Christie,A.B.G.B., Jones, E.C., Finney, R.S.H., MacG regor, W.G., Smith, J.M., Smith, W.G., Sullivan, F.M., Tarnoy, A.L., Turner, E.E., (1959): Glycyrrhetinic acid hydrogen succinate (disodium) salt, a new anti-inflammatory compound. *Lancet*., (2): 492-496.

Choudhary, A.M., Roberts, I., Nagar, A., Tabrez, S., Gupta, T., (2001): *H. pylori* related gastric outlet obstruction: is there a role for medical treatment? *J Clin Gastroenterol*; 32 (3): 272-273.

Corne, S.J., Morrisey, S.M., Wood, R.J., (1974): Proceedings; A method for the quantitative estimation of gastric barrier mucus. *Physiol.*, 242 (2) :116-117.

Egbung, G.E., Atangwho, I.J., Iwara, I.A., Odey, M.O., Ebong, P.E., (2013): Chemical composition of root and stem bark extracts of *Nauclea latifolia*. *Scholars Research Library, Archives of Applied Science Research*, 5:193-196.

Erdelmeier, C.A.J., Wright, A.D., Orjala, J., (1991): New indole alkaloid glycosides from Nauclea orientalis. *Planta Med*., 57: 149-52.

Fakheri, H., Malekzadeh, R., Merat, S., Khatibian, M., Fazel, A., Alizadeh, B.Z., Massarrat, S., (2001): Clarithromycin vs. Furazolidone in quadruple therapy regimens for the treatment of *H. pylori* in a population with a high metronidazole resistance rate. *Aliment Pharmacol Ther.*, 15 (3): 411-416 .

Gamaniel, K.S., (2005): Sub-acute Toxicity Studies on the Aqueous Extracts of *N.latifolia* in rats. *Afr.J.Trad Compl. Altern Med;* 2: 177-205.

Graham, L.P., (2005): Anti-Ulcer agents: IN: An Introduction to Medicinal Chemistry. 3ed Ed., Oxford *University Press*, pp. 642-674.

Hideyuki, S., Toshinori, K., Haruaki, I., Frank, M., Ayumi, O., Jun'ichi, K., (2003): Naucleamides A-E, New Monoterpene, Indole Alkaloids from *Nauclea latifolia* . *Chem. Pharm. Bull*. 51(1):58-61.

James, H.K., Mathieu, N.B., Mama Kone., Sebastien, D.D., (2014): Acute and sub-acute toxicity of aqueous extract of *N. latifolia* in swiss mice and OFA Rats. *Tropical J. Pharma. Res.*, 13:109-115.

Kwiecien, S., Brzozowski, T., Konturek, P.C., Konturek, S.J., Pawlik, M., Pajdo, R., Drozdowicz,D., Ptak, A., Hahn, E.G., (2001): Effect of central and peripheral actions of histamine and its metabolite N-alpha methyl histamine on gastric secretion and acute gastric lesions. *J . Physiol Pharmacol*., Pubmed., 52 (4) : 625-38.

Kusters, J.G., Van Vliet, A.H., Kuipers, E.J., (2006): Pathogenesis of Helicobacter pylori infection. *Clinical Microbiology Reviews*, 19 (3): 449-490.

Maria, A.O., Wendel, G.H., Guzman, J.A., Giordano, O.S., Guerreiro, E., (1998): Gastric cytoprotective activity of dehydroleucodine in rats. Role of nitric oxide. *Pharmacol Res.*, 37 (4): 281-284.

Megraud, F., Lehours, P., (2007): *Helicobacter pylori* detection and antimicrobial susceptibility testing. *Clin. Microbiol. Rev*. 20(2):280-322.

Merkelbach, P., Scott, I., Khalaf, S., Simpson, H.V., (2002): Excretory/secretory products of Haemonchus contortus inhibit aminopyrine accumulation by rabbit gastric glands in vitro. *Vet Parasitol*., 104 (3):217-228.

Morah, F.N.I., (1995): Naucledal and Epinaucledal from an Antiviral Preparation from *Nauclea latifolia*. *Global J. Pure Appl. Sci*., 1 (1-2):59-62.

Mukherjee, M., Bhaskaran, N., Srinath, R., Shivaprasad, H.N., Allan, J.J., Shekhar, D., Agarwal, A., (2010): Anti-ulcer and antioxidant activity of Gut Gard. *Indian J Exp Biol.*, 48(3):269-274.

Sener- Muratoglu, G., Paskaloglu, K., Arbak, S., Hurdag, C., Ayanoglu-Dulger, G., (2001): Protective effect of famotidine, omeprazole & melatonin against acetylsalicyclic acid-induced gastric damage in rats. *Dig Dis Sci*., 46 (2): 318-330.

Singh, S., Mujumdar, D.K., (1999): Evaluation of the gastric antiulcer activity of fixed oil of *Ocimum sanctum*. *Journal of ethanopharmacology*, 65: 13-19.

Ward, J. W., Elsea, J.R., (1997): Animal case and use in Drug Fate and Metabolism. Methods and Techniques, Vol. 1 Ed., New York pp. 372-390.

Xio-Bo, S., Tsukasa, M., and Haruki, Y., (1992): Anti-ulcer activity and mode of action of the polysaccharide fraction from the leaves of *P. ginseng. Planta Medica*, 58: 432-435.

Zayachkivska, O.S., Konturek, S. J., Drozdowicz, D., Konturek, P.C., Brzozowski, T., Ghegotsky, M.R.,(2005): Gastroprotective effects of flavonoids in plants extracts. *J Physiol Pharmacol*. *PubMed*. 56: 216–231.

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Table -1: Results of the different treatment groups of rats in the aspirin induced ulcer model, treatment was commenced 1st and then ulceration induced on the fifth day.

Groups	Treatment	Ulcer Score	Ulcer Index	UTR (%)	Gastric Secretion (s)			Absorbance (nm) Mucus secretion
					Vol (ml)	pH	Acid output (µEq/hr)	
I	Normal (Untreated -control)	0.0± 0	0.1± 0.78	0	0.06±0.4	2. 50 ±1.2	1.12± 0.54	0.84±0.10
II	Aspirin (200 $mgkg^{-1}$) *p.o Untreated+control*	6.0 ± 0.23	11.30±2.51**	100	0.62±1.2**	1.18±0.82	6.17±2.50	0.15±0.05
V	misoprostol 20 $mgkg^{-1}$	4.5± 1.60	10.73±1.70	95.0	0.77±1.0	1.21±1.0	5.13±3.30	0.21±0.05
VI	crude *N.latifolia* SB 50 $mgkg^{-1}$	0.5± 0.25	0.50±0.55**	4.4	0.44±3.8**	3.44±1.3	2.30±5.25	0.86±2.7**
VII	crude *N.latifolia* SB 100 $mgkg^{-1}$	4.5± 1.60	10.72±1.40*	94.9	0.56±2.4*	2.38±0.55	3.60±0.41	0.85±1.3*
VIII	butanol SB 50 $mgkg^{-1}$	0.8± 0.31	3.18±0.42*	28.1	0.55±2.8*	2.11±1.0	2.75±1.0	0.51±0.60*

- UTR (%) : Ulceration tolerant Rate is the rate at which ulcer was allowed by each test drug ; $*P \leq 0.05$;$*P \leq 0.01$.
- values are expressed as mean ± SEM; n = 5.

Table-2: Results of treatment groups in aspirin induced model, ulceration was induced 1st , then treatment commenced after the 4th day of ulceration.

groups	Treatment				Gastric Secretion (s)			
		Ulcer Score	Ulcer Index	UHR (%)	Vol (ml)	pH	Acid output (µEq/hr)	Absorbance (nm) Mucus secretion
II	Aspirin (200 $mgkg^{-1}$) *p.o* untreated +control	6.0 ± 2.30*	11.30±1.2	0	0.62± 0.25	1.18±1.5	6.17±2.5	0.15±0.11
III	Cimetidine 20 $mgkg^{-1}$	4.38±0.81*	3.68 ± 2.45	32.6**	0.60±0.01	2. 35±0.5	5.08±1.2*	0.11±0.05
IV	omeprazole 20 $mgkg^{-1}$	1.5± 0.17	10.63±1.70*	94.1	0.42±3.4*	2.17±0.35	4.20±0.78	0.10±0.01
VI	Crude *N.latifolia* SB 50 $mgkg^{-1}$	2.83± 1.50**	6.58 ±0.22	58.23*	0.47± 1.0**	3.29±0.67*	3.833±1.8*	0.22±0.10
VII	Crude *N.latifolia* SB 100 $mgkg^{-1}$	4.17±1.00*	6.82± 0.50	60.35*	0.31±0.55	5.74±1.0	4.100±0.85*	0.31±0.0

- UHR (%) : Ulceration Healing Rate is the rate at which ulcerated area was healed by each test drug.
- $*P \leq 0.05$;$*P \leq 0.01$.
- values are expressed as mean ± SEM; n = 5.

Table- 3: Results of the different treatment groups of rats in the histamine –induced ulcer model.

Groups	Treatment	Ulcer Score	Ulcer Index	UTR (%)	Gastric Secretion (s)			
					Vol (ml)	pH	Acid output (µEq/hr)	Absorbance (nm) mucus secretion
I	**Normal** Untreated – ve control	0.0± 0	0.1± 0.78	0	0.060±0.40	2. 50 ±1.2	1.12± 0.54	0.84±0.10
II	**Histamine** (200 $mgml^{-1}$) *i.p*	4.5±1.50**	9.95 ±0.33*	100	0.170±0.25*	5.183±2.5	6.167±0.35*	0.015±0.10*
VI	**crude *N.latifolia* SB** 50 $mgkg^{-1}$	0.2±0.45*	4.42±1.20	44.40	0.096±0.11**	4.767±1.5	38.467±8.34	0.067±0.05*
VII	**crude *N.latifolia* SB** 100 $mgkg^{-1}$	0.9±0.1**	8.29±1.5*	83.02	0.094±0.05*	4.500±0.5	15.0±2.56**	0.056±0.15*
VIII	**butanol SB** 50 $mgkg^{-1}$	1.0±0.7*	6.25±1.0*	62.80	0.017± 0.40*	5.930±1.9	20.167±4.75	0.047± 0.01

- Foot notes are same as shown in table-1.

Table-4: MIC and MBC results showed by crude extract of *N.latifolia* and amoxicilin

Test Sample	MIC (mg/ml)	MBC (mg/ml)
Crude extract *N.latifolia*	25	100
Amoxicilin	3.125	3.125

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Histopathology results of aspirin and histamine induced ulcer and treated groups.

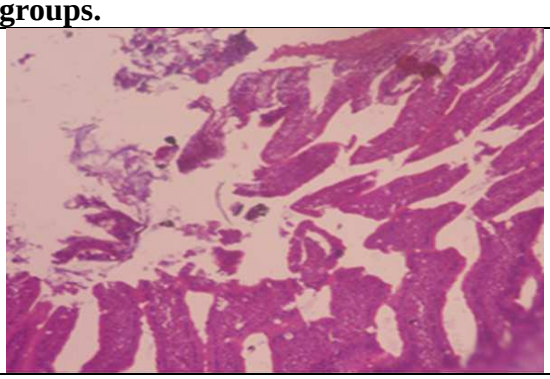

Fig 1: Normal intestinal mucosa.

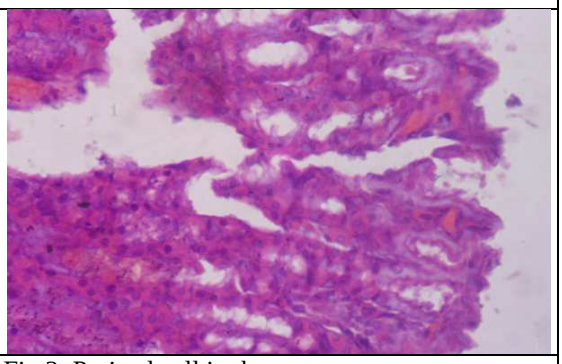

Fig 2: Parietal cell in the mucosa.

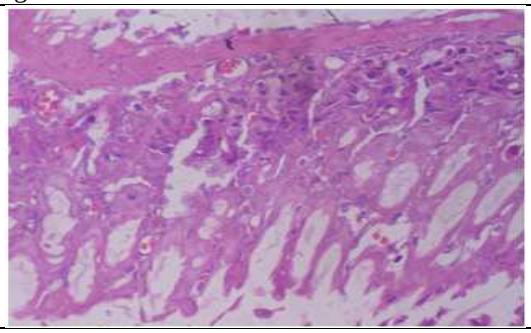

Fig 3: Inflammation of cells after some days of ulceration using aspirin.

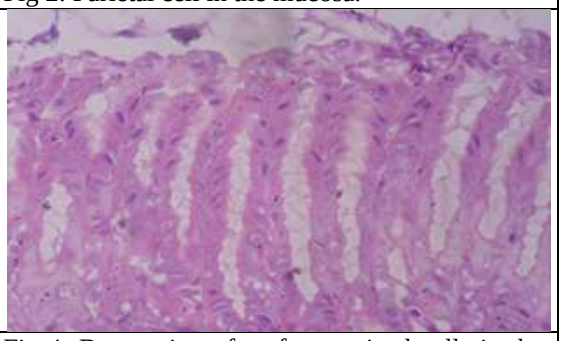

Fig 4: Destruction of surface parietal cells in the mucosa.

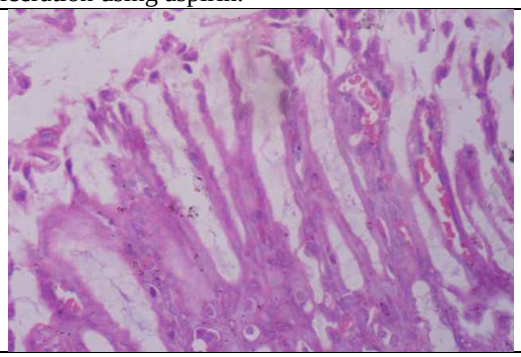

Fig 5: Increased destruction of surface parietal cells in the mucosa.

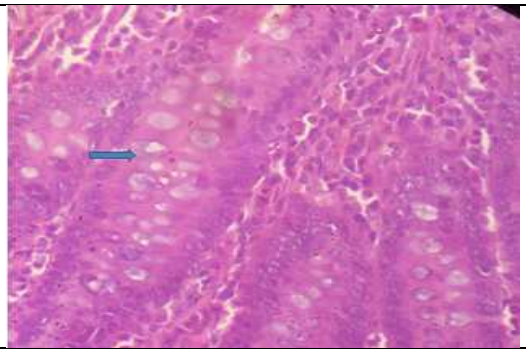

Fig 6: Treatment with crude extract *N.l* (50mg/kg), then ulcerated with aspirin (arrow;Goblet
cell filled with mucin).

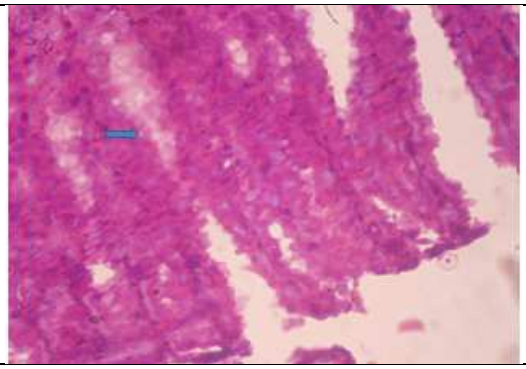

Fig 7:Treatment with Omeprazole (arrow; mucous neckglands of surface epithelium) .

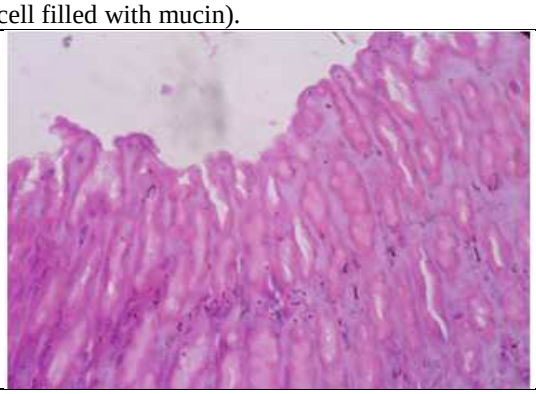

Fig 8:Treatment with crude extract *N.l* (100mg/kg) and then ulcerated with aspirin.

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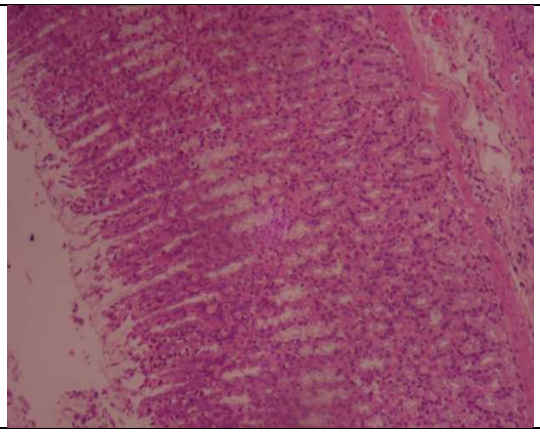

Fig 9:Treatment with butanol fraction (BNL, 50 mg/kg) and then ulcerated with aspirin.

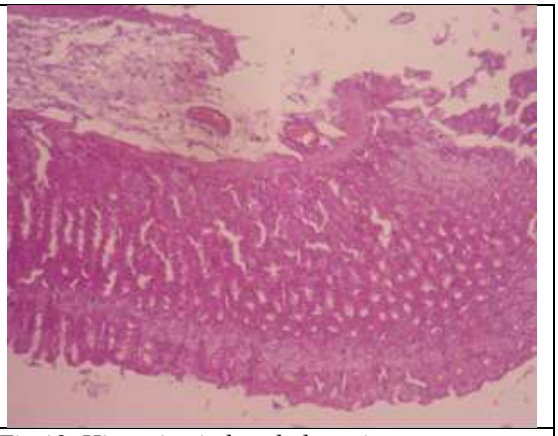

Fig 10: Histamine induced ulceration.

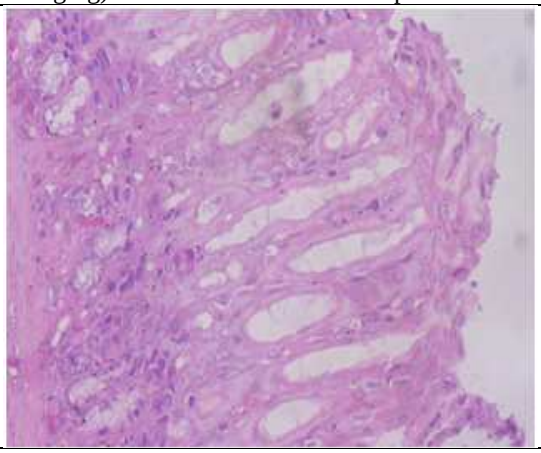

Fig 11 : Treatment with crude extract *N.latifolia* (50mg/kg) and then ulcerated with histamine.

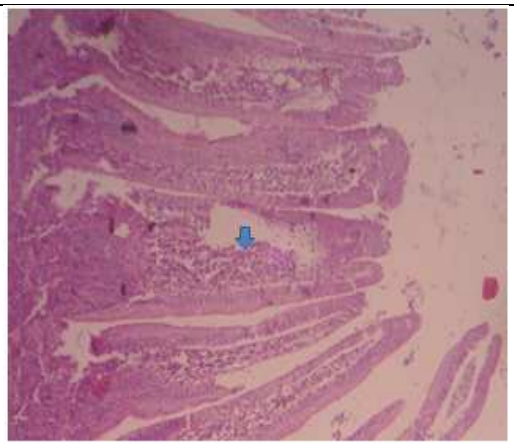

Fig 12: Treatment with crude extract *N.latifolia* 100mg/kg and then ulcerated with histamine. (arrow; infiltration of mucosal epithelium by chronic inflammatory cells).

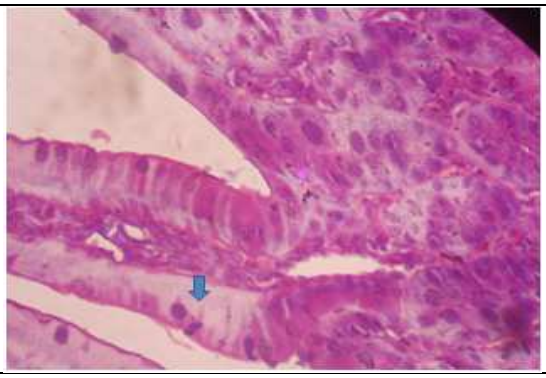

Fig 13: goblet cells being replaced by mucin like secretions when treated with 50mg/kg of crude extract of *N.latifolia*.

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