EVALUATION OF THE GASTROPROTECTIVE AND SUB-CHRONIC TOXICITY OF METHANOL STEM BARK EXTRACT OF *XIMENIA AMERICANA* LINN (OLACACEAE) IN MALE WISTAR RATS

BY

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Declaration

I declare that the work in this dissertation entitled **"Evaluation of the Gastroprotective and Subchronic Toxicity of Methanol Stem Bark Extract of *Ximenia americana*** Linn (Olacaceae) **in Male Wistar Rat**" has been carried out by me in the Department of Pharmacology and Therapeutics, under the joint supervision of Dr. J.I. Ejiofor and Dr. M.G. Magaji. The information from the literature has been properly acknowledged in the text and the list of references provided. No part of this work was previously presented for the award of another degree or diploma at this or any other institution.

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Certification

This dissertation entitled "**Evaluation of the Gastroprotective and Subchronic Toxicity of Methanol Stem Bark Extract of *Ximenia americana*** Linn (Olacaceae) **in Male Wistar Rat**" by Isaac Aksavdwa AGYIGRA. meets the regulations governing the award of the degree of Master of Science (Pharmacology) of the Ahmadu Bello University, and is approved for its contribution to knowledge and literary presentation.

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Dedication

To my Late Father Mr. Bitrus Agyigra in memory of all his supports, encouragements and prayers and my dear mother Mrs. Maimuna Bitrus Agyigra.

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I give all thanks, glory and honour to God Almighty. My appreciation goes to my supervisors, Dr. J.I. Ejiofor and Dr. M. G. Magaji for their guidance, criticisms and encouragements in the course of this work. I am highly indebted to you both for your support, scholarly contribution and keen interest in the work. I sincerely appreciate the assistance of Mal. Aliyu Ahmad and Salisu Abdullahi and all staff of the Department of Pharmacology and Therapeutics, ABU, Zaria. I also want to thank my able Head of Department, Alh. Muhammad Kabir and other staff of National Ear Care Centre, including, Alh. Jajere, Mr. Bashir Jolayemi, Madam Nkwazema, Mr. Ishaya, Mrs. Bindawa, Mr. Yusuf Olalekan Abdulakeem, Mrs. Astaharam, Mrs. Basirat, Mrs Chukuakwu, Ms Amalachukwu Okeke; and all the intern Medical Laboratory Scientists in the National Ear Care Centre. Kaduna. Lastly, I hereby acknowledge my parents and siblings for their support, prayers and contributions; and all others that supported me in one way or the other during this study. May the Lord Almighty grant you grace and mercy and reward you in your good works. Amen!

Abstract

Ximenia americana is a plant used traditionally in northern parts of Nigeria for treatment of leprotic ulcers and mouth ulcers in addition to other medicinal uses. It contains flavanoids, saponins, tannins among others that has been reported to possess gastroprotective potentials. . The aim of this study is to evaluate the gastro-protective effects and establish the toxicity profile of the methanol stem back extract of *Ximenia americana*. The oral median lethal dose of the extract was estimated in mice and rats using Lorke's method of acute toxicity test, while the composite constituents of the extract were determined using established standard preliminary phytochemical screening procedures of colour changes in test-tube chemical reactions. The antiulcer effect of the extract was evaluated using two ulcerogenic models of indomethacin and ethanol induced ulcers in rats at extract doses of 250, 500 and 1000 mg/kg body weight. Sub-chronic toxicities of the extract on some organs, haematological and biochemical parameters were also investigated following 28 days daily pretreatment in rats. Alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, steroidal glycosides, tannins and terpenoids were found to be present in the methanol stem back extract of *Ximenia americana*. The extract caused no death in mice and rats at doses of up to 5,000 mg/kg oral administration and no observable behavioural changes were seen in both animal species (mice and rats) within 24 hours. The indomethacine-induced ulcer lesions were reduced in the extract pretreated groups of rats and in a dose dependent manner that was significant ($p<0.05$) for the two higher doses of the extract (500 and 1000 mg/kg) when compared to the control group. The extract significantly ($p\leq0.05$) and dose-dependently reduced the mean ulcer spots in a similar manner as the standard agents cimetidine and misoprostol. The reduction was significant for only the two higher extract doses (500 and 1000 mg/kg). However, the ulcer lesions were more prevented in the rat groups of the standard drugs. A mean of only 3 severe ulcer spots for each of the two lower extract dose pre-treated rat groups against 9 severe ulcer spots for the normal saline control group occurred with the

indomethacin ulcerogen. However, no severe ulcer spots were found in both the cimetidine and 1000 mg/kg extract pretreated rat groups. There were no severe ulcers (≥ 3mm spots) found with the ethanol-ulcerogen in any of the groups including the normal saline control group. In conclusion the extract did not cause changes in the organ sizes in relation to the control experiment. The body weights of the extract-treated rats did also not change. However, histological examinations showed vascular congestion with polymorphonuclear cells in the kidney at the extract dose of 1000 mg/kg; consolidated areas of polymorphs infiltration at the alveoli and terminal bronchioles of the lungs; and distorted germinal centres in spleen. No remarkable changes were observed of the heart, liver, stomach and brain. Amongst the evaluated electrolytes, only Na^+, Cl^- and Ca^{2+} showed consistent but slight increases in their concentrations across the various doses of the extract, while changes in K^+ and HCO_3^- levels were neither significant nor of a consistent pattern. The liver enzymes including the aminotransferases and alkaline phosphatase were not altered. A dose dependent increase in total protein that was significant ($p<0.05$) only at 1000 mg/kg; and a reduction in albumin level that was significant at the two higher extract doses (500 and 1000 mg/kg) were observed. The changes in the urea level were inconsistent and insignificant, while the dose dependent reduction in creatinine level seen in this study was not significant at any of the doses. In conclusion, the methanol stem back extract of *Ximenia americana* possess gastro-mucosal protective properties that prevented ulceration and / or promoted ulcer healing and is also relatively systemically non-toxic.

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List of Abbreviations/Symbols

ACh	Acetylcholine
$AL(OH)_3$	Aluminium Hydroxide
ALP	Alkaline Phosphatase
ALT	Alanine Transaminase
AMP	Adenosine Monophosphate
ANOVA	Analysis of Variance.
AST	Aspartate Transaminase
Ca^{2+}	Calcium Ion
CBX	Carbonoxolone
Cl^-	Chloride ion
COX	Cyclo-oxygenase
CYP	Cytochrome P450
ECF	Extracellular Fluid
ECL	Enterochromaffin Like cells
EDTA	Ethylenediaminetetra acetic acid
EGF	Epidermal Growth Factor
EXT	Extract
FDA	Food and Drugs Administration
$FeCl_3$	Ferric Chloride
GERD	Gastroesophageal Reflux Disease
GIT	Gastro-intestinal Tract
H & E	Hematoxylin and Eosin
H^+	Hydrogen Ion
H_2	Histamine Subtype Two

H_2SO_4	Sulphuric Acid
Hb	Hemoglobin
HCl	Hydrochloric acid
HCO_3^-	Bicarbonate ion
K^+	Potassium ion
Kg	Kilogram
LD_{50}	Median Lethal Dose
LYMP	Lymphocyte
mg	Milligram
$Mg(OH)_2$	Magnesium Hydroxide
Min	Minutes
mmol	Millimole
Ml	millilitre
N	Sample size
N/S	Normal Saline
Na^+	Sodium ion
NEUT	Neutrophils
NSAIDs	Non Steriodal Anti-inflammatory Drugs
OECD	Organization for Economic Cooperation and Development
PCV	Packed Cell Volume
PGE	Prostaglandin E
pH	Hydrogen Ion Concentration.
PLT	Platelet
PO_4^{2-}	Phosphate Ion
PPIs	Proton Pump Inhibitors

RBC	Red Blood Cell
SEM	Standard Error of Mean
TCa	Total Calcium
WBC	White Blood Cell
WHO	World Health Organization
α	Significance Level
(µ/L)	Microns per Litre
(%)	Percentage

CHAPTER ONE

1.0 INTRODUCTION

1.1 Treatment of Peptic Ulcer Disease (PUD)

Peptic ulcers are parietal painful sores that occur in the epithelial lining of the upper gut (stomach, esophagus, or small intestine) due to excess gastric acid secretion (Abbas and Kumar, 2010). The development of peptic ulcer of the oesophagus (reflux of stomach acid) is rare, thus the term peptic ulcer most commonly refers to gastric and duodenal ulcers. The major difference for both ulcers is the location of where the ulcer occurs, otherwise, the risk factors, possible causes, symptoms and diagnosis are often the same. However, duodenal ulcer appears to be less harmful, but the most common type peptic ulcer (Guyton and Hall, 2006). Stomach ulcer is more harmful and if improperly treated often results in life-threatening cancerous complications of medical emergency and hospitalisations that may require gastric surgery, including abnormal bleeding, perforative holes in the walls of stomach and duodenum and/or gastric outlet obstruction (Bardhan and Royston, 2014).

The three major factors that trigger over production of acid fluid and cause peptic ulcer are *Helicobacter pylori* (*H-pylori*) infection, excessive consumption of NSAIDS and gut problems like gastrinomas (Kawamura *et al*., 2013).

Pain and vomiting are particularly, the evident symptoms and may occur either singly or concurrently (Malfertheiner *et al.,* 2009). Aside drug treatment to eradicate the underlying causative *H. pylori* and other offensive gastric mucosal microbial organisms, drugs are also used to either suppress acid secretion or increase mucosal resistance and protection and to enhance healing of ulcer wounds and/or relief symptoms (especially pain) (Anand, 2017).

The available synthetic drugs include gastric acid output inhibitors such as proton pump inhibitors [PPIs] like omeprazole or H_2-receptor antagonists like cimetidine, anti-secretory agents such as prostaglandin analogues like misoprostol, and mucosal cytoprotectors e.g. sucralfate (Fashner and Gitu 2015). Thus, there is a quest for discovering more drugs with these activities, but with better efficacy and toxicity profiles.

1.2 Statement of Research Problem

The complications of gastric ulcer are life-threatening and are of medical emergency requiring hospitalizations. The mortality rate from PUD complications is more than 10 times that of other digestive diseases with perforation causing the highest mortality worldwide (Sung *et al.,* 2010; Chung and Shelat, 2017).

In Africa, the incidence showed limited evidence that it is more common in urban areas and that its incidence is increasing further. However the highest prevalence in south part of Sub-saharan African and in the Nile-Congo watershed including Rwanda, Burundi and eastern Zaire of about 5-12%, a Tanzanian endoscopic finding revealed peptic ulcer incidence was 24.1% among adult patients with dyspepsia is of great concern (Tovey and Tunshall, 1975; Balint, 1989; Ayana *et al.,* 2014).

A 12 year retrospective study of ulcer complication in Zaria, Nigeria as at 1998 showed that perforation had an increasing frequency of occurrence (45%) followed by gastric outlet obstructions (41%) (Ameh and Nmadi, 1998). As at 2005, a 13-year review at a Nigerian hospital found obstruction to be the most common complication (56%), followed by perforation (30%) (Irabor, 2005).

The high incidence of gastric ulcer complications in older subjects of above 60 years that had been reported to be reflecting increased use of NSAIDs is a major concern considering the inevitable need of these drugs (Sung *et al.,* 2010; Liu *et al.,* 2008). It has been reported that about 10% of peptic ulcer cases are NSAIDs induced (Bhala *et al.,* 2013).

Gastric ulcer if improperly treated or left untreated results in complications and the use of the current antiulcer agents is often hampered by one adverse effect or the other; for instance:

Misoprostol has dose-related diarrhoea as common side effect and it suppresses gastric acid only at high doses. Thus, the consequent diarrhoeal effect impairs therapy compliance and/or militates against its frequent prescription. It also increases uterine tone and contractions and may cause uterine rupture, abortions or birth defects and thus, is contraindicated for pregnant women with gastric ulcers (Pasturak, 1998).

Misoprostol, a synthetic antisecretory agent of prostaglandin E_1 analogue, is highly esteemed and approved for use in the prevention of NSAIDs-induced gastric ulcers. Thus, it is mostly indicated for subjects on NSAIDs that are at high risk of NSAID-induced ulcers, especially the elderly and people with ulcer complications (More, 2002).

The FDA had also disapproved the use of sucralfate, a complex of sucrose sulfate-aluminium hydroxide, probably due to its aluminium content-related astringent properties including nausea, vomiting and constipation; as well as risk of aluminium toxicity from accumulation, hypophosphataemia from depletion of the phosphate moieties and/or drug interactions that impair concurrent use with other drugs (Joseph *et al*., 2000; Fashner and Gitu, 2015).

1.3 Justification for the Study

Although natural products are not devoid of toxic side effects there is need for continuous search for efficacious agents of natural origin with minimal toxicity, at least in part, because they are readily available and at reduced cost.

The written record of the use of plants and herbs to maintain health and cure many ailments dates back to several years (Petrovska, 2012). Most developing and 3rd world countries resort to herbal forms of treatment because of the inaccessibility of conventional health care systems, unavailability or high cost of orthodox or synthetic drugs (WHO, 2012; Ekor, 2013).

WHO had estimated that approximately 80% of the rural dwellers and up to 80% of the world's population rely on herbal medicines as alternative options for their primary health care needs (WHO, 2012).

The ethnomedicinal claims of use of the various parts of *Ximenia americana* in Africa for spasmodic bowel diseases and ulcers amongst other ailments require scientific validation.

1.4 Aim and Objectives

The aim of this research work is to evaluate the gastroprotective properties and toxicity profile of the methanol stem back extract of *Ximenia americana*.

The Specific Objectives are as follows:

i. To determine the phytochemical constituents of the methanol stem bark extract of *Ximenia americana*
ii. To establish sub-chronic toxicity profile of the methanol stem bark extract of *Ximenia americana* and determine its oral LD_{50} in mice and rats

iii. To evaluate the gastro-mucosal protective effect of the methanol stem bark extract of *Ximenia americana*

1.5 Research Hypothesis

The methanol stem bark extract of *Ximenia americana* possesses significant gastroprotective effect on gastromucosal linings from gastric acid erosion and is relatively safe.

CHAPTER TWO

2.0 LITERATURE REVIEW

2.1 Peptic Ulcer

2.1.1 Definition and types

Peptic ulcers are wounds caused by gastric acids on the linings of the upper gastrointestinal tract (GIT) including the lower oesophagus (oesophageal ulcer), stomach (gastric ulcer) and duodenum (duodenal ulcer). The ulcer is usually an open sore produced by erosion or washing off of the epithelial cells with its cutaneous, mucus or serous membrane coverings or layers (Abbas and Kumar, 2005). Duodenal ulcers are 4 times more common than gastric ulcers, but are almost never malignant (Abbas and Kumar, 2010). Curling's ulcer is a peptic ulcer with extensive burns and scalds, while that which is locally invasive to the walls of organs that erode blood vessels with evidence of intestinal bleeding (hematemesis or melaena) is termed dendritic (penetrating) ulcer. An acid-pepsin eroded damaged tissue-area is often a painful divot of a small, red crater on the inside lining of the gut as shown below;

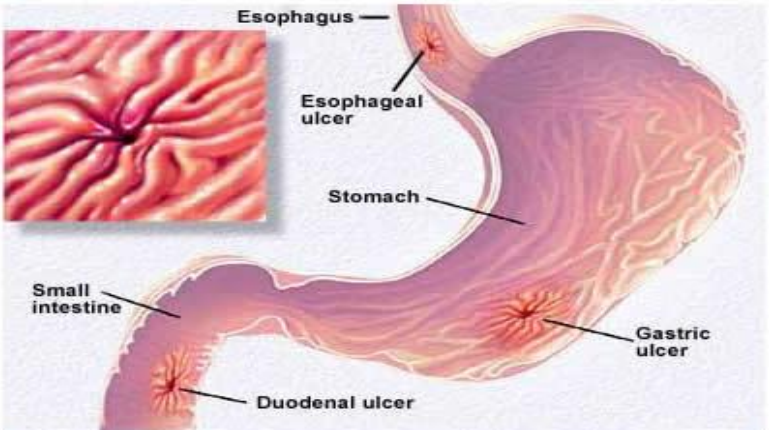


Fig. 1: Gastric mucosal ulcer patterns as illustrated by Mayo Foundation of Medical Educational Research; 2016

There is also a slowly growing and locally invasive skin ulcer commonly of the face called rodent ulcer, as well as varicose or gravitational ulcer which is an indolent or inactive type with dilated veins that could cause reversal of blood flow most commonly in the lower limbs (especially the lower third of a leg), rectum (haemorrhoids) or lower oesophagus, also known as oesophageal varices (Abbas and Kumar, 2010).

2.2 Pathogenesis of Peptic Ulcer

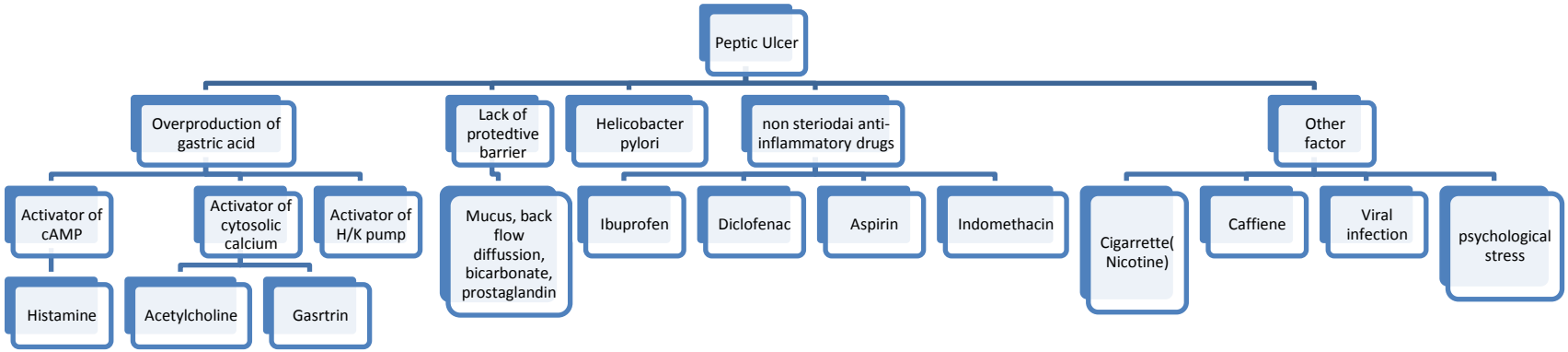


Fig. 2: Flow chart of pathogenesis of peptic ulcer

Generally, peptic ulceration results from overproduction of gastric acid and/or lack or inadequacy of the protective barrier lining of gastric mucosa (Sheila, 2010)

2.2.1 Over-production of gastric acid

The pathogenic physiology of high gastric acid secretion includes all mechanisms of parietal cell hydrogen ion release such as:

- Histamine related increases in the formation of cyclic adenosine monophosphate (cyclic AMP) which stimulates the parietal cell function.
- ACh and gastrin related increases in cytosolic calcium level that causes stimulation of parietal cell function.
- inhibition of H^+/K^+ ATPase (as with omeprazole) which also stimulates parietal cell function (Zajac, 2013)

Overproduction of gastric acid is believed to cause an imbalance in the digestive fluids (Healthwise 2014). Excess or hypersecretion of gastric acid is related to increased mass of acid-secreting parietal gastric mucosal cells, vagal hyperactivity or both. Increase in the parietal cell mass is thus synonymous to hydrogen ion release from elevated gastric acid (HCl) secretion. Ulcers result from overstretch of the cytoprotective physicochemical barriers of the gastric mucosa by the uncontrolled secretion of hydrochloric acid (HCl) and pepsin from parietal cells (acid-pepsin disease condition). Usually, the normal pumping of gastric acid is by the H^+/K^+ ATPase of the cytoplasmic tubular membranes; but on stimulation of the parietal cells, the microvilli of the expanded secretory canaliculus of these cells also tends to produce additional gastric acid resulting in hyperacidity (Sachs *et al.*, 1981). Parietal cells are stimulated by vagal impulses to release acetylcholine directly and gastrin indirectly from the gastric mucosal cells, both of which cause release of H^+ into the gastric lumen. Vagal impulses could be of disordered autonomic nervous system function from anxiety, worry or stress. This is thus the rationale in the

use of anticholinergics (e.g. propantheline) as adjunctive therapy with antacids to depress gastric motility or secretion and/or reduce pepsin production (Walan, 1984).

2.2.2. Lack or inadequacy of the protective barrier lining of gastric mucosa

The intrinsic normal gastro-mucosal acid protective mechanism is comprised of 3-defense mechanisms used to resist injuries from gastric acid and peptic activity. These are:

i. Mucus and bicarbonate secretions of the surface epithelial cells
ii. The resistance of back diffusion of acid into cells by the apical surface membrane of gastric mucosal cells
iii. The intrinsic injury resisting mechanisms of mucosal cells which extrudes back-diffused hydrogen ions by sodium hydrogen or sodium bicarbonate exchange, and which includes the platelet prostaglandin (Wallace, 2001; Rang *et al.,* 2002).

The overlying physicochemical epithelial cell barrier of the gastric mucosal wall provides defensive mechanisms (cytoprotection) against hyperacidity. This mucosal epithelial cell wall lining barriers include gastric mucus, prostaglandins, bicarbonates (HCO_3^-) and mucosal enhanced blood flow and functions as follows:

- secrete a gel-like mucus coating that protects the mucosa from autodigestion by pepsin or erosion from acid and other ingested caustic agents. (Cecil *et al.,* 2016)
- secrete bicarbonate ions that acts to neutralize acid and prevent its harsh effects. Inadequate secretion of the alkali (HCO_3^-) and / or its rapid gastric emptying results in incomplete neutralisation of the acid (HCl) (Zajac, 2013).
- cause increased blood flow for providing oxygen and bicarbonate and removing hydrogen ion and other injurious toxic agents such that their back diffusion do not occur, as well as speeding up healing of superficial lesions from mucosal ischemia of disease

conditions that contributes to gastric ulceration. Gastric ulcers damage blood vessels to reduce mucosal blood flow and resulting in predisposition to injury. The platelet prostaglandin aids mucosal blood flow and boosts the synthesis or secretion of the insoluble mucus (Cohen, 1987).

2.3 Causes of Peptic Ulcer

The factors that cause peptic ulcer, act mainly to trigger the overproduction of acid fluid or disrupt the cytoprotective barrier mechanism of the mucosal lining. These factors include:

2.3.1 *Helicobacter pylori* (*H. pylori*) bacterial infection:

H. pylori are the most common cause of duodenal and stomach ulcers. More than a quarter of people become infected with *H. pylori* at some stage in their lives and once infected, unless treated, the infection usually stays for life (Blasser *et al.*, 2007). Most people with *H. pylori* have no symptoms and do not know that they are infected and about 3 in 20 people who are infected with *H. pylori* develop an ulcer. In all, about 90% of duodenal ulcers and 70% of gastric ulcers are attributed to *H. pylori* infection (Blasser *et al.*, 2004). In the Northeastern part of Nigeria, the reported total prevalence of *H. pylori* associated gastric ulcer was 57.2%, with people in the age range of 41-50 being more susceptible (Adisa *et al*., 2011).

The exact way *H. pylori* cause problems in some infected people is not totally clear. In some people, this bacterium causes inflammation in the lining of the stomach or duodenum to disrupt the defense mucous barrier. Mild or more severe inflammation of the duodenum and/or stomach (duodenitis and gastritis) may lead to an ulcer (Ohkusa *et al.*, 2003). In some other cases, the bacteria produces mucinase which destroys the mucous barrier of the GIT from inhibition of

mucin production and also increased gastric emptying of the stomach resulting in increased gastric acid production, inflammation and ulcers (Pounder *et al.,* 1977).

Helicobacter pylori are microaerophilic spherical gram negative rod bacterium of a non-invasive spiro-bacillus type often seen in the antral flora of the stomach (Boyanova, 2011). It is often associated with metaplasia and ulceration of the antrum. Thus, individuals with antral-gastritis and dyspepsia (indigestion) are highly susceptible to *H. pylori*-induced gastric ulcer. Antral ulcer is often preceded by an initial duodenal ulcer and characterized by loss of inhibitory control of acid secretion and decreased somatostatin production (Blasser, 2004). In fact, it is reported that many patients who develop peptic ulcers also have *Helicobacter pylori* infection. *Helicobacter pylori* infection also increases both the risk and incidence of peptic ulcer disease among NSAIDs users (mostly the *H.pylori*-positive NSAIDs users) (Huang *et al.,* 2002). The presence of *H. pylori* also acts as a substrate to gastrin release with accompanying increase in gastric acid secretion (Rozengurt and Walsh, 2001). Although *H. pylori* infection is more common in developing countries, its overall prevalence rate strongly correlates with low socio-economic status, poor personal and environmental hygiene and presence of *H. pylori* positive family history typical of developing countries (Ndububa *et al.,* 2007).

The adaptive survival mechanism of *H. pylori* against its induced acid environment of the stomach is related to its production of three enzyme systems.

i. It is a urease producing colony which catalyzes the hydrolytic neutralisation of urea to erosive ammonia and carbondioxide on the mucous barrier (Weeks *et al.,* 2000). The toxic injury to the mucosa induces leucocytes chemotaxis. In addition, the gastric

virulent strains of the bacterium tend to also express cytotoxic vacuolating (Vac A and Cag) gene proteins with which stimulates the host immune system.

ii. The bacterium also produces mucolytic enzymes (proteases, lipases and phospholipases) with which it lyses the phospholipid bilayer of the epithelial cell to facilitate its penetration (Huang *et al.,* 2002)

iii. The bacterium also produces catalase enzymes with which it protects itself against the damaging effect of reactive oxygen free radicals (hydrogen peroxide, superoxides etc produced by the chemotactic leucocytes (Staurt *et al*., 2001; Abbas and Kumar, 2005).

2.3.2 The none-selectivity and dyspepsia side effects of NSAIDs and other medications

Peptic ulcer is also often associated with excessive consumption of analgesics of the nonsteroidal anti-inflammatory drugs (NSAIDs) types. Anti-inflammatory medicines such as aspirin, ibuprofen, diclofenac and others are taken by many people for arthritis, muscular pains, sprains, menstrual pains, e.t.c. Infection with ***H. pylori*** infection also require the use of anti-inflammatory medicines. It has also been reported that over 14 million Americans consume NSAIDs regularly (Deer *et al*., 2014) and that about 10% of peptic ulcer cases are related to the use of NSAIDs (Liu *et al.,* 2008). These drugs affect the stomach and duodenal lining and allow acid to cause inflammation and ulcers. The NSAIDs such as aspirin act to inhibit arachidonic cyclooxygenase 1 (COX-1) involved in the production of prostaglandins. Prostaglandins inhibit the secretion of gastric acid by blocking the formation of cyclic AMP and this effect protects the mucosal wall from acid attack. Thus, this inhibitory absence of prostaglandins causes increased gastric acid secretion. Cyclooxygenase (COX) is a prostaglandin endoperoxidase synthase enzyme

responsible for the formation of prostaglandins (prostacyclin and thromboxane) and there are COX-1 and COX-2 isoenzymes.

COX-2 is an inflammatory prostanoid mediator, selectively specific for inflammation and thus, induced or activated only by inflammatory factors (cytokines, interleukins-1, tumour necrotic factor-α) 2 (Vane and Botting, 2006); while COX-1 is none-selective and thus, tends to be inhibited by none selective analgesics like the NSAIDs, which also are used for inhibition of inflammatory mediators of the COX-2 isoform.

The inhibition of COX-1 prevents prostaglandin production and its mediated functions of anti-inflammatory, antithrombotic (platelet aggregation), antipyretic, analgesic and gastric protective functions. The inhibition of COX-1 by NSAIDs in attempts to inhibit COX-2 is thus, an unwanted or side effect of these drugs. This nonselective inhibition occurs in varying degrees (Bertollini *et al.*, 2002). Certain drugs also have the side effects of functional (non-ulcer) dyspepsia that could worsen (as risk factor) peptic ulcer. Dyspepsia is simply, indigestion either from ulcer pain-symptoms or of non-ulcer pain-type (functional dyspepsia) (Wang *et al*., 2010).

2.3.3 Health problems of the upper gut

Stomach cancer (gastrinomas) and oesophageal cancer can also trigger the overproduction of acid fluid. Zollinger-Ellison syndrome - a tumour of the pancreas also tends to cause excessive secretion of gastrin and/or gastric acid (Abbas and Kumar, 2010; Farmer, 2010).

2.3.4 Other factors that impair the mucosal protection to cause or worsen peptic ulcer include:

- Use of tobacco such as cigarette smoking: smoking stimulates gastric acid secretion due to its nicotine content that stimulates nicotinic receptors for acetylcholine binding and/or increase in gastric acid secretion (Maity *et al.,* 2003)
- Caffeine and necrositic agents such as alcohol: these are strong stimulants of gastric acid secretion.
- Psychological stress, hunger or absence of food in the GIT whereby the secreted gastric acid is in direct contact with the GIT mucosa, and this may result in ulcer
- Presence of food in the GIT also often stimulates the gastric mucosa to secrete gastrin with its consequent release of gastric juice containing HCl and which exacerbates ulcer pains.
- Viral infections especially of cytomegalovirus, herpes simplex etc (Chen *et al.,* 2005).

2.4 Risk Factors of Peptic Ulcer

Some conditions that increase the risk of developing peptic ulcers include:

1. Having *H. pylori* bacterial infection
2. Age (risk increases with age)
3. Frequent intake of alcohol
4. Having other serious digestive system diseases such as Crohn's diseases, ulcerative colitis and gastroparesis
5. Too much consumption of NSAIDs such as naproxen, aspirin, and ibuprofen
6. Family history of peptic ulcer (Abbas and Kumar, 2010)

2.5 Clinical Features of Peptic Ulcer

2.5.1 The symptoms of peptic ulcer include:

1. Abdominal pain – especially in the upper section of abdomen (most common symptom)
2. Bloating, heartburn, nausea and vomiting
3. Loss of appetite and indigestion (dyspepsia)
4. In severe case, there is a chance of bloody vomiting or passage of bloody stools (Abbas and Kumar, 2010)

Pain and vomiting are particularly evident and may occur either singly or concurrently. The pain is predominant at the epigastric regions due to the high concentration of sensory nerve fibres from the vagus nerve in these areas. The pain may be of a gnawing type, burning type, or dull aching pain of considerable severity. In complicated situations, haemorrhage, passage of bloody stools and weakness may occur; wounds may become very deep to affect the adjacent organs like the pancreas and liver. Stomach cancer, gastric lymphomas or mucosal associated lymphoid tissue disease may also occur (Nuhu and Kasama, 2008). Severe nausea and vomiting may lead to fluid deficits and metabolic alkalosis.

2.5.2 Complications of peptic ulcer

1. Gastric obstruction: Gastric outlet-obstruction can affect the passageway from stomach to the duodenum
2. Perforation: This is a hole that can occur in the walls of the stomach or on the anterior portion of the duodenal bulb and which is usually a medical condition
3. Penetration: Ulcers that penetrate posteriorly into the pancreas pose continuous radiating severe pain unrelievable with antacids (Søreide *et al.*, 2015)

4. Intractable ulcers: Ulcers that are unable to heal and remaining symptomatic after intensive medical regimen or those with frequent recurrences (Walter *et al.,* 1999).

2.6 Prevalence of Peptic Ulcer

It is estimated that 10% of the population have peptic ulcer disease. Approximately two-thirds of the World's population is infected in *H. pylori* (CDC, 2002). The prevalence of *H. pylori* infection worldwide varies greatly among countries and among population groups in the same country. The infection is more common in developing countries where the prevalence rate ranges between 70 and 90% as compared to 20–50% in developed countries. The overall prevalence rate of *H. pylori* infection strongly correlates with low socioeconomic status, low living standards, poor personal and environmental hygiene, presence of *H. pylori*-positive family members and increasing age (Ndububa *et al.,* 2001).

In the united state it is estimated that 20% of individual under the age of 40 are infected and half of individual over the age of 60 are infected (NIH, 2002), complications such as hemorrhage (73 %), perforation (9 %), and obstruction (3 %) was reported of peptic ulcer disease indicating that the frequency of these complications of PUD vary geographically (Wang *et al.,* 2010).

The incidence in Africa showed limited evidence that it is more common in urban areas and that its incidence is increasing further. However the highest prevalence in south part of Sub-saharan African in the Nile-Congo watershed including Rwanda, Burundi and eastern Zaire of about 5-12%, a Tanzanian endoscopic finding revealed peptic ulcer incidence of 24.1% among adult patients with dyspepsia is of great concern (Tovey and Tunshall, 1975; Balint, 1989; Ayana *et al.,* 2014).

A 12 year retrospective study of ulcer complication in Zaria, Nigeria as at 1998 showed that perforation had an increasing frequency of occurrence (45%) followed by gastric outlet obstructions (41%) (Ameh and Nmadi, 1998). As at 2005, a 13-year review at a Nigerian hospital found obstruction to be the most common complication (56%), followed by perforation (30%) (Irabor, 2005).

The age of acquisition is of *H pylori* is unknown. In a prospective study, immunoglobulin G antibodies to *H. pylori* were measured in 143 children under the age of 20 years. 91% of 43 randomly chosen subjects over 10 years had antibodies to *H. pylori*. 69% had antibodies, including 58% of those aged less than 1 year. *H. pylori* infection is acquired at an early age, and in this study was not associated with any other pathology (Holcombe *et al.,* 1993).

Various studies on *H. pylori* showed prevalence rates between 73.0% and 94.5% among patients with dyspepsia. The study was endoscopy-based with the use of histology to detect *H. pylori*, and also serology-based which yielded prevalence rates of 80.0% and 93.6% among patients with dyspepsia respectively (Olokoba *et al.,* 2013). These prevalence rates are similar to those of other investigators in regions of Nigeria and Mustapha *et al*., (2006) using histology following Haematoxillin and Eosin, with modified Giemsa staining of antral endoscopic biopsies in Maiduguri, North-eastern Nigeria found a prevalence rate of 84% for *H. pylori* among Dyspeptic Patients.

Conversely, (Adisa *et al.,* 2011) studied *Helicobacter Pylori* associated gastritis in North-Eastern Nigeria and recorded the prevalence of gastritis to be 94.9% of patients while *H. pylori* associated gastritis were 57.2%. The age group of patients with the highest prevalence (26%) of

H. pylori associated gastritis was 41-50years. Specific diagnosis of *H.pylori* associated gastritis is crucial in the prevention of gastric cancer.

Similarly, Ndububa *et al.,* (2007) found a prevalence rate of peptic ulcer associated with *H. pylori* to be 73% in Ile-ife, South-west Nigeria using histology and Campylobacter-like organism (CLO) - urease test on gastric mucosal biopsies.

Furthermore, using serology to detect antibodies against *H. pylori* was found to have the prevalence rate of 94.5% in Ibadan, South-west Nigeria. The high prevalence rates found for *H. pylori* infection among dyspeptic patients by various investigators may be due to early acquisition of the organism, similarities in the age of the patients enrolled, similarities in geographical location, socio-cultural practices, environmental and living conditions (Olokoba *et al.,* 2006)

2.7 Management of Peptic Ulcer

The prognosis of peptic ulcer is pretty good if it is completely cured, but if left untreated or incompletely treated, it tends to reoccur. Peptic ulcer is managed both non-pharmacologically and pharmacologically (Andrew *et al.,* 2015).

2.7.1 Drugs used in treatment of peptic ulcer (pharmacological management)

The cornerstone for ulcer therapy is to accelerate healing via reduction in gastric acidity and/or inhibition of inflammatory erosion of protective mucosal barriers (cytoprotection). However, drugs are also used in relieving symptoms (especially of pain), preventing complications and reoccurrence and eradicating *H. pylori* bacterium (antibacterial and antibiotics) and other underlying causes of acid secretion (Louw, 2006; Fashner and Gitu, 2015). The basic ulcer drug treatments or therapy include the use of:

2.7.1.1 Antibacterials and antibiotics: The following antibacterial agents are used; (clarithromycin (biaxin), amoxicillin, metronidazole (flagyl) and tetracycline: This is often the first line drug management and which most often are used in combination with antiulcer drugs (Fashner and Gitu, 2015).

2.7.1.2 Gastric antacids (acid neutralisers for pain relief): There are two types of antacids: is Systemic e.g. sodium hydrogen trioxocarbonate (iv) - $NaHCO_3$ and Non-systemic e.g. magnesium hydroxide - ($Mg(OH)_2$, aluminium hydroxide ($Al(OH)_3$, magnesium trisilicate ($MgSiO_3$) antacids. Antacids counteract acidity and their efficacy is based on the inherent ability to react with and neutralise gastric acid (Zajac, 2013). Thus, antacids are used in neutralising gastric acidity in order to relieve ulcer pains and to act on pyloric sphincter to induce release of gastrin which boosts the flow of gastric juice to increase gastric motility and intragastric pH (Gulia, and Choudhary, 2011). Magnesium-containing antacids may cause diarrhoea, and brands with calcium or aluminium may cause constipation. However, continuous use of antacids for prophylaxis is not recommended. Antacids are given about 1 hr after meals and they act for 1-2 hours. The timing of medication is critical in ulcer therapy because a meal itself neutralises acidity; thus, antiulcer medications should not be given with a meal. A double dose of antacids is often taken just before bed time (Farmer, 2010).

2.7.1.3 Gastric acid output inhibitors (proton-pump inhibitors- PPIs and H_2-receptor antagonists): Inhibitors of H^+/K^+ ATPase proton pump (proton-pump inhibitors – PPIs) like omeprazole is of the family of benzimidazole derivatives, found in the world health organisation's list of essential medicines for peptic ulcers that do not respond to H_2-receptor antagonists (Quadder *et al.,* 2006**;** Zajac *et al.,* 2013). They cause pronounced

and long-lasting reduction of gastric acid production and thus, are usually better and often used first before the H_2 - receptor antagonists. They are currently the most potent inhibitors of acid secretion available. The H^+K^+ ATPase proton pump of the apical membrane of parietal cells controls the terminal step in acid (H^+) secretion into the stomach lumen. The PPIs are prodrugs, which when activated by acid react covalently with the cysteine ring of H^+/K^+ ATPase (Zajac *et al.,* 2013). Being that uncharged molecules readily crosses the lipoid membranes of parietal cell canaliculus which is of an acidic environment, these drugs tend to get protonated into active form which then binds convalently and irreversibly to the gastric proton pump to irreversibly deactivate the enzyme to block or inhibit its functions. Other proton pump inhibitors (PPIs) are lansoprazole, pantoprazole, esomeprazole and rabeprazole (BNF, 2004).

Histamine subtype II (H_2) receptor antagonists or blockers: – cimetidine, ranitidine, famotidine and nizatidine. These are competitive H_2-receptor antagonists. H_2 histaminic receptors are found in the enterochromaffin like cells (ECL cells) that secret gastric juice in the stomach. Histamine in small doses often has profound effects on gastric secretion of these cells. The clinical use of H_2-receptor antagonists for peptic ulceration therapy is based on their capacity to inhibit gastric acid secretion. These drugs prevent the (Enterochromaffin like) ECL cells' released histamine from binding on the parietal cell H_2 receptors to cause its stimulation of acid secretion, thus reducing gastric acidity (except for gastrin and ACh related secretion) (Rossi, 2005). The H_2-antagonists offer several advantages over antacids, including longer duration of action (6–10 hours), greater efficacy, and ability to be used prophylactically (Ho *et al.,* 2014).

Cimetidine (tagamet) - the prototypical H_2 antagonist is far more efficacious than anticholinergics and is used in duodenal ulcers, gastrinoma and gastroesophageal reflux. It is absorbed orally, has a plasma half life of 2 h and excreted mainly unchanged in urine (Rang, 1999; Reldoffi, 2008). H_2 antagonists are in general, well tolerated, with few and infrequent side effects except for cimetidine, which causes gynecomastia (may bind to androgen receptor sites- causing loss of libido and impotence), galactorrhea, granulocytopenia, agranulocytosis, mental confusion, restlessness, seizures, reduced sperm count, hypotension, diarrhoea, headache and dizziness (Rossi, 2005). It also alters cytochrome P450 (CYP) metabolism as an inhibitor of several P450 enzymes and thus, reduces the metabolism of many drugs to increase their serum concentrations to toxic levels (Humprey and Merritt 1999). The more recently developed H_2-receptor antagonists (rantidine and famotidine) seem more effective than cimetidine and allegedly has fewer side effects and less likely to alter CYP metabolism (Thomson and Mahachai, 1996).

Ranitidine Bismuth Citrate (RBC) can also be used in combination with other drugs in the treatment of peptic ulcer and the regimens used include: RBC + Amoxicillin + Clarithromycine; or RBC + Clarithromycin + Metronidazole (BNF, 2004; Katzung, 2004). The FDA also approved bismuth based triple therapy consisting of bismuth subsalicylates, metronidazole and tetracycline. There is also a quadruple treatment regimen of bismuth-based therapy for improving the efficiency of the triple regimen. Quadruple regimens are complex and are recommended as second line treatment after failure of regimens containing Clarithromycin (McCoughlin *et al.,* 2005). This is because *Helicobacter pylori* is known to develop resistance to metronidazole and clarithromycin which are the two key antibiotics used in many of the combination therapies. The

resistance to metronidazole is more prevalent (10-50%) than the resistance to clarithromycin (Osato *et al*., 2001).

2.7.1.4 Anticholinergic drugs (antagonists of M_1 receptors) - propantheline, pirenzepine, Telenzepine: These are depressors of gastric motility and secretion. Parietal cells are also stimulated by vagal impulses to release ACh directly and gastrin indirectly from the gastric mucosal cells, both of which cause release of H^+ into the gastric lumen. ACh mediates rest-and-digest state (as opposed to fight-or-flight) and thus, increase in its release means increase in gastric motility and digestion. Anticholinergics depress gastric motility (spasm) and secretion and reduce production of gastric acid and pepsin. Propantheline may be used as adjunctive therapy with antacids, but not as a sole agent. Pirenzepine (gastrozepin) cannot diffuse through the blood brain barrier and so has no effects on the brain and spinal cord (Stolerma and Ian, 2010). Anticholinergics as antiulcer drugs are given 30 minutes before meals should not be given with a meal (Tang *et al.*, 2007).

2.7.1.5 Anti -secretory agents: - Misoprostol (a synthetic prostaglandin E_1 (PGE_1) analogue, enprostil) Misoprostol has anti-secretory and protective properties that promotes healing of peptic ulcer and thus, used to prevent and treat stomach ulcers (Katzung, 2004). It is also medically used to induce labour, treat postpartum bleeding from insufficient uterine contraction or used concurrently with mifepristone or methotrexate to induce abortion (Pasturak, 1998). It is one of the most important medications of basic health system in the world health organization's list of essential medications (WHO, 2014). Oral misoprostol is commonly associated with dose-related diarrhoea, abdominal pain, nausea, flatulence, headache, dyspepsia, vomiting, and constipation (Ratnaike and Jone. 1998). It also causes

uterine rupture and thus, not used by pregnant women to reduce risk of NSAID-induced gastric ulcers because it increases uterine tone and contractions and/or abortion or birth defects (Week and Alfirevic, 2006).

Misoprostol is approved for use in the prevention of NSAIDs-induced gastric ulcers and thus, mostly indicated for subjects on NSAIDs that are at high risk of NSAID-induced ulcers, such as the elderly and people with ulcer complications. It is also sometimes co-prescribed with NSAIDs (e.g. diclofenac with arthrotec) to prevent the gastric ulceration. Misoprostol acts upon gastric parietal cells to inhibit gastric acid secretion by G-protein coupled receptor-mediated inhibition of adenylate cyclase, which leads to decreased intracellular cyclic AMP levels and decreased proton pump activity at the apical surface of the parietal cell (Hawkey *et al*., 1998). Misoprostol is used at high doses to achieve gastric acid suppression. Although, at lower doses, it stimulates increased gastro-mucosal secretion and blood flow that increases mucosal integrity, this lower-dose effect is not pronounced. Its multiple daily doses with its diarrhoeal side effect also impairs therapy compliance and militates against its frequent indication or use as first line drugs for gastric ulcer (Hawkey *et al.,* 1998; More, 2002).

2.7.1.6 Mucosal Cytoprotectors

Sucrose sulphate-aluminium complex (sucralfate) is a complex of aluminum hydroxide and sulphated sucrose used in ulcer management. It is used for gastroeosophageal reflux disease (GERD), especially during pregnancy, in which it is the first-line drug therapy combined with lifestyle and diet modification. It is also the preferred agent to H_2 antagonists for stress-induced peptic ulcer prevention (prophylaxis) (Mendenhall, 2014; Fashner and Gitu, 2015). Although sucralfate is not FDA approved for peptic ulcer, it is

used primarily as a maintenance therapy for resolved duodenal ulcers and sometimes, for active peptic ulcers other than that of NSAIDs (Fashner and Gitu, 2015).

Sucralphate binds to the ulcer lesion to create a physical barrier protection from stomach acid and also promotes bicarbonate production and acts like an acid buffer cytoprotectant (Maton, 2003). It is a locally acting substance that forms a cross-linking, viscous, paste-like material on reaction with the HCl in the stomach. Its' acid buffering activity for as long as 6 to 8 hours after a single dose is due to its high affinity for defective mucosa (McEvoy, 2007). It also attaches to proteins on the surface of ulcers, such as albumin and fibrinogen, to form stable insoluble complexes that serve a protective barrier function at the ulcer surface, preventing further damage from acid, pepsin and bile. It is also thought that sucralfate also stimulates the production of prostaglandin E_2, epidermal growth factors (EGF), platelet growth factors and gastric mucus (Korman *et al.*, 2003). Sucralfate is considered a non-systemic drug excreted primarily unchanged in urine (McEvoy, 2007). Constipation is the most common side effect; others are flatulence, headache, hypophosphatemia, xerostomia (dry mouth), and bezoar formation. The drug is not used in chronic kidney failure (for aluminium accumulation and toxicity), in children and pregnancy Category B subjects for lack of safety and efficacy studies (Farmer, 2010). Its shortcomings include aluminium toxicity that could lead to renal failure and tetratogenicity (Steiner *et al.*, 1992)

2.7.1.7 Surgery:

Surgery is recommended and resorted to only in cases of complications that do not respond to standard treatment (Alexander-Williams, 1991).

2.7.2 Non Pharmacological management

i. Rest: This includes adequate sleep and relaxation. Bed rest is highly recommended and sometimes sedatives can be used to promote sleep.

ii. Diet: Small frequent feedings is encouraged in ulcer patients because presence of food in the stomach usually serves as acid buffer and helps to protect ulcer wounds. Spices, hot pepper and late night snacks as well as coca cola and caffeine drinks like coffee (or other strong stimulants of gastric acid secretion) should be as these promote gastric inflammation. Fatty foods tend to cause reflux into the duodenum and are therefore, should restricted (Andrew *et al.*, 2015).

iii. Avoidance of certain substances such as smoking which delays healing of ulcer pains as well as alcohol and aspirin which are associated with gastric ulcer.

2.7.2.1 Surgery:

Surgery is recommended and resorted to only in cases of complications that do not respond to standard treatment (Alexander-Williams, 1991).

2. 8 Medicinal Plants with Anti-ulcer Potentials

Medicinal plants reported to have anti-ulcer effects include:

i. Liquorice (*Glycyrrhiza glabra*): This has been used as antiulcer agent since early 1970s due to its glycyrrhizin component. Glycyrrhenitic acid was isolated from licorice and the steroid-like structure of the glycyrrhenitic acid derivatives has a serious side effect of causing electrolyte imbalance (Sanjai, 2005; Beaumont and Maccaferri, 2011). *Carbenoxolone (CBX)* sodium: a glycyrrhetinic acid derivative with a steroid-like structure, similar to substances found in the root of the glycyrrhiza glabra with

pirenzepine anticholinergic has a rather limited efficacy in speeding healing of chronic gastric ulcers (Sanjai, 2005). It either acts by inhibiting the growth of *H.pylori* or providing soothing effect on inflamed and irritated mucous membranes and stimulating mucus secretion which protects ulcer site from gastric acid (Murugesh, 2004; Beaumont and Maccaferri, 2011).

ii. There are also reports of the use of the leaves of *Ficus capensis Thumb.* (Moraceae) as antiulcer agent (Emeje, 2004).

iii. The aqueous extract of unripe fruit of *Carica papaya* had also been reported to possess antiulcer properties through its ability to inhibit gastric acid secretion and to reduce pepsin activity (Bamidele *et al.,* 2013).

iv. The n-butanol portion of the dried flower buds of *Syzygium aromaticum* L. of the family Myrtaceae commonly known as clove and esteemed as a flavouring agent in tobacco are also reported to possess anti-secretory type of anti-ulcerogenic activity in rats (Okasha *et al.,* 2007; Magaji *et al.,* 2007).

v. The methanolic extract of plant berries of *Solanum nigrum* L. of the family Solanaceae commonly known as Blacknight shade is also reported to possess antiulcer activity and/or gastroprotective effect on aspirin-induced ulceration in rats (Aastha *et al.,* 2012).

vi. *Aloe vera* (L.) Burm.f. commonly known as Aloe had also been reported to show antiulcer activity in ethanol-induced ulcer model **(**Gopinathan and Naveenraj, 2013**).**

vii. There are also reports of use of dried banana powder (*Musa paradisiacal*), chamomile (*Anthemis nobilis*), and Neem (*Azadirachta indica*) (Dharmani and Gautam, 2006; Bandyopadhyay, 2004).

2.9 The Plant - *Ximenia americana*

2.9.1 Taxonomic nomenclature

Plant Name: *Ximenia americana* Linn

Order: Santalales

Family: Olacaceae

Genus: *Ximenia*

Specie: *Ximenia americana*

Common Names: English: yellow, wild, sour or monkey plum, sea lemon, false sandal wood; Hausa: 'Tsada'**;** Igbo: 'Anya Nwona'**;** Yoruba: 'Igo' (Adamu *et al.*, 2005)

2.9.2 Description, Origin and geographical distribution

The family of olacaceae is a group of flowering plants seen mostly as small sprawling trees or shrubs of about 50 – 70 cm high. It is commonly found in woodlands and grassy savannah around river banks. It has about 8 known species including *X. roiigi, X. aegyptiaca, X. parviflora, X. coriaceae, X. caffra, X. aculeata and X. americana* (Monte *et al.*, 2012).

The fresh leaves are oval shaped, bright green and have a strong smell of bitter almonds. The Fruits are lemon-yellow or orange-red drupes of about 3 cm long and 2.5 cm thick. Flowers are fragrant, white, yellowish-white or pinkish colour. The plant has spiny slender branches that are sometimes semi-parasitic on self or on roots of other trees and it is a drought resistant hedge tree (Shantha *et al.*, 2012).

X. americana Linn is originated from Australia and Asia, but is now widely distributed in many tropical regions of Africa, India, New Zealand, Central America and South America. The trees

grow mostly in tropical countries and are found in abundance across Southern Africa. It grows on many soil types; but, often on poor and dry loamy soil (Debela *et al.,* 2012).



Plate 1: *Ximenia americana* in its natural habitat

2.9.3 Uses of *Ximenia americana*

2.9.3.1 Non-medicinal Use

The fruits are pleasantly eaten or used to make juice, jams and jellies and sometimes used in preparation of intoxicating drinks. **The leaves** are edible, but contain cyanide and need to be thoroughly cooked. In Australia, the leaves are reported to be strongly cyanogenic, but in Asia, the young leaves are eaten after thorough cooking, but are however not eaten in large amounts (Wikipedia, 2010). The leaves are sometimes crushed and used as flavouring agent for its bitter almond smell. The **seed pulp** contains hydrocyanic acid and is often not eaten raw. Economically, the plants are cultivated as hedge trees for shade. The root are used in tanning hides and is thus applied in leather making, while the bark is used to strengthen indigo dyes. The seed oil contains about 99% saturated and monounsaturated fatty acids which makes it stable to

oxidation and useful in cosmetics as ointment for dry skin and hair conditioner (Sallamander, 2010). The oil improves the functions of the sebaceous tissues and is particularly popular as a massage treatment for dry and chapped skin. Glyceride blends containing ximenic acid from *X. americana* are useful for preparation of food supplements, including margarine, chocolate, ice cream, mayonnaise, cheese, dry soups, drinks, cereal bars and sauces and snack bars. There is also blend compositions of the plant used for health purposes to delay onset of symptoms related to disorders of insulin resistance diabetes, development of Alzheimer's disease, memory functions improvement, lowering of blood lipid levels lowering, anticancer effects or skin anti-ageing effects (Lall and Kishore, 2014).

2.9.3.3.Medicinal Use

The reported varied therapeutic potentials of the various parts of the plant indicated use of the **Leaves and twigs** for treating fever, cold and headache; and as infusion for eye wash (conjunctivitis), angina, toothache and constipation. It is also used as laxative and poison antidote. When cooked as vegetable at 100 ppm, the leaves also caused 100% mortality of *Bulinus globus*, the vector in the transmission of schistosomiasis (James *et al.,* 2008); **root-leaf decoction** for febrifuge, diarrhoea, jaundice, and relief of febrile headache (James *et al.,* 2008, Geyid *et al.,* 2005); **roots** for skin problems, headache, sleeping sickness, oedema, dysentery, mouth ulcers, venereal diseases, guinea worm, leprosy, haemorrhoids and as antidote in poison (Ogunleye and Ibitoye, 2003; Okigbo *et al.,* 2009); **fruits and seeds** for colds, laxative, dropsy, rheumatism and diabetes. A decoction of the roots or fruits is used to treat dysentery in calves. The fruit is useful in treating habitual constipation and it acts as a vermifuge (Maikai, *et al.,* 2008); **seeds** for purgatives and as emetics; the seed oil is used medicinally for hepatitis, kidney problem and abdominal pain (Desissa and Binggeli, 2002). Women also use the pressed oil as

contraceptives. The seed oil is applied to skin cuts or used after bone surgery to prevent infections (James *et al.,* 2008); **stem bark** is used as tea for astringent purposes and as an agent against excessive menstruation or as powder to treat stomach ulcers, febrile headaches, kidney and heart complaints. The plant is extensively used among the Hausa/Fulani's in treating malaria and other microbial infections (*E. coli, P. aeruginosa and C. albicans*) (Ogunleye and Ibitoye, 2003). It is also used for leprotic ulcers and other skin infections of mixed origin (Ogunleye and Ibitoye, 2003). The plant is known for its anti-snake venom and anti-trypanosomal activities among the Hausa Fulani's (Maikai, *et al.,* 2008). The plant is also alleged to have antineoplastic, anti-inflammatory, pesticidal, hepatic and haematological effects in African traditional medicine (Monte *et al.*, 2012).

2.10 Previous Pharmacological Studies on *Ximenia americana*

The analgesic properties of the aqueous stem bark extract of *Ximenia americana* has been ascertained in hyperthermic rats against lysine-acetylsalicylate (Aspegic) treated controls was reported to have anti-pyretic activity (Siddaiah *et al.*, 2009; Soro *et al.*, 2009).

The *in-vitro* antitrypanosomal activity of the methanolic and aqueous extracts of stem bark of *Ximenia americana* was evaluated *on Trypanosoma congolense* using blood from highly infected mice. The stem bark methanol extract of *Ximenia americana* also showed antiviral effect against measles virus *in-vitro by* plaque reduction neutralisation assay (Maikai *et al.,* 2008). Hepatoprotective activity of the leaf extract of *Ximenia americana* had also been reported from studies on acetaminophen-induced hepatotoxicity in rats (Venkateshwararao *et al.,* 2011). The haematological effects of the leaves, stem bark and root have also been previously reported (James *et al*., 2008).

Phytochemical screening of the methanolic extract of the leaves and its antidiabetic activity in rats was reported to posses alkaloids, flavanoids, proteins, phenolic compounds, terpenoids, saponins, steroidal glycosides and tannins as well as having significant antidiabetic activity (Siddaiah *et al.*, 2011).

CHAPTER THREE

3.0 MATERIALS AND METHODS

3.1 Materials

3.1.1 Collection and identification of plant material

The whole *Ximenia americana* plant was collected in June, 2014 from Tashan-yari area of Makarfi Local Government Area of Kaduna State, Nigeria. The plant was identified and authenticated by a taxonomist, Mallam Sanusi of the Department of Biological Sciences, Ahmadu Bello University, Zaria by comparing with an existing voucher number 099 and as well deposited.

3.1.2 Experimental animals

Adult male Swiss albino mice (18-29 g) and adult male Wistar rats (100-200 g) obtained from the Animal House Facility of the Department of Pharmacology and Therapeutics, Faculty of Pharmaceutical Sciences, Ahmadu Bello University, Zaria were used for the study. The animals were allowed to acclimatise to Ahmadu Bello University Pharmacology Animal House conditions with food and water provided *ad-libitum* except when an experiment requires fasting of the animals. All experiments were conducted in accordance with the regulation for the Care and Use of Laboratory animals as accepted internationally (NIH, rev 1996).

3.1.3 Equipment and instruments

i. Weighing balance (Wet. Avery Ltd, Birmingham, England, TH-5000)
ii. Heamatocrit centrifuge (Denley, BS400, UK)
iii. Ion Selective Electrode-Electrolyte analyser (Pioway, XI-921D, Japan)
iv. Haematology analyser (Pioway HY-3400, Japan)
v. Centrifuge (Techmel and Techmel, TT-645P, UK)

Other Materials include mortar and pestle, refrigerator spatula, animal cages, animal feeds, needles and syringes, cotton wool, cannular, scissors, pasteur pipette, evacuated (EDTA) tubes and other sample bottles, capillary tubes, marker and masking tape.

3.1.4 Drugs, Chemicals and Solvents

i. Omeprazole Capsule 20mg (Hovid BHD, Malaysia)
ii. Misoprostol 200ug (Cytotec Pharmaceutical Lab. Germany)
iii. Cimetidine Capsule 200mg (Paucodine / Pauco Pharmaceutical Ind. Nig. Ltd.)
iv. Indomethacin capsule 25mg (M/S Aphantee Pharm. Nig. Ltd)
v. Ethanol and methanol (BDH Chemical Ltd., Poole England)
vi. Absolute Formaldehyde (BDH Chemical Ltd., Poole England)
vii. Chloroform (BDH Chemical Ltd., Poole England)
viii. Distilled water and normal saline (0.9 g NaCl in 100 ml of Distilled Water)

3.2 Methods

3.2.1 Preparation of plant extract

The stem bark of *Ximenia americana* were scraped off from the plant stems and dried under shade until a constant weight was obtained and then crushed into powder of 759 g. Soxhlet extraction method was used the extraction, the obtained powdered material (759 g) of *Ximenia amemerican*a was packed into a filter thimble underneath a conical flask containing the solvent (absolute methanol) which is heated, to evaporate and condense through the condenser into the timble for 72 hrs extraction. The mixture was then filtered and a filtrate was obtained and concentrated to dryness using a rotary evaporator over water bath at 45°C a dark-brown methanol

extract was obtained packed into an airtight bottle stored and kept about 25°C. The percentage yield of the extract was calculated as:

$$\text{Percentage yield (\%)} = \frac{\text{Weight of extract (g)}}{\text{Weight of dried stem bark powder (g)}} \times 100$$

(Sultana *et al.*, 2009)

3.2.2 Preliminary phytochemical screening

The method of Trease and Evans (2002) was used to screen the extract for the previously reported constituents including carbohydrates, alkaloids, tannins, sterols, terpens, saponins, steroidal glycosides as outlined below:

3.2.2.1 Carbohydrates (Molisch'reagent test)

Few drops of Molisch' reagent was added to 5 ml of the extract in a test tube and conc. H_2SO_4 (1 mL) was added down the test tube side. Formation of a purple ring interphase beneath the aqueous layer confirms the presence of carbohydrates (Silva *et al*., 1998).

3.2.2.2 Alkaloids (Mayer's reagent test)

A little portion of the extract dissolved in 5 mL of water was mixed with equal volume of 1% aqueous hydrochloric acid (HCl) and stirred over water bath for 10-15 minutes and then filtered; 1mL of the filtrate was mixed with few drops of Mayer's reagent; and another 1mL of the filtrate with Wagner's reagent; and yet another 1ml with Drangendorff's reagent. The mixtures were checked for a white precipitate, red precipitate and rose red precipitate, respectively which signifies the presence of alkaloids (Evans, 1989).

*3.3.3.3 Steroidal Glycosides (*Liebermann-Burchard's test)

The extract (5 g) was dissolved in 10 mL of distilled water in a test tube and was evaporated on a boiling water bath at (55°C) to dryness. The residue was dissolved in 0.5 mL chloroform in a test

tube. Then Conc.H_2SO_4 acid (2 ml) taken with a pipette was dropped at the bottom of the test tube for Liebermann-Burchard's reaction. Separation of the two liquids by a reddish brown ring colour indicates the presence of steroids and triterpenes (Brain and Turner, 1975).

3.2.2.4 Anthraquinones (Borntrager's test)

The extract (3 g) was dissolved with 10 mL of benzene and filtered to obtain a filtrate which was mixed with ammonia solution (5 mL of 10%) and stirred; another 3 g of the extract boiled with 5 mL of 10% hydrochloric acid for 3 minutes was filtered hot and allowed to cool. The filtrate mixed with 5 mL of benzene produces a benzene layer which was filtered off and half the volume of the residue mixed with 10% ammonia solution and shaken gently. A red colour in the ammonia (lower) phase for both the filtrate and residue indicates the presence of free and combined anthraquinones, respectively (Sofowora, 1993).

3.2.2.5 Saponins (Frothing test)

Little portion of the extract dissolved in 10 mL of distilled water and shaken gently for 30 seconds which foams vigorously for 30 minutes indicating the presence of saponin (Silva *et al*., 1998).

3.2.2.6 Flavonoids (Shinoda and Sodium Hydroxide Test)

i. Shinoda Test: The extract (2 g) dissolved in 2 mL of 50% methanol and warmed over water bath was filtered. Magnesium metals (4-5 pieces) and few drops of concentrated hydrochloric acid (HCl) were added to the filtrate and observed for the pink colour of flavonoids.
ii. Sodium Hydroxide Test: Few drops of aqueous sodium hydroxide added onto 5 mL of the extract was also observed for a yellow colour of flavonoids. (Mahran, 1980)

3.2.2.7 Tannins ($FeCl_3$ Test)

The extract (3 g) boiled with 10 mL of water was cooled and filtered. A drop of 1% $FeCl_3$ solution added to 1 mL of the filtrate was checked for blue-black precipitate of tannin (Evans, 1996).

3.3 Acute Toxicity Study

Lorke's method (1983) was adopted for the acute toxicity test in mice and rats. Thirteen (13) of each animal species were used. Nine (9) of rats/mice in 3 groups of 3 animal per group for the three graded doses of 10, 100, 1000 mg/kg were treated orally per body weight and observed for 24 hours for signs of changes in the behavioral pattern and/or death. In a 2^{nd} phase of the experiment, the remaining 4 rats/mice in each of the 4 groups of one animal per group, respectively were given lower or higher doses of *Ximenia americana* depending on occurrence of death or no death in the first phase and observed again for 24 hr. The oral median lethal doses were then calculated as the geometric mean of the highest non-lethal and the lowest lethal doses as follows;

$\mathrm{LD50} = \sqrt{maximum\ non lethal\ dose \times minimum\ lethal\ dose}$ for both animal species. (Lorke, 1983)

3.4 Assessment of Mucosal Cytoprotective effect of *X. americana* Stem Bark Extract for Ulcer Inhibition in Rats

The method of indomethacin ulceration of Djahangiuri (1969) was used. Twenty five (25) rats deprived of food for 24 hr were divided into five (5) groups of 5 rats each and the various groups were treated per kg body weight for 30 minutes with normal saline (1 ml/kg), cimetidine (100 mg/kg), *Ximenia americana* at doses of 250, 500 and 1000 mg/kg. Indomethacin (100 mg/kg per

body weight) was administered to all the groups of rats for 6 hr ulcer-induction after which the rats were anaesthesised with chloroform and the abdomen incised to isolate the stomach into 2% v/v formaline overnight. The stomachs were then cut open along the greater curvature and spread out on filter papers to count the total number of lesions as well as the number of ulcer spots of larger diameter ≥ 3.

In another group of experiment, the method of ethanol-induced ulcers of Robert, (1979) was also used. Twenty five (25) rats deprived of food for 24 hr were divided into five (5) groups of 5 rats per group and treated per kg body weight for 30 minutes with normal saline (1 mL/kg), misoprostol (100 mg/kg), *Ximenia americana* extract at doses of 250, 500 and 1000 mg/kg. Absolute ethanol (1 mL) was then administered to all the rats in each group for an hour to induce ulcers, after which the rats were sacrificed under chloroform anaesthesia and the abdomen incised as previously to isolate the stomachs which were then washed and spread open on filter papers to count the total number of lesions as well as the number of ulcer spots of larger diameter ≥ 3.

3.5 Subacute Toxicity Study

Twenty (20) rats were divided into 4 groups of 5 rats per group and treated daily per body weight with normal saline (1 mL/kg), *Ximenia americana* extract at doses of 250, 500 and 1000 mg/kg respectively for 28 days. The animals were sacrificed on the 29^{th} day and some organs (brain, liver, heart, kidneys, lungs, spleen and stomach) were isolated, weighed and placed into 10% formaline, (the brain in 20%) for histological examinations. Blood samples were also collected both into EDTA tubes and non-heparinised tubes for haematological and biochemical (liver function and kidney) tests respectively.

4.0 RESULTS

4.1 Extract Yield

A yield of 22.79 % w/w crude methanol extract was obtained from the powdered stem bark of *Ximenia americana* plant for the study.

4.2 Phytochemical Constituents

The preliminary phytochemical screening of the methanol stem bark extract of *Ximenia americana* revealed the presence of phytochemical constituents as listed in the table below.

Table 4.1: Phytochemical constituents in the methanol stem bark extract of *Ximenia americana*

Constituents and test type	Remark
Alkaloid (Mayer's reagent test)	+
Anthraquinone (Borntrager's test)	+
Carbohydrates (Molisch's reagent test)	+
Flavonoids (Shinoda's test)	+
Saponins (frothing test)	+
Steroidal glycosides (Liebermann-Burchard's test)	+
Tannins ($FeCl_3$ test)	+
Terpenoids (Liebermann-Burchard's test)	+

Key: (+) Present

4.3 Acute Toxicity Study

No death was recorded in the first-phase of the study in both rats and mice. In the second phase, doses of 1600, 2900 and 5000 mg/kg were used and no death was also recorded. The oral median lethal dose (LD_{50}) for the methanol stem-bark extract of *Ximenia americana* was therefore, estimated to be greater than 5,000 mg/kg in both mice and rat and no signs of behavioural changes were also observed.

4.4 Mucosal Cytoprotective Effect of *X. americana* Stem bark Extract on:

1. Indomethacin-induced Ulcer

The extract significantly ($p \leq 0.05$) and dose-dependently reduced the mean ulcer spots in a similar manner as the standard agent cimetidine. The reduction was significant only for the two higher doses of the extract (500 and 1000 mg/kg). However, the ulcer lesions were more prevented in the rat groups of cimetidine which showed mean ulcer spots of 0.6 ± 0.4 as against 1.80 ± 0.9 of the 1000 mg/kg extract dose. A mean of only 3 severe ulcer spots for each of the two lower extract doses pre-treated rat groups against 9 severe ulcer spots for the normal saline control group occurred with the indomethacin ulcerogen with no severe ulcer spots in both the cimetidine and 1000 mg/kg extract pretreated rat groups.

2. Ethanol-induced Ulcer

The ethanol-induced ulcer lesions were also reduced in the extract treated groups and similarly, the reduction in the ulcer lesions was significant in rats of the two higher dose levels of the extract as with indomethacin-induced ulcer; and both doses prevented the occurrence of lesions in the same manner. The standard antiulcer drug (misoprostol) used for this study also inhibited

the ulcer spots as expected. There were no severe ulcers (≥ 3mm spots) found in any of the groups including the normal saline control group.

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Table 4.2: Effect of *X. americana* stem bark extract on indomethacin-induced mucosal lesions in rat

Treatments Per kg	Mean ± SEM of ulcer spots	No. of ulcer spots ≥ 3mm (index of severity)
N/ S (1 mL)	9.20 ± 1.40	9
XA 250mg	6.60 ± 1.07	3
XA 500mg	4.60 ± 0.51*	3
XA 1000mg	1.80 ± 0.86*	0
Cimetidine 100mg	0.60 ± 0.40*	0

Data presented as mean±SEM; Statistical tool: one way ANOVA and * = $p \leq 0.05$ (Dunnett post-hoc test); XA= *Ximenia americana* extract; N/S= normal saline**;** n=5

Table 4.3: Effect of *X. americana* stem bark extract on ethanol-induced mucosal lesions in rat

Treatments Per kg	Mean ± SEM of ulcer spots	No. of ulcer spots ≥ 3mm (index of severity)
N/ S (1 ml)	7.60 ± 0.86	0
XA 250mg	5.20 ± 0.58	0
XA 500mg	2.40 ± 0.24*	0
XA 1000mg	2.40 ± 0.40*	0
Misoprostol 100mg	0.80 ± 0.50*	0

Data presented as mean±SEM; Statistical tool: one way ANOVA and * = $p \leq 0.05$ (Dunnett post-hoc test); *Ximenia americana* extract; N/S= normal saline**;** n=5

4.5 The Subacute (28 days) Toxicity Evaluation of *Ximenia americana* Stem Bark Extract on Body and Organ Weights, Blood components and some Biochemical Parameters in Rat

Slight variations of either an increase or decrease in the body and organs weights were observed, but none was significant ($p \leq 0.05$) at all the three dose levels of *Ximenia americana* extract used in the study. There was no significant alteration in the concentration of the liver enzymes and blood components. Slight increases in the concentrations of sodium ion, chloride ion and total calcium were observed at all the three doses of the extract. The level of total protein was increased in a dose dependent manner and was significant ($p \leq 0.05$) only at 1000mg/kg with respect to the control group; but there was no significant difference ($p \leq 0.05$) amongst the treatment groups. The albumin level was reduced in a dose dependent manner and significant ($p \leq 0.05$) for the two higher extract dose groups with respect to the control group. There were no consistent changes in the urea level and the dose dependent reduction in the level of creatinine was not significant at any of the doses.

Table 4.4: Weekly body weight changes of rats following 28 days daily pretreatment with *Ximenia americana* extract

Treatments Per kg	Mean ± SEM of body weights in 4 days weekly intervals				
	DAYI	DAY8	DAY15	DAY22	DAY29
N/ S (1 mL)	122± 8.00	145± 10.20	149± 10.84	160± 11.18	161± 9.51
XA 250 mg	108± 2.50	122± 2.64	140± 8.94	148± 5.00	141± 1.68
XA 500 mg	116± 9.11	142± 10.26	144± 11.60	147± 13.40	158±12.78
XA 1000 mg	120± 6.60	136± 7.25	134± 10.06	141± 10.06	144± 12.32

Data presented as mean±SEM; Statistical tool: one way ANOVA and * = $p \leq 0.05$ (Dunnett post-hoc test); XA = *Ximenia americana* extract; N/S= normal saline**;** n=5

Table 4.5: Changes in organ weights in relation to body weight of rats pretreated with *Ximenia americana* extract for 28 days

Treatments (Per kg)	Organ Weights (g)						
	Kidneys	Spleen	Liver	Brain	Lungs	Stomach	Heart
N/S (1 ml)	0.68±0.02	0.46±0.02	3.74±0.14	0.86±0.05	1.4±0.15	0.9±0.04	0.52±0.12
XA 250 mg	0.68±0.03	0.58±0.48	3.64±0.08	1.03±0.05	2.2±0.80*	0.9±0.04	0.43±0.05
XA 500 mg	0.63±0.03	0.55±0.03	3.65±0.18	1.06±0.05	1.5±0.18	1.0±0.08	0.50±0.05
XA 1000 mg	0.66±0.06	0.52±0.07	3.54±0.09	1.06±0.06	1.5±0.20	0.9±0.07	0.32±0.04

Data presented as mean±SEM; Statistical tool: one way ANOVA and * = $p \leq 0.05$ (Dunnett post-hoc test); XA = *Ximenia americana* extract; N/S= normal saline**;** n=5

Table 4.6: Changes in haematological parameters of rats pretreated with *Ximenia americana* for 28 days

Treatments (Per kg)	Per µL of			Hb (g / dl)	PCV (l/L)	Percent (%) of		
	WBC $\times 10^9$	RBC $\times 10^{12}$	PLT $\times 10^{12}$			NEUT	EOS	LYM
N/ S (ml)	20.12 ± 0.77	5.31± 0.28	205± 19.04	13.16± 0.75	42.00 ± 2.65	14.00± 0.80	10.20± 1.50	76.00± 2.24
XA 250 mg	14.72 ± 2.12	5.56± 0.22	204± 11.23	13.33± 0.42	44.00 ± 2.00	12.30± 1.11	11.00± 2.00	77.00± 3.00
XA 500 mg	13.00 ± 3.12	5.00± 0.38	185± 20.00	12.35± 1.09	39.00 ± 3.00	11.0± 1.41	11.00± 2.00	78.30± 3.10
XA 1000 mg	14.26 ± 1.84	5.51± 0.17	220± 3.66	13.24± 0.45	44.40 ± 2.00	12.00± 0.86	11.00± 2.00	77.11± 3.00

Data presented as mean±SEM; Statistical tool: one way ANOVA and * = $p \leq 0.05$ (Dunnett post-hoc test); XA = *Ximenia americana* extract; N/S= normal saline**;** n=5

Table 4.7: Changes in electrolyte concentrations and H^+ (pH) of body fluid in rats pretreated with *Ximenia americana* for 28 days

Per kg treated Rat groups	Na^+ (mmol/L)	K^+ (mmol/L)	Cl^- (mmol/L)	HCO_3^- mmol/L)	Ca^{2+} (mmol/L)	TCa (mmol/L)	pH
N/ S (ml)	133.00± 1.62	15.10± 1.03	97.40± 1.02	19.00 ± 1.14	1.08± 0.02	2.10 ± 0.04	7.49± 0.02
XA 250mg	139.00± 1.89	12.40± 0.47	102.30± 1.65*	18.00 ± 0.71	1.14± 0.02	2.23± 0.04*	7.66± 0.02
XA 500mg	139.00± 1.97	13.40± 1.92	101.00± 0.48	20.30± 0.85	1.08± 0.02	2.11± 0.03	7.51± 0.02
XA 1000mg	135.00± 1.24	12.00± 0.95	100.40± 0.98	18.00± 1.22	1.09± 0.01	2.13± 0.02	7.59± 0.06

Data presented as mean±SEM; Statistical tool: one way ANOVA and * = $p \leq 0.05$ (Dunnett post-hoc test); XA= *Ximenia americana* extract; N/S= normal saline**;** n=5

Table 4.8: Changes in liver enzymes, plasma proteins and kidney excretory parameters in *Ximenia americana* pretreated rats for 28 days

Per kg treated Rat groups	Liver enzymes (µ/L)			Plasma proteins (g/dL)		Kidney function tests (mmol/L)	
	AST	ALT	ALP	Total proteins	Albumin	Urea	Creatinine
N/ S (ml)	76.2 ± 9.00	43.00 ± 5.20	84.40± 2.00	84.40 ± 1.12	36.00 ± 1.00	7.78. ± 0.13	134.00 ± 55.2
XA 250mg	68.00 ± 14.14	38.00± 7.00	87.30 ± 2.00	86.30 ± 1.50	32.30± 0.63	7.52 ± 0.33	123.00 ± 10.00
XA 500mg	75.0 0± 4.40	34.00 ± 4.00	88.30 ± 1.25	90.00 ± 3.30	27.30 ± 2.00 *	6.05 ± 0.77	114.00 ± 13.40
XA 1000mg	85.00 ± 9.44	38.20± 6.31	84.00 ± 2.00	99.00 ± 2.16*	22.00 ± 1.00 *	9.22 ± 0.93	113.00 ± 29.00

Data presented as mean±SEM; Statistical tool: one way ANOVA and * = $p \leq 0.05$ (Dunnett post-hoc test); XA= *Ximenia americana*; N/S= normal saline**;** n=5

4.6 Histological effects of *Ximenia americana* extract on some rat tissues following 28 days pretreatment

Histological examination showed that the extract at all doses did not cause any pathological changes in the brain, heart, liver and stomach. However, vascular congestion with polymorphonuclear cells infiltration was seen in the kidney at the extract dose of 1000mg/kg. The lung has consolidated areas of polymorphonuclear cells infiltration at the alveoli and terminal bronchioles, while the spleen had distorted germinal centres.

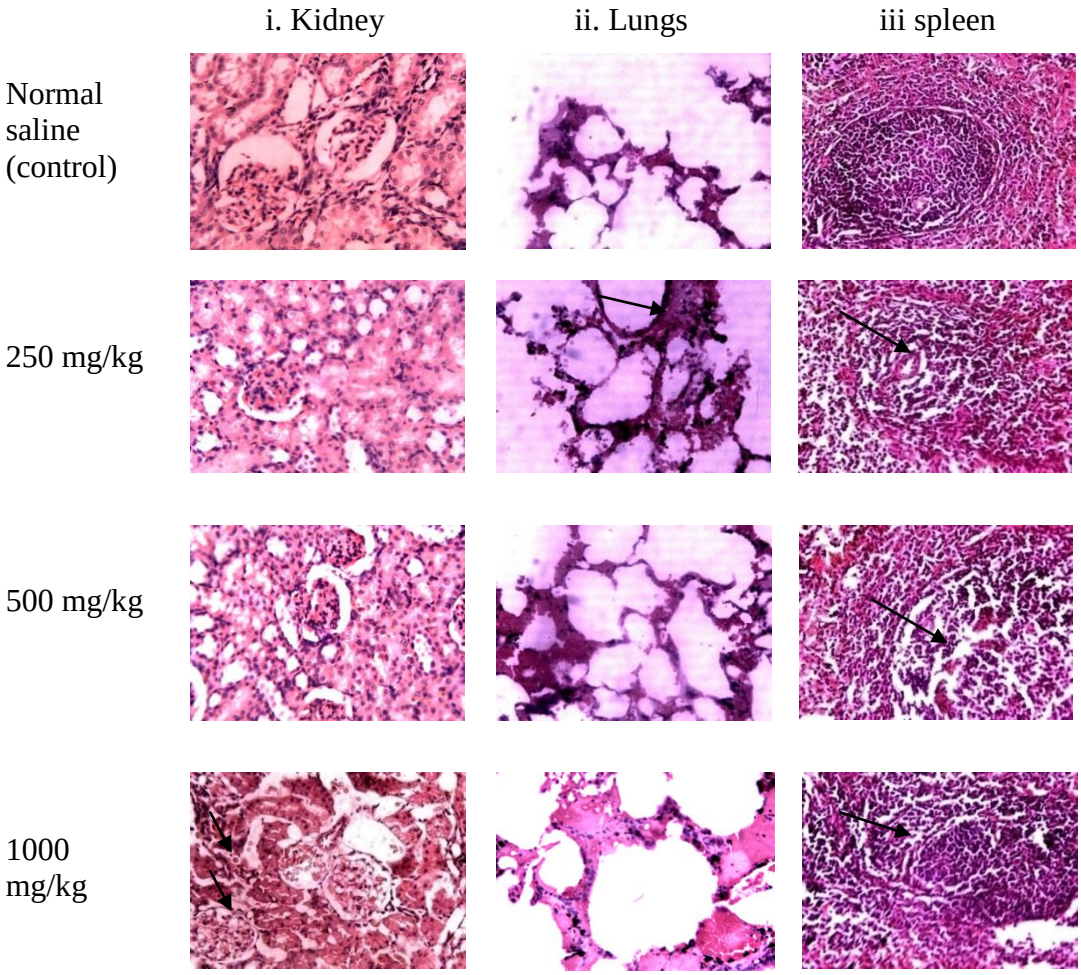


Plate ii: Photomicrograph of tissue sections (H and E at ×250) of rats following 28 days

treatment with normal saline and *Ximenia americana* stem bark extract:

i. Vascular congestion with polymorphonuclear cells infiltration in the kidneys at the extract dose of 1000 mg/kg
ii. Consolidation of polymorphonuclear cell infiltration in the alveoli and terminal bronchioles of the lungs at the extract dose 250 mg/kg
iii. Distorted germinal centre in the spleen at all the extract doses

CHAPTER FIVE

DISCUSSION

A large proportion of people now use plants and herbs to maintain health and cure many of the ailments of both mankind and animals. The beneficial and toxicity potentials of herbal preparations are often related to the active principle contained in the plants (Aliyu *et al*., 2010; Ekor, 2013). The preliminary phytochemical screening of the methanol stem bark extract of *Ximenia americana* locally used as an anti-ulcer agent showed presence of alkaloids, anthraquinones, flavonoids, saponins, tannins, terpenoids and carbohydrates. This finding is consistent with other works reported in the literature (Maikai *et al*., 2009). Some of these constituents reported to possess anti-ulcer effects include flavonoids and other phenolic compounds which prevents gastric mucosal lesions and thus, provide the cytoprotective type of antiulcer effect (Zayachikivska *et al*., 2005; Barro *et al*., 2008). Saponins, tannins and terpenoids also possess certain properties related to anti-ulcer effect (Boralli and Isso, 2000).

Assessing the safety of drugs in biological systems is often initiated from an acute toxicity study (Mukinda and Syce, 2007). This study revealed that the methanol stem bark extract of *Ximenia americana* caused no death in mice and rats at doses of up to 5,000 mg/kg and no observable behavioural changes. Thus, the extract was relatively not harmful in its short term (within 24 hours) oral exposure to both animal species.

The antiulcer effect of the extract was assessed in ulcerogenic rats of indomethacin and absolute ethanol-induced model. The cytoprotective barrier mechanisms of the mucosal linings of rats are known to be disrupted resulting in overproduction of acid fluids with ulcers (Bower *et al.,* 1977). The extract of *Ximenia americana* at doses of 500 and 1000 mg/kg significantly ($p \leq 0.05$) and dose-dependently reduced the mean ulcer spots as with the standard drugs, cimetidine and

misoprostol. However, it was only at the 1000 mg/kg dose group of the indomethacin ulcer model that severe ulcer lesions did not occur, similar cimetidine standard antiulcer drug. Each of the two lower extract dose pretreated rat groups showed three (3) mean severe ulcer spots against a mean of nine (9) for the normal saline control group. This is an indication of ulcer protection. This study showed that the higher the dose of the extract, the more the protection against ulcer, but there may be concerns of possible dose-related toxic effects. The absence of severe ulcers in any of the rat groups of ethanol-induced ulceration including the normal saline control group seemed to suggest that it is a milder ulcerative agent than indomethacin, which produced severe ulcer lesions in the rats at lower extract doses of 250 and 500 mg/kg body weight of rats and also in the normal saline control group. Thus, the indomethacin ulcerative mechanism of causing accumulation of cyclic AMP from blockade of prostaglandin production (Bower *et al.*, 1977), may have caused significant increase in gastric acid attack of the mucosal wall barriers.

Overproduction of acid could be in the form of inhibition of GIT mucin production as with the mucinase activity of *H. pylori* or increased gastric emptying of the stomach with consequent incomplete neutralisation of the acid (HCl); which consequently cause irritation and/or inflammation of the mucosa. Thus, in addition to boosting production of prostaglandins for hyperacidity protection, potential antiulcer agents could also cause mucosal enhanced blood flow and/or secretion of gastric mucous and bicarbonate which constitute the defensive barrier mechanism of the mucosal epithelial cell wall lining which helps resist acid attack (cytoprotection) and maintain the integrity of the mucosal wall (Cohen, 1987). Agents for ulcer therapy could therefore be of cytoprotective for increased acid resistance or acidity inhibitors to prevent acid accumulation.

The extract did not cause changes in the organ sizes in relation to the control experiment. However, organ weight assessment study is usually not adequate enough for predicting pathological effects. Functional and structural changes in tissues could still occur without altering the organ weights. Although the histological examination also showed no serious toxic effects or tissue damage, some abnormal effects were observed such as vascular congestion with polymorphonuclear cells seen in the kidney at the highest extract dose of 1000 mg/kg, consolidated areas of polymorphs infiltration at the alveoli and terminal bronchioles of the lungs and distorted germinal centres in spleen were also seen. Body weights of the extract-treated rats did also not change and thus, indicating lack of fatty degeneration (emaciation) (Sansone and Sansone, 2014) or blockade of free flow of body fluids that could lead to oedematous accumulation (Garbella *et al.,* 2011). The occasional slight and insignificant fluctuation in the number of blood components observed might be related to the mononuclear cellular infiltration that were observed in the histology of some of the organs, this study is consistent with (Aminu, 2016) which reported histological changes of some organs such lungs, kidney, spleen and the liver as evidence that suggest toxicity.

Alterations in the serum electrolyte concentrations were slight and none was of a consistent pattern except for sodium (Na^+), chloride (Cl^-) and calcium (Ca^{2+}) ions which showed a slight but consistent increase across the various doses of the extract. Sodium ion (Na^+) is largely associated with Cl^- and HCO_3- and is often available both as NaCl and $NaHCO_3$; such that in the regulation of acid-base equilibrium, Cl^- and HCO_3- concentrations alter concurrently with Na^+. The increase seen in both Na^+ and Cl^- in this study might probably be related to this concurrency in function (Thompson *et al.,* 1987). Many electrolytes are micronutrients available in minute amounts for optimal functioning of the body system; and their depletion or increase causes a

shift in their basal levels resulting in deviation in the steady state ionic concentration of body fluid (Graber and Corish, 1991). Significant alteration in extracellular fluid (ECF) ionic concentration of electrolytes poses life-threatening toxicity. Electrolytes (sodium, potassium, chloride, bicarbonate and calcium ions) has been implicated in a case report of electrolyte abnormalities in pyloric obstruction resulting from peptic ulcer which alter acid-base balance (Lans *et al.,* 1953; Castellanos, 2017)

Sodium ion (Na^+) is usually the predominant cation of the ECF, while Cl^- (with HCO_3-) is the anion. Under normal physiological conditions, the kidney makes the necessary physiological adjustment that effectively maintains body fluid equilibrium. Thus, the minimal increase seen in both ions could probably be due to the observed histological changes in the kidneys of the methanol stem bark extract-treated rats. Bicarbonate (HCO_3-) ion is an important regulatory anion of the pH of body fluid and its absorption as with Cl^- is also facilitated by increased Na^+ concentration. Thus, the observed reduction in pH that occurred might have resulted from this slight change in ionic concentration which also in part might have been responsible for the antiulcer effect of this plant extract (Lans *et al.,* 1953). Adequate secretion of the alkali (HCO_3^-) and / or its reduced gastric emptying increases neutralisation of acid (HCl). Most plasma Ca^{2+} exists as both protein bound and complexes of citrate and PO_4^-, and it is only the remaining diffusible ionised Ca^{2+} concentration that often exerts physiological effects and its reduction tends to result in hypocalcemia (Watt, 2013). The slight consistent increase in Ca^{2+} seen in this study may suggest lack of adverse effect of the extract on Ca^{2+} concentration that could distort calcium-dependent functions of the nerves and muscles. Changes in the total plasma calcium-concentration could also occur from alterations in plasma proteins (Antoniucci *et al.,* 2007). For instance, reduced protein-bound calcium occurs in hypoproteinemia. Thus, the observed

increased Ca^{2+} concentration may be linked to the dose dependent increase in total protein that occurred in this study, though significant only at 1000 mg/kg, similar relation was found between total calcium and albumin and total protein in *Aotus nancymai* (Weller *et al.,* 1990)

As with the liver enzymes, a measure of total protein and albumin in blood also assesses the liver and kidney functions. Although, there was no significant change in the concentrations of aminotransferases and alkaline phosphatase, suggestive of no liver injuries in the rats pretreated with the extract; there was a dose dependent increase in total protein that was significant only at 1000 mg/kg and a reduction in albumin level that was significant at the two higher extract doses (500 and 1000 mg/kg). Plasma proteins provide the osmotic pressure required in the formation and absorption of tissue fluids and this function is often reduced in a damage or diseased liver (Watt, 2013). The level of albumin which is usually higher in concentration in blood plasma than globulin (Bertholf, 2015) was found to have been reduced in this study. Since the histological examination of the liver showed no remarkable alteration, the cause for this change in protein and albumin level is not known, except that slight alteration occurred in the kidney which could also cause changes in serum total protein and albumin level (which was conversely reported by Manojlovic *et al*., (1993) that changes in serum total protein and albumin levels are the most prominent in alterations in nephrotic syndrome and uraemia in kidney disease and liver cirrhosis patients

The tonicity (osmolarity) of the ECF is also partly dependent on the urea and creatinine levels, the retention of which cause disturbances in the body biochemistry. Both are waste products of protein metabolism, but the changes in the urea level in this study were inconsistent and insignificant, while the dose dependent reduction in creatinine level seen in this study was not significant at any of the doses. Urea is the main urinary chemical waste, while creatinine in

biofluids increases only in conditions of rapid muscle breakdown as in fevers and starvation (Carls *et al.*, 2008).

CHAPTER SIX

6.0 SUMMARY AND CONCLUSION

6.1 Summary

The secondary metabolite constituents found to be present in the stem bark methanol extract of *Ximenia americana* were alkaloids, anthraquinones, carbohydrates, flavonoids, saponins, steroidal glycosides, tannins and terpenoids.

The methanol stem bark extract of *Ximenia americana* caused no death in mice and rats at doses of up to 5,000 mg/kg and no behavioural changes were also observed from its oral exposure in mice and rats within 24 hours suggesting that the extract is practically non toxic.

This study showed that the stem bark extract of *Ximenia americana* possesses significant protective antiulcer activity in ulcerogenic rats in both indomethacin- and ethanol- induced models.

The extract at all tested doses caused no changes in organ-sizes or body-weights following 28 days treatment in rats and also did not cause any pathological change in the anatomy of the brain, heart, liver and stomach tissues studied.

However, the kidney at the extract dose of 1000mg/kg showed vascular congestion with polymorphonuclear cell infiltration. There were consolidated areas of polymorphs infiltration at the alveoli and terminal bronchioles of the lungs, while the spleen had distorted germinal centres.

Amongst the evaluated electrolytes, only Na^+, Cl^- and Ca^{2+} showed a consistent but slight increases in their serum concentrations across the various doses of the extract, while changes in K^+ and HCO_3^- levels did not show in a consistent pattern.

The liver enzymes (aminotransferases and alkaline phosphatase) were not altered, but a dose dependent significant increase in total protein was seen at 1000 mg/kg; while reduction in albumin level that was significant at the two higher extract doses (500 and 1000 mg/kg).

The changes in the urea level were inconsistent and insignificant, while the dose dependent reduction in creatinine level seen in this study was not significant at any of the tested doses of the extract.

6.2 Conclusion

In conclusion, the methanol stem back extract of *Ximenia americana* possess significant gastro-mucosal protective effect probably through cytoprotective mechanism; and is relatively non-toxic orally in mice and rats.

6.3 Recommendations

i. There may be need for chronic toxicity study to establish the effect of long term use of this plant part

ii. To carry out the quantitative phytochemical screening as to establish the phytoconstituent with the optimum antiulcer principle

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Appendix



Plate A Rats stomach showing congested serosa lesion

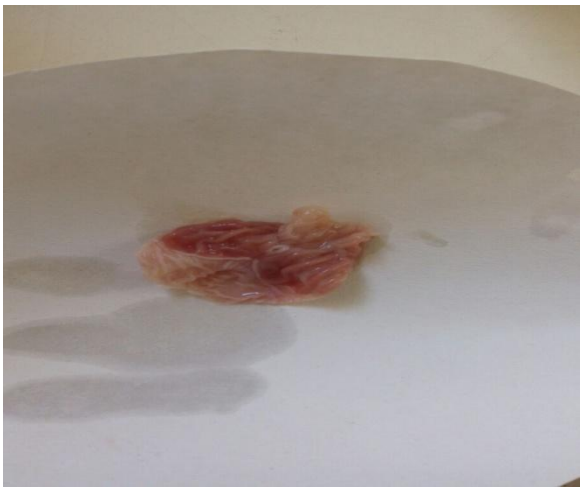


Plate B Opened rats stomach with no ulcer

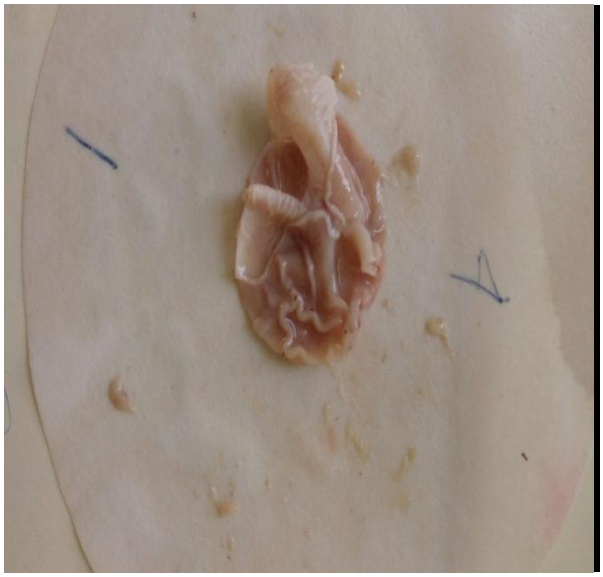


Plate C

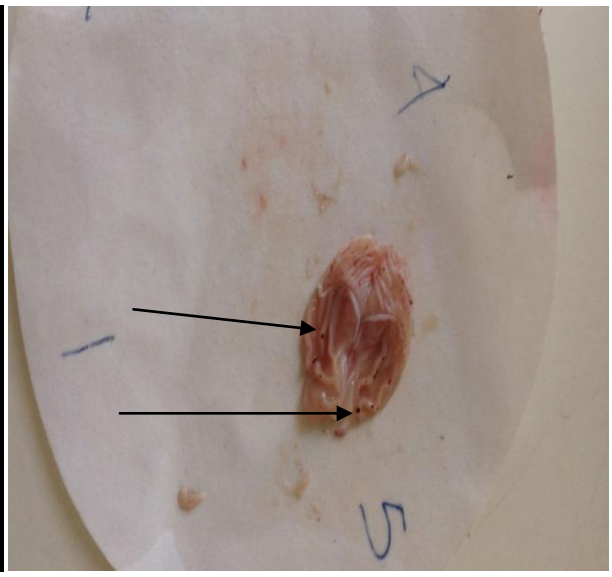


Plate D

Opened rats stomach with few ulcer lesions

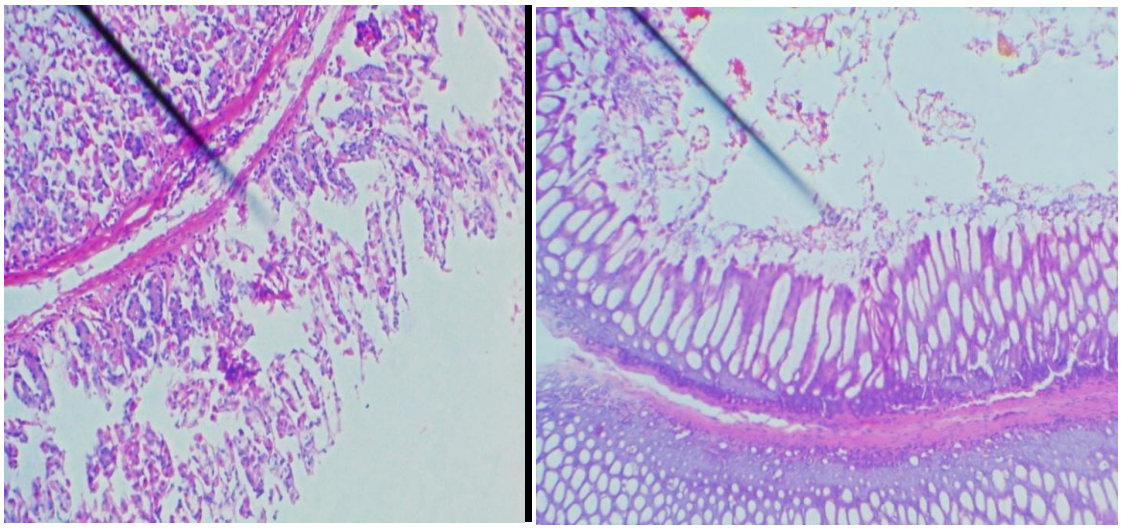


Plate E: **Plate F**

Plate E and F: Photomicrograph of rat stomach showing deep ulcerated mucosa underlying a connective tissue H and E ×10

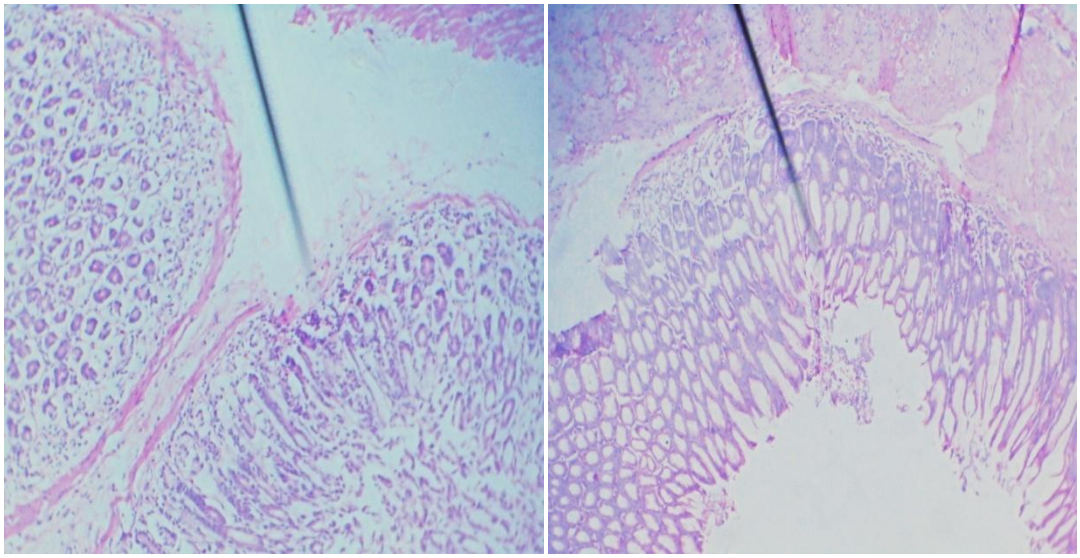


Plate G **Plate H**

Plates G and H: Photomicrograph of rat stomach showing gastric crypt and ulcerated mucosa H and E ×10