GASTROPROTECTIVE POTENTIAL OF AQUEOUS AND METHANOLIC LEAF EXTRACTS OF *VITEX DONIANA* ON ASPIRIN-INDUCED GASTRIC MUCOSAL DAMAGE AND SECRETIONS IN ALBINO RATS

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SEPTEMBER, 2015

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M.Sc/SCIEN/41757/2012-2013

A DISSERTATION SUBMITTED TO THE SCHOOL OF POSTGRADUATE STUDIES,
AHMADU BELLO UNIVERSITY, ZARIA
IN PARTIAL FULFILMENT OF THEREQUIREMENT FOR THE AWARD OF MASTERS DEGREE IN BIOCHEMISTRY

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ABTRACT

The study was designed to evaluate the gastro-protective activity of aqueous and methanolic extracts (250 mg/kg and 500 mg/kg) of *V. doniana* on aspirin-induced gastric mucosal damage in albino rats. A total of 49 adult male albino rats were used, which were randomly divided into 7 groups of 7 rats per group. Aqueous and Methanolic leaves extracts of *V. doniana* were administered for 14 days before induction of gastric mucosal damage. Ulcer index, preventive index, gastric volume, gastric pH, total acidity, gastric wall mucus and antioxidant activity of the plant extracts were determined using standard procedures. Both doses (250 mg/kg and 500 mg/kg) of aqueous extracts (9.25 ± 0.96^{b}, 9.00 ± 2.28^{b}) and methanolic extracts (4.43 ± 1.72^{a}, 8.50 ± 1.73^{b}) showed a significant ($p<0.05$) decrease in ulcer index when compared to the control group (0). The lower dose of both extracts showed significant higher preventive index when compared to the standard drug cimetidine (50 mg/kg). Total acidity and gastric juice volume in the aqueous (36.88 ± 2.92^{b}, 43.83 ± 3.75^{c}) (2.04 ± 0.21^{a}, 2.96 ± 0.66^{b}) and methanolic (32.70 ± 2.61^{a}, 38.58 ± 3.11^{b}) (1.91 ± 0.48^{a}, 2.28 ± 0.72^{ab})extract treated group showed a significant ($p<0.05$) decrease when compared to the control group (31.07 ± 1.31^{a} and 1.60 ± 0.30^{a}). There was no significant difference in the gastric pH of all the treated group when compared to the control. Gastric mucus significantly ($p<0.05$) decreased in the diseased group compared to normal control. There was a significant ($P<0.05$) decrease in Malondialdehyde (MDA) activity in both extract (25.58 ± 0.74^{c}, 28.17 ± 0.39^{d})(17.47 ± 0.87^{b}, 24.94 ± 0.15^{c})and a significant ($p<0.05$) increase in catalase activity (39.54 ± 1.42^{a}, 41.90 ± 1.12^{a}) (61.31 ± 1.46^{c}, 52.15 ± 8.37^{b}) in the extract treated group compared to the control group (11.14 ± 0.79^{a} and 68.49 ± 1.75^{d}). The histopathology of the stomach in the extract treated groupshowed slight and moderate ulceration when comparedto the control group. Chromatographic techniques were carried out on methanolic extract which showed the best gastro-protection. *In-vitro* antioxidant activity using 2,2-diphenyl 1-picryl

hydrazyl (DPPH) scavenging activity was carried out on the different fractions obtained from column chromatography, fraction one which had the highest DPPH scavenging activity was further partially characterized by Fourier Transform Infrared Spectrophotometer (FTIR), the fraction was found to contain hydroxyl groups, halides, alcohols, phenols alkenes, carboxylic acid and alkane functional groups. Therefore the result suggests that the leaves of *V. doniana*posses gastro-protective potential and antioxidant properties justifying their use in folk medicine.

1.0 CHAPTER ONE

1.1 Introduction

Gastric ulcer occurs mainly in the stomach. It is a major health hazard in terms of both morbidity and mortality. It is a multifaceted disease with a complex etiology that is not fully understood, it is considered something of medical enigma (Gary *et al*., 1992). Patients who suffer from this disease are normal or below normal secretors of pepsin and hydrochloric acid (Gary *et al*., 1992)

Gastric ulceration is one of the common diseases affecting millions of people in both developed and developing countries of the world. About 80% of the populations of the developing countries suffer from this disease (Moss and Sood, 2003). It is generally accepted that the disease develops as a result of an imbalance between the protective and aggressive factors of the stomach (Piper and Stiel, 1986). Predisposing factors of gastric ulcer include *Helicobacter pylori* infection, excessive use of non-steroidal anti-inflammatory drugs, cigarette smoking, stress and excessive alcohol consumption (Berenguer *et al.*, 2006).

The stomach is an organ of the digestive system, located in the abdomen just below the rib and on the left side. Swallowed food is squeezed down the oesophagus and pushed through a sphincter (small muscle ring) into the stomach, where it is mixed with powerful gastric juices containing enzymes and hydrochloric acid. The stomach is a muscular bag, so it can churn the food and break it down mechanically as well as chemically. Once the food is in the consistency of a smooth paste, it is squeezed through a second sphincter into the first part of the small intestine (duodenum). The lining of the stomach – the mucosa or gastric epithelium – is layered with multiple folds. Ulcers occur in this lining (Laine *et al.*, 2008).

The gastric mucosal barrier is the property of the stomach that allows it to contain acid, if this barrier is broken by acetylsalicylic acid (aspirin), the acid diffuses back into the mucosa where it can cause damage to the stomach itself, this is referred to as gastric mucosal damage. The barrier consists of three protective components which include:

i. A compact epithelial cell lining. Cells in the epithelium of the stomach are bound by tight junctions that repel harsh fluids that may injure the stomach lining.
ii. A special mucus covering, derived from mucus secreted by surface epithelial cells and Foveolar cells. This insoluble mucus forms a protective gel-like coating over the entire surface of the gastric mucosa. The mucus protects the gastric mucosa from auto-digestion by e.g. pepsin and from erosion by acids and other caustic materials that are ingested.
iii. Bicarbonate ions, secreted by the surface epithelial cells. The bicarbonate ions act to neutralize harsh acids.

Gastric secretions are secretions in form of juice produced by the stomach. They include mucus, hydrochloric acid, enzymes (pepsinogen) and some hormones (gastrin). Modern drugs which are currently used in the treatment and prevention of gastric ulcer (antacids, histamine antagonists, proton-pump inhibitors e.t.c.) are characterized by numerous side effects such as arrhythmias, hyperplasia and haematopoietic changes (Thirunavukkarasu *et al.,* 2009). More so, cases of high cost, relapses after treatment, drug interactions associated with the use of these drugs have further undermined the usefulness of these products. Thus, the development of new anti-ulcer

drugs and the search for novel therapy for gastric ulcer has been extended to products of natural origin (Hostettmamm *et al.,* 2000).

1.1.1 Symptoms of gastric ulcer

A dull and gnawing ache has been described to be the most common symptom (Linder and Wilcox, 2001), which comes and goes for several days or weeks and occurs 2 to 3 hours after meal. The pain appears in the middle of the night on empty stomach which can be relieved by food intake. Other symptoms include loss of appetite, weight loss, bloating, burning, nausea and vomiting. While some individuals experience only very mild or no symptoms at all (Roy *et al.,* 2001; Park *et al.,* 2009). Emergency symptoms include the bloody or black stools that may accompany sudden and persistent stomach pain. Bloody vomit or vomit that looks like coffee may also accompany (Pilotto *et al.,* 2000). There could be signs of serious problems such as perforation where the ulcer burrows through the stomach or duodenal walls and bleeding could be initiated when acid or other ulcer breaks a blood vessel. Obstruction may be caused when the ulcer blocks the path of food trying to leave the stomach (Asefa, 2000; Pilotto *et al.*, 2000).

The usefulness of plant in the management of several human ailments cannot be overemphasized. This is justified by the fact that medicinal plants have been proved to be effective and safer remedies for treating human diseases. About 90% of the African population still relies exclusively on plants as a source of medicine (Hostettmamm *et al.,* 2000), thus the need to validate this finding through the present research.

Aspirin is a potent non- steroidal anti-inflammatory drug (NSAID) that is used for the treatment of rheumatoid arthritis and related diseases as well as the prevention of cardiovascular thrombotic diseases. Gastric ulcer associated with the use of aspirin is a major problem. Many factors such as gastric acid and pepsin secretion, gastric microcirculation, prostaglandin E_2 (PGE_2) content (Laine *et al.,* 2008), and pro-inflammatory cytokines interleukin (IL)-1β and tumour necrosis factor (TNF)-α play important roles in the genesis of gastric mucosal damage, and its subsequent development (Wallace, 2008). It stimulates HCl secretion and cause weakness of mucous gel layer. NSAIDs block COXI activity and reduce gastric mucosal prostaglandin which results in decrease mucosal blood flow, decrease secretion of mucus and bicarbonate.

Vitex doniana, a member of *Verbenaceae* family is a medium-sized deciduous tree with a heavy rounded crown and a clear bole up to 5m. It is widely distributed in the Eastern and Western parts of Nigeria. The plant is commonly called Black plum (English), Dinya (Hausa), Ori-nla (Yoruba), and Ucha koro (Igbo).

Vitex doniana has numerous applications in traditional medicine. Various parts of the plants are potent in the management and treatment of several disorders (Atawodi, 2005). Locally, the leaf is acclaimed to have anti-ulcer effects while the paste made from the stem bark is used in wound healing (Burkill, 2000). Validation of these claims through scientific research has become extremely imperative as this will pave way for wider acceptability and usage of anti-ulcer therapy from this plant by the majority of the ever increasing human population and hence reduced incidence of gastric ulcer.

1.2 Statement of Research Problem

The incidence of gastric ulcer in both developing and developed countries are due to the incidence of many aggravating factors such as *Helicobacter pylori* infection, excessive use of non-steroidal anti-inflammatory drugs, stress, excessive alcohol consumption etc (Berenguer *et al.,* 2006). Although there are various drugs for the treatment of ulcer, they are not affordable to many poor people especially those in developing countries also the rural population in various parts of the world do not have adequate access to high quality drugs for the treatment of gastric ulcer, and so depend on plants for treatment. Therefore, there is need to seek for less expensive but effective treatment strategies.

1.3 Justification of the Study

Synthetic drugs used in the treatment and prevention of gastric ulcer have a number of pitfalls such as arrhythmias, gynaecomastia, hyperplasia and hematopoietic changes etc (Thirunavukkarasu *et al.,* 2009). Medicinal plants are effective in the treatment of human diseases. *Vitex doniana* is one of such plants which have been used to cure human diseases and have also been demonstrated to posses some level of in vitro trypanocial activity against *Trypanosoma brucei brucei* (Atawodi, 2005). Its leaves have been used locally in the treatment of ulcer. Thus, there is need to validate these findings as a way to boost acceptability and hence usage of anti-ulcer therapy from leaves of *Vitex doniana.*

1.4 Aim and Objectives

1.4.1 Aim

The aim of the study is to investigate the gastro-protective potential of the aqueous and methanolic leaf extract `of *Vitex doniana* against experimentally induced gastric ulcers.

1.4.2 Objectives

i. To evaluate the safety dose of *Vitex doniana* leaf extract on albino rats.

ii. To determine the gastro protective potentials of aqueous and methanolic leaf extracts of *Vitex doniana.*

iii. To determine the *in-vivo* antioxidant activity of both extracts

iv. To partially purify the extract with the best gastro protective activity using chromatographic method.

v. To determine the *in-vitro* antioxidant activity of the different fractions and to partially characterize the fraction with highest antioxidant activity.

1.5 Hypothesis

Vitex doniana leaf extract does not possess gastro-protective effect on aspirin induced gastric mucosal damage in albino rats.

CHAPTER TWO

2.0 Literature Review

2.1 Medicinal Plant

Medicinal herbs are an indispensable part of the traditional medicine, practiced all over the world due to easy access, low cost and ancestral experience. The indigenous system in medicine makes a substantial contribution to the public health in Nigeria and other developing countries; they formed the basis of health care throughout the world (Ahmad *et al.*, 2006). Millions of household

in rural and urban areas consume traditional diet, use home remedies and health customs based on principles of traditional medicine. In traditional medicine several plants and herbs have been used to treat gastrointestinal disorder, including peptic ulcer. Some of such plants which include:

Leucas lavandulifolia commonly known as Gumma belonging to the family *Labiatae*, reports have shown that the methanolic extract of this plant has anti-ulcer activity by the significant reduction in ulcer index as well as gastric acid output in Indomethacin and pylorus ligation induced ulcer models. The plant exhibited ulcer protection in a dose dependent manner (Jeetendra., *et al* 2010).

Research has also shown that ethanolic extract of *Momordica dioca* on pylorus ligature and aspirin induced ulcer produced a decrease in the ulcer number and increase in percentage of ulcer protection by 72.09% and that the activity of plant would probably be due to the compounds present in the plants such as flavonoids and triterpenes. The methanolic extract of *Solanum nigrum* has been reported to possess antiulcer activity on aspirin induced ulceration with respect to its antioxidant status in the gastric mucosa by exerting gastro protective effect by its free radical scavenging action (Aastha *et al.*, 2012).

Ethanolic extract of *Passiflora foetida has also been found to* p*osses'* antiulcer and antioxidant activities, it was shown to inhibit ulcer index, increase gastric pH and also reduce the lipid peroxidation and alkaline phosphatase levels when compared to toxicant group. Histopathological evaluation of alcohol and aspirin-induced ulcer models showed perforated ulcer, deep ulceration of granular epithelium and loss of the histological structure, almost

reducing the sub-mucosa but the extract showed partial healing of ulcer with few inflammatory cells (Sathish., *et al.,* 2011). Research on the ethanolic leaf extract of *Musa paradisiaca* has shown to also posse's gastro protective effect on gastric mucosa (Mbagwu *et al.,* 2011).

Another specie *Vitex negundo,* according to research done by Yakaiah *et al.*, (2013), it was reported that the ethanolic extract of leaves of *Vitex negundo* on pylorus ligature and aspirin induced ulcer, showed protective effect against total acid, free acid, gastric volume and ulcer index and also increases pH and percentage of protection against ulcers in both models and related its protective effect to the presence of flavonoids, terpenoids, tannins, and triterpene which are known as an anti-ulcer agent and their action has been mentioned to be due to activation of cellular proteins, reduction of mucosal prostaglandin metabolism, cytoprotective actions and reduction of gastric vascular permeability and their ability to scavenge free radicals.

A study on the methanloic extract of the stem bark of *Cissus quadrangularis* on aspirin induced ulcer model revealed that the plant promotes ulcer protection by the decrease in ulcer index, gastric secretions and increase in the glycoprotein level, gastric mucin content and non-protein sulphydryl (NPSH) concentration. They concluded that the protective effect of the extract may be due to its antisecretory and cytoprotective property (Mallika *et al*., 2006).

2.1.1 *Vitex doniana*

Vitex doniana commonly known as Black Plum or African olive (Glew *et al.*, 1997) belongs to the family Verbenaceae. It is one of the most abundant and widespread tree species occurring in savannah regions and West Africa. It comprises of about 150 species and is pantropical with a few species in temperate regions. Its importance can be attributed to its statuses a multiple-use plant. *Vitex doniana* is a tree plant with a chromosome number 2n=32 (Burkill., 2000). It is one of the most abundant and widespread tree species occurring in savannah regions often in wet areas along rivers and on termite mould up to 200m altitude. It occurs in regions with mean annual rainfall of 750-2000mm (Burkill., 2000). it is commonly found in alluvial soil. In central Africa it is often the first species to be established when gallery forest evolves in low lying areas in the savanna. They are found in northern, eastern and western Nigeria.

Earlier reports have shown that aqueous root bark of the plant can be used for the treatment of anemia (Abdulraham *et al.*, 2010), methanolic stem bark extract can be used for the treatment of gastroenteritis, diarrhoea, dysentery (Kilani, 2006) and aqueous leaves and stem bark extract for the treatment of liver disorder (James *et al.*,2010).

2.1.2 Description of the plant

Vitex doniana is a deciduous tree, usually 4-8m high, occasionally up to 15m, with a dense rounded crown. Its bark is light grey with numerous vertical fissures and branchlets which is not hairy but rather smooth. The leaves are long stalked with 5-7 leaflets which are usually widest towards the tip and more or less hairless. The flowers are numerous, white, tinged purple, 3-12cm in diameter usually borne in short, stout axillary cymes on a long stalk. The calyx and pedicels are densely hairy. The fruits are ellipsoid to oblong, up to 2.5cm long, clasped by a

calyx cup, they are usually green when young, turning purplish-black on ripening with a starchy black pulp. (Maundu *et al.,* 2005).

Vitex doniana has been used as a source of food. The cooked young leaves are eaten as a vegetable or used in sauces. The blackish pulp of the fruits is edible and sweet, and eaten raw, it is often used to make jam. A beverage is made from the fruit juice, and boiled fruits are the basis for alcoholic liquor and wine (Okigbo, 2003). The seeds inside the fruit stone are also edible. Research conducted by Egbekun, *et al.,* (1996) was reported that syrup similar to honey was produced from the fruit and that physicochemical and sensory results showed that it can be substituted for other syrups as a nutritive sweetener.

Apart from *Vitex doniana* been used as a source of food, it has been found to posse numerous applications in traditional medicine. The leaf sap is used as an eye drop to treat conjunctivitis and other eye complaints. A leaf decoction is applied externally against headache, stiffness, measles, rash, fever, chickenpox, and internally as a tonic and to treat respiratory diseases (Ky, 2008). The pastes of pounded leaves and bark are applied to wounds and burns. Leaf infusions are added to alcoholic drinks to make them stronger. A root decoction is administered orally to treat rachitis, gastro-intestinal disorders, jaundice, and as an anodyne (Ky, 2008). Powdered bark added to water is taken to treat colic, and a bark extract is used to treat stomach complaints and kidney troubles. Research done by Ladeji and Okoye, (1996) showed that the stem bark extract of the tree can be used for the control of hypertension and also posse anti – hepatotoxic effect, also Ladeji, *et al.,* (2005) reported its use against leprosy and liver diseases and the bark extract of the plant containing much more potassium and phosphate than calcium, magnesium, zinc and iron,

therefore, it was concluded that, the use of *Vitex doniana* to control postpartum bleeding after child birth was justified.

The dried and fresh fruits are eaten against diarrhea, and as a remedy against lack of vitamin A and B (Ky., 2008). In Ghana it is used for treatment of colds and cough in children and its bark in treatment of sterility (Abbiw, 1990). Various parts of the plant are used by traditional medicine practitioners in Nigeria in the management and treatment of several disorders which include rheumatism, hypertension, cancer, and inflammatory diseases (Olusola *et al.*, 1997; Owolabi *et al.*, 2011).

2.1.3 Reports on the Chemical constituent of *Vitex doniana*

According to research done by Agbede and Ibitoye, (2007) it was found that the mean values for the proximate composition of *Vitex doniana* fruits was found to contain: moisture 487.7±0.5, ash 52.7±0.1, fat 30.0±0.4, fibre 67.3±0.7, proteins 72.8±0.1 and carbohydrate 289.5±0.8 g kg^{-1}. Though it had lower vitamin C content when compared with other well known fruits (Lanntech, 2007), it is safe for consumption and could be a cheap source of raw material for juice production (Nnajiofor, 2003).

The macro-mineral contents of *Vitex doniana* fruits was found to contain: calcium, magnesium, sodium, potassium and phosphorus. These macro-mineral are present at higher values than those reported for some well known fruits and consequently the consumption of the fruit would help to meet the recommended levels of these minerals especially among the school children and adult weighing between 70-100 kg (Lanntech, 2007). It is also a rich source of crude fiber which is an

important part of diet which decreases serum cholesterol levels, hypertension, diabetes, colon and breast cancer (Ishida *et al.,* 2000).

Phytochemical reports on *Vitex* species indicate that they are rich sources of ketosteroids, iridiods, terpenoids and flavonoid glycosides (Ono *et al.,* 2000). The phytochemical analysis of aqueous leaves extract of *V. doniana* revealed the presence of alkaloids, terpenoids, flavonoids, saponins, tannins, phenols and absence of resins, steroids and glycosides (Ukwuani *et al.,* 2012) while that of ethanolic extract of the stem bark reviewed the presence of flavonoids, tannins, steroids, saponins, carbohydrate, terpenes and cardio-active glycosides (Abdurahaman *et al.,* 2012).

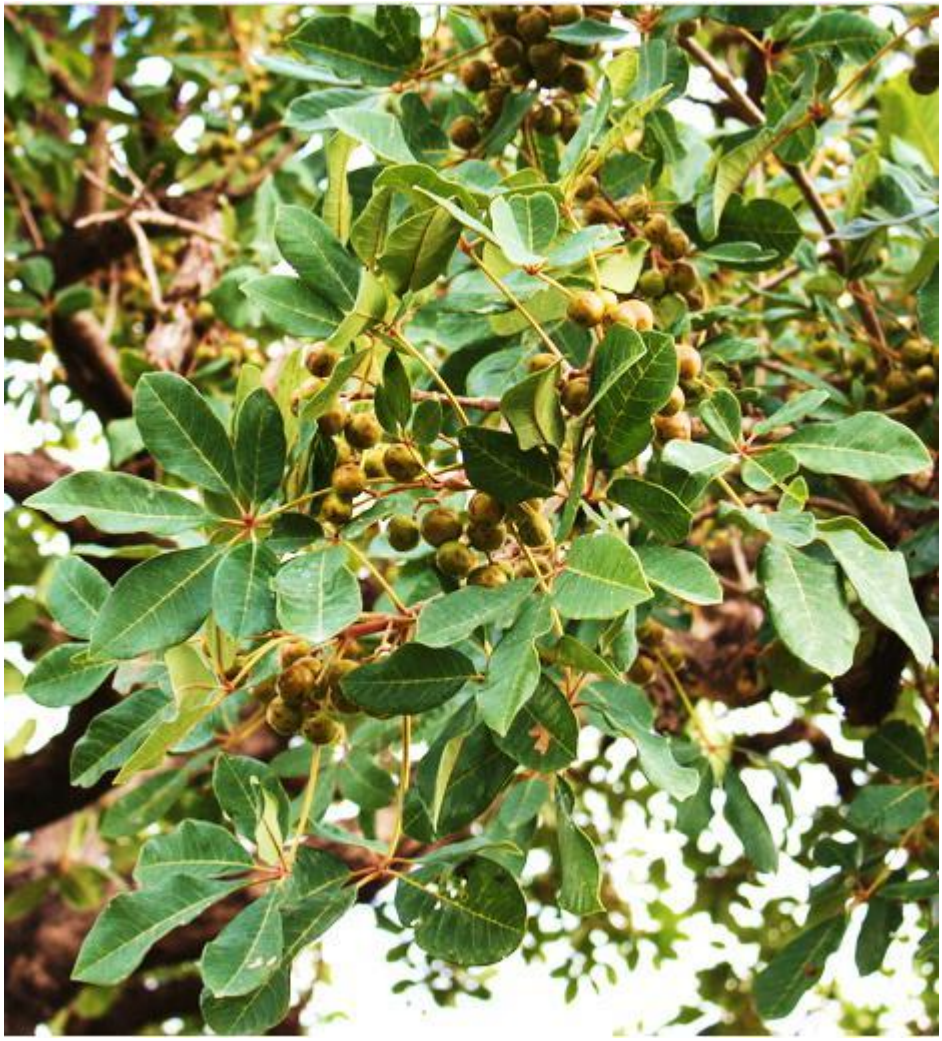

Plate 2.1: Photograph of *Vitex doniana* leaves collected from Basawa, Zaria, July 2014

2.2 Aspirin-A Non-steroidal Anti-Inflammatory Drug

Aspirin as well as other Non-steroidal anti-inflammatory drug (NSAIDs) are widely used for the treatment of pain and inflammation worldwide (Huang *et al.*, 1996). They are available over the

counter with widespread use for various indications. The use of aspirin is associated with well recognized risks of gastrointestinal toxicity (Singh and Triadafilopoulus, 1996). The various mechanisms of action of NSAID induced gastric injury include:

a. Reduction of the hydrophobicity of the mucous gel layer by changing the action of surface active phospholipids.
b. Suppression of prostaglandin synthesis.
c. Damage of the gastric epithelium by intracellular accumulation of these drugs in an ionized state.
d. Injury due to neutrophils adherence to the endothelium of gastric micro-circulation (Fromm, 1987).

While the above-mentioned mechanisms likely contribute to the toxicity of NSAIDs in the stomach and in the small intestine, where entero-hepatic recirculation of some NSAIDs leads to repeated exposure of the epithelial cells to these drugs, they are unlikely to be the sole mechanism for ulcer formation (Reuter *et al.*, 1997). For example, gastric ulcers occur when NSAIDs are administered parienterally. It is possible that an NSAID that is excreted in bile may reflux into the stomach and then cause damage to the epithelium. However, it has been demonstrated that aspirin, which is not excreted in bile can induce gastric ulcers when administered intravenously in cats (Whittle *et al.*, 1985; Brune *et a.l*, 1993). Further supporting an important contribution of nontopical actions of NSAIDs to ulcer formation is the observation that the incidence of significant gastric ulceration and bleeding is not appreciably reduced when the NSAIDs are enteric-coated to prevent direct contact of the NSAID molecule with the gastric

mucosa or when the NSAIDs are formulated as a prodrug that is inactive until metabolized in the liver (Carson *et al.*, 1987; Hawthorne *et al.*, 1991).

The most important of the systemic effects of NSAIDs, in terms of inducing gastric ulceration, is their ability to suppress prostaglandin synthesis. The evidence that this is the primary mechanism underlying the ulcerogenic effects of NSAIDs includes the following: (*i)* observations that prostaglandins modulate many components of mucosal defense. (*ii)* A good correlation between the degree of suppression of gastric prostaglandin synthesis by various NSAIDs at various doses, and their ability to induce injury in the stomach (Wallace and McKnight, 1993). (*iii)*. A good temporal correlation between the first manifestation of damage following NSAID administration and the suppression of mucosal prostaglandin synthesis (Wallace and McKnight, 1990). On the other hand, gastric prostaglandin synthesis can be markedly suppressed without ulceration ensuing (Wallace *et al.*, 2000). In all likelihood, therefore, it is the case that suppression of gastric prostaglandin synthesis renders the mucosa more susceptible to the damaging effects of luminal agents (acid, pepsin, ethanol, etc.) including in some cases, the NSAID itself. This notion is perhaps best supported by the observations that extensive epithelial damage, created by a topical irritant (hypertonic saline), would normally heal without any deeper mucosal injury or heamorrhage. However, administration of an NSAID, or even very brief interruption of mucosal blood flow, results in a rapid transformation of the superficial injury into heamorrhagic, erosive damage penetrating through the full thickness of the mucosa. This effect of NSAIDs can be prevented by administration of a prostaglandin (Wallace *et al.*, 1990). Apart from aspirin been a cause of mucosal damage there are other causes of mucosal damage, they include:

Heavy Cigarette smoking is a risk factor for gastric ulcer. Epidemiological and experimental studies have found a close association between cigarette smoking and peptic ulcer diseases in humans and animals (Piper *et al.,* 1982; Iwat *et al.,* 1995). The frequency of peptic ulcer in smokers is double that in non-smoker. Nicotine from cigarettes bas been thought to aggravate peptic ulcer formation (Endoh and Leung., 1994). However, there are numerous unidentified compounds present in cigarette smoke. The ulcerogenic activity of smoking may involve (1) reduction of prostaglandins (PG) E2 levels in human (Quimby *et al.,* 1986), it has been known that PG can protect the gastric mucosal barrier (2) neutrophil infiltration that induce microcirculatory abnormalities (Quimby *et al.,* 1986).

Helicobacter which was first discovered in 1982 was found to be an important factor in the pathogenesis of peptic ulcer diseases (Warren and Marshall., 1983). It was originally named *Campylobacter pyloric*, but later it was renamed as *Helicobacter pylori* because there are some characteristic features that distinguish it from other Campylobacter species (Kato *et al.,* 1992). The association of *H. pylori* infection and active chronic gastritis and peptic ulcer disease was well established for more than a decade. The relationships between *H. pyloric* and peptic ulcer and the importance of eradication of *H. pyloric* from the gastro duodena mucosa of patients with peptic ulcer were confirmed by numerous clinical trials. Nowadays, proton pump inhibitor (omeprazole) based triple therapy is the most commonly accepted eradication regime.

Bile is another cause of gastric mucosal damage. It disrupts the gastric mucosal barrier and causes back-diffusion of hydrogen ions. The constant bathing of the gastric mucosa with bile results in chronic gastritis and the development of gastric ulcer (Schindlbeck *et al.,* 1987).

Gastric hypo-secretion and poor sphincter formation of pylorus may explain increased gastric bile acid concentrations in the fasting stomach of patients with gastric ulcer (Schindlbeck *et al.*, 1987).

2.3 Secretion of Hydrochloric acid

The parietal cells secretion is an isotonic solution of essentially pure HCl that contains 150mEq of chloride ions (Cl^-) and 150mEq of proton/hydrogen ions (H^+) per liter. The pH of this solution could be as low as 0.87, the concentration of H^+ being a million times higher than that of plasma (Altman, 2001). Carbonic anhydrase enzyme has been found to be abundantly present in the gastric parietal cells which combine carbon dioxide and water forming carbonic acid from where bicarbonate ion (HCO_3^-) is exchanged with plasma chloride. Hydrogen ion is pumped out against the concentration gradient into the gastric lumen by H, K-ATPase that is located in the apical membrane of the parietal cells. The parietal cells are polarized with their basolateral membrane in contact with interstitial fluid and the apical membrane facing the lumen of gastric glands. Energy is required to pump H^+ out of the parietal cells in exchange with K^+ and this is produced by hydrolysis of ATP. The formation of cyclic-AMP in the process activates expulsion of Cl^- along with H^+ into the gastric lumen through channels (Guyton, 1991).

2.4 Mucosal Defense

Mucosal defense is a term used to describe the various factors and components that permit the mucosa to remain intact despite its frequent exposure to substances with a wide range of temperature, pH, and osmolarity, as well as to substances with detergent or cytotoxic actions, and bacterial products capable of causing local and systemic inflammatory reactions .It is important

to realize that the gastric mucosa is not impervious to damage by these agents. The use of the term *gastric mucosal barrier* has often been misconstrued to suggest that this tissue is impenetrable. Mucosal injury occurs regularly, but does not lead to clinically significant disruption of the function or even the "barrier" properties of the tissue. The reasons for this include the fact that there are several layers to mucosal defense, with secondary components becoming more important when more superficial components are breached, and because of a very rapid process of repair when damage to the epithelium occurs (Wallace and Granger, 1996). Moreover, the various components of mucosal defense can be modulated by a number of endogenous substances, including prostaglandins. The net result is that the systemic circulation is protected from invasion by microbes, microbial products, and other toxins. On the other hand, there are certain circumstances in which mucosal defense is impaired, such as after administration of NSAIDs, thereby rendering the mucosa more susceptible to injury. The various levels of mucosal defense can be viewed in a structural sense, starting at the lumen and moving into deeper levels of the tissue. Which of these components will participate in a defensive response depends on the intensity of the challenge and the extent to which any toxin is able to diffuse into the mucosa.

2.4.1 Luminal factors

Gastric juice contains a number of elements capable of reducing bacterial colonization of the stomach, including acid, immunoglobulin, and lacto-ferrin. Few microbes can survive in the acid secreted by the stomach. The importance of acid as a defensive factor is evident from the observations that hypochlorhydria and achlorhydria increase the risks and exacerbate the severity of bacterial and certain parasitic infections (Giannella *et al.*, 1973). Bacterial count in the

stomach and duodenum is inversely related to the level of gastric acid secretion (Gray and Shiner, 1967). The mucus that is secreted on the surface of the stomach acts both as a lubricant, to reduce physical damage to the epithelium by ingested materials, and as a trap for bacteria (Belley *et al.*, 1999). Thus mucus can diminish the ability of bacteria to gain access to the epithelium. Ironically, it is the mucus layer in the stomach (primarily in the antrum) that is the site of colonization by *H. pylori* (Walsh and Peterson, 1995). Mucus performs an important structural role in creating an unstirred layer on the mucosal surface which supports maintenance of a near-neutral pH at that surface as well as acting as a physical barrier against luminal pepsin (Allen and Flemstrom, 2005).

Different forms of mucus produced by mucous neck and surface epithelial cells may contribute to the creation of a stable layer of mucus on the epithelial surface (Ho *et al.*, 2004; Morris *et al.*, 1984). Bicarbonate secreted by the epithelium can be concentrated within the surface mucus, creating a microenvironment with a pH closer to neutrality than that found in the luminal gastric juice (Chu *et al.*, 1999; Garner *et al.*, 1984).

Mucus has been suggested to retard the diffusion of protons which would further aid in maintaining a favourable pH at the apical surface of the epithelium. Lichtenberger and colleagues in 1983 demonstrated that the surface of the stomach is hydrophobic, and therefore a barrier to acid back-diffusion, because of the presence of a surfactant-like layer of surface active phospholipids. Disruption of this layer using aspirin or bile salt resulted in elevated diffusion of acid into the mucosa and to mucosal necrosis (Goddard *et al.*, 1990).

2.4.2 The epithelium

Several layers of mucosal defense, experimentally reducing the effectiveness of the mucus-bicarbonate layer on the epithelial surface does not usually result in epithelial damage (Wallace, 1989). This may be in part related to the inherent ability of gastric epithelial cells to remain intact and functional when continuously exposed to high concentrations of acid. Sanders *et al.,* 1985 demonstrated that the apical membrane of cultured cells was highly resistant to damage by acid. Exposure of the apical surface of these cells to a solution of pH 2 for more than 4 hours did not damage the cells. However, the basolateral membrane of these cells was very sensitive to acid, being damaged when exposed to a solution with a pH of only 5.5. These observations suggest that the apical membrane of gastric epithelial cells is highly resistant to high concentrations of acid. This hypothesis is supported by the findings of Boron *et al*., (1994), who performed studies using rabbit gastric glands, they concluded that the apical membrane of parietal and chief cells was exceptionally resistant to diffusion of hydrogen ions.

A further feature that makes the gastric epithelium resilient to injury is its relative "youth"; that is the human gastric epithelium is renewed every 2–4 days (Wright, 1984). The ability to replace older cells on a continuous and rapid basis without there being a significant break in epithelial continuity and barrier function can be attributed to the process of extrusion of cells as they undergo apoptosis. The cells surrounding the apoptotic cell gradually pinch in at the base of that cell until the apoptotic cell is no longer attached to the basement membrane (Harding and Morris, 1977).

2.4.3 Mucosal blood flow

With adequate vascular perfusion, epithelial damage does not generally progress to necrosis of deeper layers of the mucosa. The entire luminal epithelium can be destroyed in rat, and there is little macroscopic evidence of the injury, other than extensive mucus release. Microscopically, there is clear evidence of destruction of the epithelium, but remarkably, reestablishment of epithelial continuity can be seen within minutes to hours of induction of the damage. This rapid repair has been termed "restitution," and it involves the migration of healthy epithelial cells from the gastric pits over the denuded basement membrane (Lacy and Ito, 1984). This is another element of mucosal defense for which there is good evidence of modulation by prostaglandins. Even in the presence of very high levels of hydrochloric acid in the stomach (i.e., pH <1), the pH within the mucoid cap can be maintained close to neutrality. As the basement membrane is highly sensitive to damage by acid, this protection is crucial to permit restitution to occur. The maintenance of the relatively high pH microenvironment is dependent on undisturbed mucosal blood flow. If blood flow to the stomach is interrupted, then the pH within the mucoid cap drops precipitously and heamorrhagic lesions form. The gastric mucosa can be exposed to high concentrations of acid without significant epithelial injury occurring. Part of the reason for this is that the mucosal vasculature responds very quickly to the presence of acid in the superficial mucosa, so as to buffer, dilute, and remove the acid (Bruggeman *et al.*, 1979).

2.4.4 Inflammation

Superficial injury to the gastric mucosa also triggers an acute inflammatory response, characterized by increase in blood flow, as well as plasma exudation and recruitment into the

mucosa of leukocytes. The objective of this response is to minimize tissue injury, facilitate repair of damaged tissue, and prevent entry into the systemic circulation of foreign substances, including microbes and microbial products (Wallace and Granger, 1996). This inflammatory response is coordinated via the release of an array of soluble mediators, from cells such as mucosal mast cells that act as "sentinels" within the mucosa, while the acute inflammatory response is aimed at reducing mucosal injury, there are circumstances in which this response can be deregulated and can contribute to mucosal injury. Interestingly, NSAIDs can trigger some of the elements of an acute inflammatory response, and this contributes to their ability to cause mucosal injury (Wallace and Granger, 1996).

2.4.5 Ulcer healing

When the above-mentioned components of mucosal defense are insufficient to limit injury to the mucosa, an ulcer is formed. Repair of ulcers is a highly regulated and complicated process that involves inflammation, cell proliferation (particularly at the ulcer margin), formation of granulation tissue at the base of the ulcer, and angiogenesis (new blood vessel growth). In response to ulceration, a new type of cell appears in the ulcer margin which secretes large amounts of epithelial growth factor (EGF), acting as a potent stimulus for re-epithelialization (Wright *et al.*, 1990). Glandular structure is gradually reestablished, along with the mucosal microcirculation. Platelets contribute significantly to ulcer healing, at least in part through the delivery of numerous growth factors that can promote angiogenesis and epithelial cell proliferation (Wallace *et a.l*, 2006). Some of the clinical benefit of drugs that suppress gastric acid secretion may be related to a facilitation of platelet aggregation.

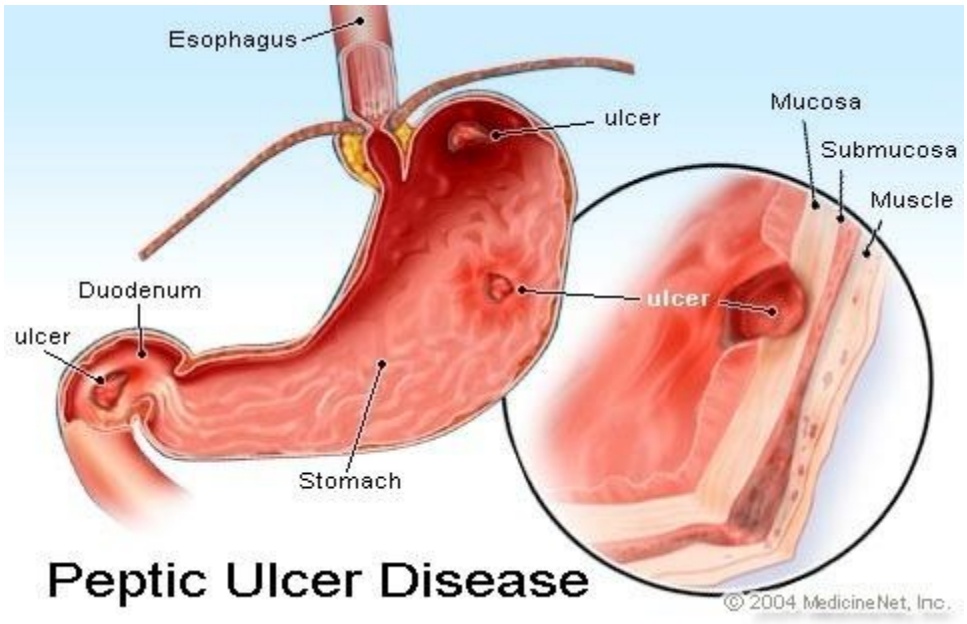

Figure 2.1: Stomach showing peptic ulcer disease (medicinenet.inc. 2004)

2.5 Diagnosis of Gastric Ulcer

2.5.1 Endoscopy

This is a diagnostic approach that makes use of an endoscope; the procedure is used to evaluate the oesophagus, stomach and duodenum. It is a thin flexible tube with a light source and video camera on one end that can be passed through the mouth and throat in order to look at the inside of the upper digestive tract. The video camera attached to the endoscope projects images on a computer screen that allows the doctor to see ulcers, tissue growth, and other possible problems. When combined with a biopsy, endoscopy is the most accurate procedure for detecting the presence of peptic ulcers, bleeding and stomach cancer, or confirming the presence of *H. pylori*.

2.5.2 Breath test

This test can be used to monitor the effects of ulcer treatment as well as to diagnose the presence of *H. pylori*. Urea labeled with carbon 13-C is given, *H. pylori* produces urease, which will break down the urea in the test dose to ammonia and carbon dioxide containing the labeled carbon. The carbon dioxide containing the labeled carbon can then be detected in the patient's breath.

2.5.3 Barium contrast x-ray

In this process, liquid form of barium is given to patient to take by mouth or is injected in the stomach and duodenum portions, after which imaging is done. White barium liquid creates a contrasting effect, which helps in getting clear x-ray images. This procedure is useful to identify the size and severity of the ulcer. Also duodenum ulcer that are difficult to observe by means of endoscopy are diagnosed with barium contrast x-rays.

2.5.4 Blood test

Blood tests are used to measure antibodies of *H. pylori* and results are available in minutes. Diagnostic accuracy is reported to be 80-90%. One of such important test is called enzyme-linked immunosorbent assay (ELISA).

2.6 Treatment and Management of Gastric Ulcer

Most anti-ulcer drugs target gastric acid secretion and mucosal defense mechanisms. Classes of these drugs include proton pump inhibitors, histamine (H2) receptor antagonist, antibiotics (amoxicillin, tetracycline) and cytoprotective agents (misoprostol).

2.6.1 Histamine receptor antagonist

The most commonly used H_2 antagonist includes cimetidine, ranitidine, famoditine; they are analogs of histamine which competitively inhibit acid secretion by eliminating the direct and synergistic influence of histamine on gastric and acetylcholine-stimulated acid secretion. The mechanism of action of these drugs involves blocking of the parietal cells which make the acid in the stomach. These cells found in the stomach lining are stimulated in a number of ways to produce acid, one way is by histamine. These drugs block the action of histamine on the parietal cells. The increased intra-gastric pH associated with the effect of H2-receptor antagonist may reduce absorption of drugs that require an acid medium for dissolution or absorption (e.g. ketoconazole ampicillin). Despite the differences in the potencies of the H2 antagonist, these drugs have been shown to be equally effective in promoting mucosal healing (Matz, 2006).

2.6.2 Proton pump inhibitors

Proton-pump inhibitors (PPIs) are a group of drugs whose main action is a pronounced and long-lasting reduction of gastric acid production. They are the most potent inhibitors of acid secretion available. Proton pump inhibitors act by irreversibly blocking the hydrogen/potassium adenosine triphosphatase enzyme system (the H^+/K^+ ATPase, or, more commonly, the gastric proton pump) of the gastric parietal cells (Zajac, 2013). The proton pump is the terminal stage in gastric acid secretion, being directly responsible for secreting H^+ ions into the gastric lumen, making it an ideal target for inhibiting acid secretion. Targeting the terminal step in acid production, as well

as the irreversible nature of the inhibition, results in a class of drugs that are significantly more effective than H_2 antagonists and reduce gastric acid secretion by up to 99% (Matz, 2006).

Unlike H_2 –receptor antagonists, renal impairment has minimal or no effect on the pharmacokinetics of proton pump inhibitors making dosage adjustments unnecessary. PPIs stop cells in the lining of the stomach producing too much acid. This can help to prevent ulcers from forming or assist the healing process. By decreasing the amount of acid, they can also help to reduce acid reflux-related symptoms such as heartburn. Proton pump inhibitors may diminish the absorption of drugs that require an acidic environment for dissolution of absorption. Omeprazole and pantoprazole are proton pump inhibitors (Matz, 2006).

2.6.3 Cyto-protective drugs

Antibiotics are prescribed in appropriate dosage to eradicate/kill the bacteria, example include metronidazole, tetracycline and amoxicillin (Podolski,1996). Misoprostol is a synthetic prostaglandin E1 analog which stimulates gastric mucosal defense mechanism and at higher dosages also inhibits gastric acid secretion. It has been shown to be as effective as other antiulcer drugs in healing GIT ulcers but its lack of a demonstrated advantage over H_2- receptor antagonists and side effects have prevented the use of misoprostol as initial antiulcer therapy.

2.7 Involvement of Reactive Oxygen Species in Gastric Ulceration

Uncontrolled hydrochloric acid secretion and ulceration in the stomach due to various factors is a serious global problem today. Among various causes of gastric ulceration, lesions caused by stress, alcohol consumption, helicobacter pylori infection and use of NSAIDS, have been shown to be mediated largely through the generation of reactive oxygen species(ROS) especially hydroxyl radicals (OH). (Bandyopadhyay *et al*, 2002).

Antioxidants are substances that can lessen or combat the cellular damage done by free radicals. This definition explains the positive physiological role of many substances regarded as antioxidants such as superoxide dismutase, catalase, among others. Lipid peroxidation is one of the biochemically measurable processes by which free radicals causes membrane damage, cell damage and tissue injury. The level of antioxidant defense systems has been found to greatly decrease in disease states. Although oxidation reactions are crucial for life, they can also be damaging; hence, plants and animals maintain complex systems of multiple types of antioxidants, such as glutathione, vitamin C, and vitamin E as well as enzymes such as catalase, superoxide dismutase and various peroxidases. Low levels of antioxidants, or inhibition of the antioxidant enzymes, cause oxidative stress and may damage or kill cells. Reactive oxygen species, generated in the cells of aerobically respiring organisms due to many factors, have been implicated in pathogenesis of many human sufferings like Parkinsons, Alzheimer diseases and many other neurodegenerative conditions (Halliwell and Gutteridge, 1989). The seemingly paradoxical consequences of the beneficial and harmful effects of oxygen (O_2) have been shown for several decades (Halliwell and Gutteridge., 1984). While more than 95% of the O_2 taken in by the aerobic organism is fully reduced to water (H_2O) during the process of mitochondrial respiration, a small percentage (<5%) of the O_2 consumed is converted is semi reduced species,

i.e. the superoxide anion radical, hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-). These species are collectively referred to as reactive oxygen species (ROS) which can be highly toxic, and their interaction often with cellular macromolecules bring about oxidative damage (Sies., 1993).

Involvement of ROS in the pathogenesis of gastric ulceration was first evident from the studies on ischemia-reoxygenation induced gastric mucosal injury (Yuda, 1993). A growing body of experimental and clinical evidence suggests that gastric mucosal damage by ethanol, non steroidal anti-inflammatory drugs and by helicobacter pylori is mediated through reactive oxygen species (Phull *et al.,* 1995; Yoshikawa *et al.,* 1987). Moreover ROS may play an important role in gastric ulceration induced by several kinds of stress. ROS also decreases the level of endogenous antioxidants such as GSH, tocopherol and ascorbate, and make the mucosa more prone to oxidative damage (Phull *et al.,* 1995). The pathogenesis of gastric mucosal lesions by water immersion restraint stress and burn shock in rat is associated with increased lipid peroxidation (Yoshikawa *et al.,* 1986). Systemic administration of glutathione or superoxide dismutase prevents water immersion stress and drug induced ulceration. Cold restraint stress alters the level of various damaging and cytoprotective factors of rat gastric mucosa to cause gastric ulceration (Das and Banerjee., 1993). Although the involvement of ROS in gastric lesions caused by various types of stress has been reported, detailed investigation on the role of ROS in cold restraint stress induced gastric ulceration has been limited. Very few studies have been undertaken to show the causal role of any specific oxygen derived free radical in mediating gastric damage during stress. Stress induced gastric ulceration which is associated with increased lipid peroxidation and depletion of endogenous GSH is due to increased formation of O_2,

activation of SOD and inactivation of gastric peroxidase (GPO) - a condition suitable for generation of H_2O_2 and formation of more reactive hydroxide which causes antioxidant depletion and lipid peroxidation (Das *et al.,* 1997). The cyto-protective enzyme prostaglandins synthatase (PGS) has also been shown to be inactivated leading to decreased defense against the aggressive factors (Das and Banerjee., 1993). Lipid peroxidation caused by OH is increased in gastric lesions induced by ethanol, indomethacin, ischemia-reperfusion, water immersion, and burn shock (Vaananann *et al.,* 1991; Yoshikawa *et al.,* 1993).

Stress causes both sympathetic and parasympathetic stimulation of the stomach which induces an increased motility and muscular contraction leading to vascular compression and mucosal ischemia (Kitagaw *et al.,* 1979; Hass and Moss., 1973). Sympathetic stimulation also causes direct arteriolar vasoconstriction, thus greatly reduces the blood flow to the stomach leading to local hypoxia and near or actual ischemia. The ischemic condition increases the leakage of O_2 from mitochondrial electron transport chain and facilitates the availability of redox active copper or iron. Increased O_2 production leads to increased level of H_20_2 (by the action of SOD), which in conjugation with O_2 generates OH via the metal catalyzed Heber-Weiss reaction. Hydroxyl radicals thus generated oxidizes important cellular constituents such as structural and functional proteins, membrane lipids, and depletes glutathione. Lipid peroxidation causes loss of membrane fluidity, impaired ion transport and membrane integrity and finally loss of cellular functions (Smith *et al.,* 1987). Stress also causes inactivation of prostaglandin synthetase leading to decreased biosynthesis of prostaglandin-the master molecule for gastro protection against all forms of damage to the mucosa (Das and Banerjee., 1993).

Ingestion of ethanol is the predisposing cause of acute hemorrhagic gastric erosion in humans. Ethanol lowers the concentration of non-protein sulphydryls especially glutathione, thereby exerting ulcerogenic effect by increasing ROS formation (Szelenul and Brune., 1988; Pihan *et al.,* 1987).

Non-steroidal anti-inflammatory drugs (NSAIDs) such as aspirin, indomethacin, ibuprofen, which are commonly used as pain killer in the treatment of rheumatoid arthritis and many other acute and chronic inflammatory conditions cause gastric mucosal damage (Ivey, 1988). The best studied drug, aspirin inhibit prostaglandin synthesis, interferes with protective mechanism such as mucus and bicarbonate secretion, surface epithelial hydrophobicity and mucosal blood flow (Langman *et al.,* 1991). The changes permit back-diffusion of acid through the breached surfaces to destroy cells, capillaries and vein causing hemorrhagic ulcer. Enhancement of leukotriene synthesis by NSAIDs exhibits sufficient damaging effect. Aspirin also decreases mucosal ATP synthesis and cell turnover process (Ivey, 1988). The changes brought about by NSAIDs, as described above, in totality can induce gastric damage through the generation of ROS and inhibiting cell proliferation.

NSAID also inhibits gastric peroxidase and may increase mucosal H_2O_2 and OH level to cause oxidative mucosal damage (Vaananann *et al.,* 1991; Levi *et al.,* 1990).Anti-thyroid drugs especially mercaptomethylimidazole (MMI), which is used to treat hyperthyroidism is a potent inducer of gastric acid secretion. The effect is partially mediated through increased liberation of histamine and possibly through increased level of H_2O_2 by irreversible inactivation of gastric peroxidase (Bandyopadhyay *et al.,* 1993)

CHAPTER THREE

3.0 Materials and Methods

3.1 Materials

3.1.1 Chemicals and Reagents

Magnesium chloride, sucrose, alcian blue, methanol, thiobarbituric acid, hydrogen peroxide, trichloro acetic acid, Diphenylpicryl hydrazine (DPPH) were purchased from Sigma Chemical Company St. Louis U.S.A and all other chemicals were of analytical grade.

3.1.2 Equipments

The major equipment used for the study were dissecting set, electrical weighing balance, centrifuge (Labofuge 300 centrifuge-Heracus), spectrophotometer (Jenway 6305 spectrophotometer).

3.1.3 Experimental animals

A total of 49 apprently healthy Wistar rats weighing between 150-200g (6-8 weeks) old were obtained and kept in well aerated laboratory cages in the animal house, Department of Pharmacology, Ahmadu Bello University, Zaria. They were allowed to adjust to laboratory environment for a period of 2 weeks before the commencement of the experiment. The animals were fed with growers mash from the vital feeds company and water was provided *ad libitum* during the stabilization period. The animals were divided into extract treatment groups and the control groups.

3.1.4 Plant sample collection and identification

The leaves of *Vitex doniana* was collected from Zaria, Kaduna State and authenticated at the Herbarium unit in the Department of Biological Sciences, Ahmadu Bello University, Zaria where voucher number V/N1162 was obtained.

3.2 Methodology

3.2.1 Preparation of plant sample

The collected plant samples were rinsed in clean water and air dried at room temperature. The dried plant samples were pulverized into powder using mortar and pestle, the powder obtained was used to prepare the extracts.

3.2.2 Aqueous extract preparation

To 500 grams of powdered leaves, 2.5L portion of distilled water was added and stored at room temperature for 48 hours and shaken at intervals. At the end of the extraction, the crude extract was filtered using muslin cloth and Whatmann filter paper No.1. The aqueous extract obtained was concentrated to dryness at 45°C using water bath. The dried extract was stored in an air tight sample bottle until required for analysis.

3.2.3 Methanolic extract preparation

To 500 grams of powdered leaves, 2.5L portion of methanol was added and kept at room temperature for 48 hours and shaken at intervals. At the end of the extraction, the crude extract was filtered using muslin cloth and Whatmann filter paper No.1. The methanolic extract obtained was concentrated to dryness using rotary evaporator. The dried extract was stored in an air tight sample bottle until required for analysis.

3.2.4 Lethal dose studies

The median Lethal dose (LD_{50}) of the plant extract was done in-order to select a suitable dose for the evaluation of the effects of the extracts. This was done by the method described by Lorke, (1983). In the initial stage, rats were divided into 3 groups of 3 animals each and were treated with 10mg, 100mg and 1000mg of the extract per kg body weight orally; they were observed for 24hrs for signs of toxicity, including death. In the final phase, rats were divided into 3 groups of one rat each the animals were administered higher doses (1600, 2900 and 5000 mg/kg) of the extract and then observed for 24 hours for behavior as well as mortality. Then the LD_{50} was calculated by the formula:

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

D_0 = Highest dose that gave no mortality,

D_{100} = Lowest dose that produced mortality.

3.3 Animal Grouping

The rats were randomly divided into 7 groups, with seven animals per group. The extracts were reconstituted in distilled water, and administered orally on daily basis for 14 days.

Group 1: Normal control was fed normal chow and water *ad libitum.*

Group 2: Animals were administered a single dose of aspirin orally (400 mg/kg).

Group 3: Animals were given aqueous extract (250 mg/kg b.w)

Group 4: Animals were given aqueous extract (500 mg/kg b.w)

Group 5: Animals were given Methanolic extract (250 mg/kg b.w)

Group 6: Animals were given methanolic extract (500 mg/kg b.w)

Group 7: Animals were given standard drug Cimetidine (50 mg/kg b.w)

3.3.1 Induction of gastric ulcer

The animals were administered with both extract of *Vitex doniana* and the standard drug for 14 days, after which the animals were fasted 48 hours in separate cages with raised wide meshed wire bottom to avoid coprophagia (Basso *et al.,* 1983) to ensure complete emptying of the stomach, the animals were however allowed free access to water (modified method of Urushidani *et al.,* 1979). One hour before the experiment the water was removed from the cages and the rats weighed, and the extract administered accordingly.

3.3.2 Collection of gastric secretion

Under light anesthesia (Chloroform) the abdomen was shaved and midline incision was made. The duodenum was identified and the junction between the pyloric bulb and the duodenum was picked up gently. A pyloric ligature was made with a thread, care being taken to avoid damage to the blood vessels or traction on the stomach. The abdomen was then closed by suturing, cleaned thoroughly with saline. The animals (rats) were allowed to recover in their individual cages, after recovery aspirin was administered to the animals.

Four hours after the pyloric ligation, rats were euthanized by chloroform anesthesia. The abdomen was opened; the stomach was removed and washed with saline. The stomach was opened along the greater curvature and the gastric content drained in to a centrifuge tube.

3.4 Determination of Gastric Ulcer Parameters

3.4.1 Ulcer index

Gastric lesions in the glandular regions of the stomach were identified in the gastric mucosa as elongated black red lines parallel to the long axis of the stomach. The length (mm) of each lesion

was measured using a thread and ruler (mm) and lesion index was calculated by adding the length of all lesions in the fundic region of the stomach (Szabo *et al.,* 1985).

3.4.2 Preventive Index (PI)

This was calculated according to the method of (Hano *et al.,* 1976) as follows

$$P.I = \frac{\text{mean ulcer index (Aspirin group)} - \text{mean ulcer index (test group)}}{\text{Mean ulcer index (Aspirin group)}} \times 100$$

3.4.3 Determination of gastric juice volume

Gastric juice was collected and centrifuged at *2028g* for 10 minutes. The resulting supernatant was collected and volume determined and expressed in milliliter (Kikuko *et al.,* 1996).

3.4.4 Determination of gastric juice pH

One milliliter of the supernatant liquid was diluted to 10ml using distilled water. The pH of the solution was determined with the aid of a digital pH meter (Moore, 1968) modified by Safwan *et al.,* 2011.

3.5 Biochemical Analysis

3.5.1 Estimation of total acidity

Ten milliliter (10ml) of gastric juice sample from the stomach of the animal was pipetted into a 250ml conical flask 2-3 drops of phenolphthalein indicator was added and titrated against 0.1M

NaOH till a faint pink colour is obtained. The value obtained was used to gmeasure the total acidity using the formular below (Kulkarni, 2005).

$$\text{Acidity} = \frac{\text{Volume of NaOH x Normality of NaOH}}{0.1} \times 100\text{mEq/L/100g}$$

3.5.2 Estimation of gastric wall mucus

Gastric wall mucus was determined according to the modified procedure of Corne *et al* (1974). The glandular segments of the stomach was excised and weighed. Each segment was transferred immediately to 10 ml of 0.1% w/v alcian blue solution (in 0.16 M sucrose solution buffered with 0.05 ml of sodium acetate at pH 5). Tissue was stained for 2 h in Alcian blue and excess dye was removed by two successive rinses with 10 ml of 0.25 M sucrose, first for 15 and then for 45 minutes. Dye complexes with the gastric wall mucus were extracted with 10 ml of 0.5 M $MgCl_2$ which was shaken intermittently for 1 min at 30 min intervals for 2 h. Four milliliter of blue extract was shaken vigorously with an equal volume of diethyl ether. The resulting emulsion was centrifuged at 3600 rpm for 10 min and the absorbance of aqueous layer was recorded at 580nm and the amount of alcian blue extracted was calculated from the calibration curve as micrograms of alcian blue extracted per weight (g) of stomach glandular tissue.

3.6 In-vivo Antioxidant Activity

3.6.1 Catalase activity

Catalase activity was determined by the method described by Aebi (1984). Tissue homogenate 10µL (100-150 µg protein) was added to 2.8ml of 50Mm potassium phosphate buffer (pH of 7.0) in 3ml cuvette. The reaction was initiated by adding 0.1ml of fresh 30Mm $2H_20_2$ and the decomposition rate of $2H_20_2$ was measured at 240nm for 5mins on a spectrophotometer. A molar extinction coefficient of $0.041mM^{-1}cm^{-1}$ was used to measure catalase activity which was expressed as µmole H_20_2 decreased/min/mg protein. The catalase concentration was calculated as follows:

Catalase concentration = Absorbance / E

3.6.2 Lipid peroxidation by measuring the malondialdehyde (MDA) level

Lipid peroxidation as evidenced by the formation of TBARS was measured by the modified method of Niehaus and Samuelson (1968) and described by Akanji *et al* 2009. To 0.15ml of tissue homogenate, 0.25M sucrose solution was treated with 2ml of (1:1:1 ratio) TBA-TCA-HCL reagent (0.37%TBA, 0.25N HCL and 15% TCA) and was placed in water bath for 1h at 90^oC. The mixture was cooled and centrifuged at 3000rpm for 5mins. The absorbance of the pink supernatant was measured against a reference blank using spectrophotometer at 535nm. The MDA activity was calculated as follows:

Molar extinction of MDA = I.56 x 10^5 $cm^{-1}m^{-1}$

MDA concentration = Absorbance/ I.56 x 10^5 $cm^{-1}m^{-1}$

MDA activity (µmol/mg of protein) = MDA concentration/ mg of protein

3.7 Partial Purification and Fractionation of Extract

The extract that showed the best gastro protective effect (Methanolic extract of *Vitex doniana leaves*) was fractionated using a suitable solvent system (ethyl acetate, methanol and water).

3.7.1 Thin Layer Chromatography (TLC)

TLC was carried out to determine the best solvent system (ethyl acetate, methanol and water) to be mounted on the column chromatography. A thin layer chromatographic plate pre-coated with silica gel was used. The extract was dissolved and spotted on the plate. The plates were placed in the chromatographic tanks which contained the different solvent system (ethyl acetate, methanol and water) at a ratio of 10:1.5:1 to develop. Thereafter the plates were removed, air dried and sprayed.

3.7.2 Column chromatography

Silica gel was used as the stationary phase. The slurry was prepared by mixing silica gel and the solvent system (ethyl acetate, methanol and water). The column was eluted gradiently with the solvents and fractions of 20mls were collected and concentrated at room temperature. Thin layer was used to monitor the column and pool the fractions together.

3.8 In-Vitro Antioxidant Activity

The In-vitro antioxidant property of the partially purified pooled fractions was determined using 2,2-diphenyl 1-picryl hydrazyl (DPPH) scavenging activity according to the method of Chan *et al.,* 2007. Different dilutions of the methanolic extract (0.2-1.0 mg/ml) were prepared. DPPH solution was also prepared by dissolving 6.0mg of DPPH in 100 ml methanol. Then, 1ml of extract from each dilution was added into the test tube containing 2 ml of DPPH solution.

Control was prepared by adding 1ml of methanol to 2ml of DPPH solution. Ascorbic acid at various concentrations (0.02-0.1mg/ml) was used as standard. The mixture was shaken vigorously and was left to stand in the dark for 30 min. The absorbance of the resulting solution was measured spectrophotometrically at 517 nm. A blank was prepared without adding extract. IC_{50} (Inhibitory concentration to scavenge 50% free radicals) was also determined. Lower absorbance of the reaction mixture indicates higher free radical scavenging activity. All samples were analysed in triplicates. IC_{50} value denotes the concentration of sample required to scavenge 50% of the DPPH free radicals. The scavenging activity of each extract on DPPH radical was calculated using the following equation:

Scavenging activity (%) = (1– absorbance of test/absorbance of control) × 100

IC_{50} was calculated from equation of line obtained by plotting a graph of % inhibition against sample concentration (mg/ml).

3.9 Histological Analysis of the Tissues

Following 24 hours fixation in 10% formal-saline, the stomach tissue were dehydrated through ascending grades of alcohol (70%, 90%, 95% and absolute 98%) for 2 hours each. This was followed by clearing in xylene (to remove alcohol) and the tissues were subsequently passed through a molten paraffin wax (impregnation) for 2 hours. The tissues were then immersed in a mould containing molten paraffin wax, which was allowed to solidify (embedding) while the tissue was inside. The tissues trapped in the wax were then trimmed for sectioning in a microtome machine at 3 µm. The sections were then finally stained with the staining agents, haematoxylin and eosin, for microscopy (Fereshteh *et al.*, 2009).

3.9.1 Statistical analysis

Data were expressed as Means ± SD. The data was analysed using the analysis of variance (ANOVA). The differences in mean were compared using Duncan Multiple Range Test. $P < 0.05$ was considered significant.

CHAPTER FOUR

4.0 Results

4.1 Lethal Dose Determination of *Vitex doniana* leaf extract

There was no mortality within 24 hours after oral administration of 10, 100, 1000, 1600, 2900 and 5000 mg/kg body weight of extracts to the rats in both the first and second phases respectively as shown in Table 4.1. Therefore, the LD_{50} value was estimated to be > 5000 mg/kg making the plant safe for use

4.2 Anti-ulcerogenic Study

4.2.1 Effects of aqueous and methanolic leaves extract of *vitex doniana* on ulcer index Pretreatment with 250 mg/kg and 500 mg/kg of both aqueous(9.25 ± 0.96^{b},9.00 ± 2.28^{b})and methanolic (4.43 ± 1.72^{a}, 8.50 ± 1.73^{b}) extract significantly ($p<0.05$) decreased ulcer index when

compared with the control group as shown in Table 4.2 also there was significantly ($p<0.05$) higher ulcer index in the disease control group (17.50 ± 2.65) when compared to the normal control which had zero ulcer index showing that both extract was effective in reducing the ulcer index.

4.2.2 Effects of aqueous and methanolic leaves extracts of *vitex doniana* on preventive index

Pretreatment with both extract inhibited gastric ulceration. The lower dose of methanolic extract showed the highest preventive index (74.68%) which was quite close to that of the standard drug (75.71%) showing the protective ability of the extract against severe damage, as shown in Figure 4.1,

Table 4.1: Lethal dose determination of the leaf extracts of *Vitex doniana* in albino rats for 48 hours.

Group	Dose (mg/kg)	No of animals	Mortality 24hrs	Lethal dose (mg/kg)
Phase 1	10	3	0	> 1000
	100	3	0	> 1000
	1000	3	0	> 1000
Phase 2	1600	1	0	> 5000
	2900	1	0	> 5000
	5000	1	0	> 5000

Table 4.2: Ulcer index of animals pretreated with aqueous and methanolic extracts of *Vitex doniana*

Treatment groups N=7	Dose (mg/kg)	Ulcer index (mm)
Group 1(Normal control)	Normal saline	0
Group 2(Aspirin only)	400	17.50 ±2.65[c]
Group 3(Aqueous)	250	9.25 ±0.96 [b]
Group 4(Aqueous)	500	9.00 ±2.28[b]
Group 5(Methanolic)	250	4.43 ±1.72[a]
Group 6(Methanolic)	500	8.50 ±1.73[b]
Group 7(Cimetidine)	50	4.50 ±2.08[a]

Values expressed as mean ± Standard deviation. Values with different superscripts down the column differ significantly ($p<0.05$)

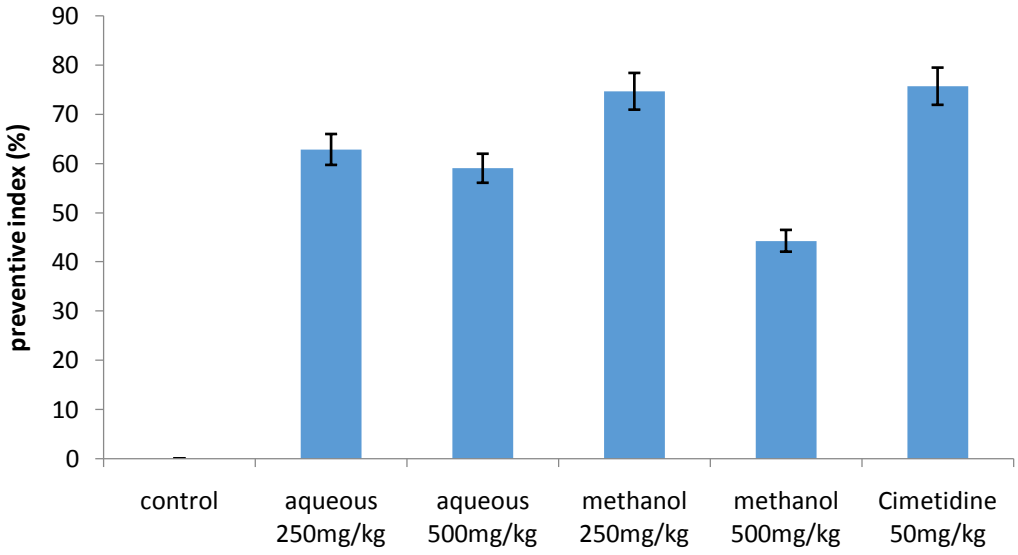

Figure 4.1: Effect of aqueous and methanolic extract of *Vitex doniana* on the Preventive index

4.2.3 Effect of aqueous and methanolic extract of *Vitex doniana* on total acidity

There was no statistical significant difference between the aqueous extract group of 500mg/kg (43.83±3.75) and the disease control group (44.92 ±3.76^{c}), but there was a significant ($p<0.05$) decrease in the 250mg/kg aqueous extract (36.88±2.92). There was a significant ($p<0.05$) decrease in both doses of methanolic extract (32.70±2.61, 38.58± 3.11) when compared to the control group (31.07±1.31) as shown in Table 4.3 showing the protective and reducing ability of the extract on total acidity against the mucosal lining

4.2.4 Effects of aqueous and methanolic leaves extracts of *Vitex doniana* on the gastric juice volume and pH.

There was a significant ($p<0.05$) increase in the gastric juice volume of the disease control group (3.78±0.84) when compared to the normal control group (1.60±0.30), but there was a significant ($p<0.05$) decrease in the gastric juice volume of both doses of aqueous extract (2.04±0.21, 2.96±0.66) and methanolic extract (1.91±0.48, 2.28±0.72) when compared to the control group (Table 4.4), it was shown that this might be due to the anti-secretory ability of the

extract to reduce the volume of gastric juice. There was no statistical significant difference in gastric pH of all the groups

4.2.5 Effects of aqueous and methanolic extracts of *Vitex doniana* on the gastric mucus

There was a significant ($p<0.05$) decrease in gastric mucus (1.21±0.20) of the disease control group when compared with the normal control group (3.22±0.26).

Table 4.3: Total Acidity of animals pretreated with aqueous and methanolic leaves extract of *V.doniana*

Treatment groups N=7	Dose mg/kg	Total acidity mEq/L
Group 1(Normal control)	Normal saline	31.07 ± 1.31^{a}
Group 2(Aspirin only)	400	44.92 ± 3.76^{c}
Group 3(Aqueous)	250	36.88 ± 2.92^{b}
Group 4(Aqueous)	500	43.83 ± 3.75^{c}
Group 5(Methanolic)	250	32.70 ± 2.61^{a}
Group 6(Methanolic)	500	38.58 ± 3.11^{b}
Group 7(Cimetidine)	50	30.17 ± 1.76^{a}

Values expressed as mean ± Standard deviation. Values with different superscripts down the column differ significantly ($p<0.05$)

Table 4.4: Gastric volume and pH of animals pretreated with aqueous and methanolic leaves extract of *vitex doniana*

Treatment groups N=7	Dose mg/kg	Gastric mucus ml/4hrs	Gastric pH
Group 1(Normal control)	Normal saline	1.60 ±0.30[a]	3.42 ±0.61[ab]
Group 2(Aspirin only)	400	3.78 ±0.84[c]	2.34 ±0.79[a]
Group 3(Aqueous)	250	2.04 ±0.21[a]	2.88 ±0.51[ab]
Group 4(Aqueous)	500	2.96 ±0.66[b]	3.01 ±0.41[ab]
Group 5(Methanolic)	250	1.91 ±0.48[a]	3.24 ±0.44[b]
Group 6(Methanolic)	500	2.28 ±0.72[ab]	2.94 ±0.48[ab]
Group 7(Cimetidine)	50	1 .82 ±0.39[a]	3.26 ±0.35[b]

Values expressed as mean ± Standard deviation. Values with different superscripts down the column differ significantly ($p<0.05$)

Table 4.5: Gastric mucus of rats pretreated with aqueous and methanolic extracts of *vitex doniana*

Treatment groups N=7	Dose mg/kg	Gastric mucus mg/g tissue
Group 1(Normal control)	Normal saline	3.22 ± 0.26^{cd}
Group 2(Aspirin only)	400	1.21 ± 0.20^{a}
Group 3(Aqueous)	250	2.89 ± 0.29^{c}
Group 4(Aqueous)	500	2.37 ± 0.72^{b}
Group 5(Methanolic)	250	3.04 ± 0.23^{c}
Group 6(Methanolic)	500	2.41 ± 0.22^{b}
Group 7(Cimetidine	50	3.48 ± 0.13^{d}

Values expressed as mean ± Standard deviation. Values with different superscripts down the column differ significantly ($p<0.05$)

However there was a significant increase in the gastric mucus of both doses of aqueous extract (2.89±0.29, 2.37±0.72) and methanolic extract (3.04±0.23, 2.41±0.22) respectively when compared to the control group, but there was no statistical difference in the lower dose (250mg/kg) of both extract when compared to the normal control (Figure 4.5) showing that the extract had the ability to increase mucus secretion and scavenge free radicals capable of causing damage to the lining of the mucosa.

4.3 In-vivo Antioxidant Activity

4.3.1 Effects of aqueous and methanolic extracts of *vitex doniana* on the MDA and catalase activity.

There was a significant ($p<0.05$) decrease in the catalase activity of the disease control group (36.81±0.90) when compared to the normal control (68.49±1.75), but there was no significant difference between the disease control group and both doses of aqueous extract treated group (39.54±1.42, 41.90±1.12). There was a significant ($p<0.05$) increase in both doses of methanolic treated group (61.31±1.46, 52.15±8.37) when compared to the control group (Table 4.6). In the MDA activity there was a significant ($p<0.05$) increase in the disease control group (34.69±1.54)

when compared to the normal control (11.14±0.79). However there was a significant ($p<0.05$) decrease in all the extract treated group when compared to the control group, the effect of these extract on this markers shows that the plant has antioxidant property.

Table 4.6: MDA and Catalase activity of animals pretreated with aqueous and methanolic extracts of *V. doniana*

Treatment groups N=7	Dose mg/kg	MDA µmol/mg protein	Catalase µmol/mg protein
Group1(Normal control)	Normal saline	11.14 ± 0.79^{a}	68.49 ± 1.75^{d}
Group 2(Aspirin only)	400	34.69 ± 1.54^{e}	36.81 ± 0.90^{a}
Group 3(Aqueous)	250	25.58 ± 0.74^{c}	39.54 ± 1.42^{a}
Group 4(Aqueous)	500	28.17 ± 0.39^{d}	41.90 ± 1.12^{a}
Group 5(Methanolic)	250	17.47 ± 0.87^{b}	61.31 ± 1.46^{c}
Group 6(Methanolic)	500	24.94 ± 0.15^{c}	52.15 ± 8.37^{b}
Group 7(Cimetidine)	50	11.25 ± 1.09^{a}	63.26 ± 0.67^{cd}

Values expressed as mean ± Standard deviation. Values with different superscripts down the column differ significantly ($p<0.05$)

4.4 In-vitro Antioxidant Activity

4.4.1 DPPH (2, 2-diphenyl 1-picryl hydrazyl) radical scavenging activity

The above results from this present study showed that methanolic extract had a better gastro-protective effect compared to aqueous extract. Methanolic extract was further fractionated using thin layer and column chromatography, 7 bands were obtained (Appendix IV) and bands close to each other were pooled together into 5 different fractions. The solvent system used was ethyl-acetate, methanol and water in a ratio of 10:1.5:1. DPPH Scavenging activity was carried out on the various pooled fractions.

Figure 4.2 shows the $1C_{50}$ (inhibitory concentration) of the various pooled fractions. DPPH radical scavenging activity of fraction 1 was considerably stronger than other fractions when compared to the standard (ascorbic acid). The lower IC_{50} value reflects a higher radical scavenging activity. The IC_{50} value for ascorbic acid was found to be 0.47 mg/ml, fraction one 0.52mg/ml, fraction two 0.86 mg/ml, fraction three 0.69mg/ml, fraction four 0.79 mg/ml, fraction five 0.75 mg/ml. Therefore fraction one showed a lower IC50 when compared to the standard, making fraction 1 a better free radical scavenger.

4.5 Histopathological Findings for Gastro-protective study

Histopathological examination carried out on the stomach tissues of the various groups' shows the photomicrograph of the stomach tissue of (A) the normal control group revealing a normal and intact mucosal layer. However, photomicrograph of (B) disease control group showed a stomach tissue with necrosis and disruption of the entire mucosa structure. Pretreatment with 250 mg/kg aqueous extract showed a deep ulceration on the mucosal lining. Rats pretreated with 500 mg/kg aqueous extract showed ulceration with necrosis of the apical mucosa and ulceration into the mucosa. Pretreatment with 250mg/kg methanolic extract showed a slight ulceration of the mucosal lining. Pretreatment with 500 mg/k.g of methanolic extract showed moderate ulceration of the stomach. Pretreatment with standard drug cimetidine 50 mg/kg indicated highly reduced ulcerations of the mucosal lining.

4.6 Partial Characterization of the Most active Fraction using Fourier Transform Infrared Spectrophotometer (FTIR)

The FTIR of fraction 1 where 11 peaks were present in which 8 of the peaks when compared with the standard library had close similarity indices with the following compounds Alkyl halides, Ethers, Alcohols, Alkenes, ketenes, Alkanes, Carboxylic acids and Amides showing the functional group present in the extract as shown in Table 4.7

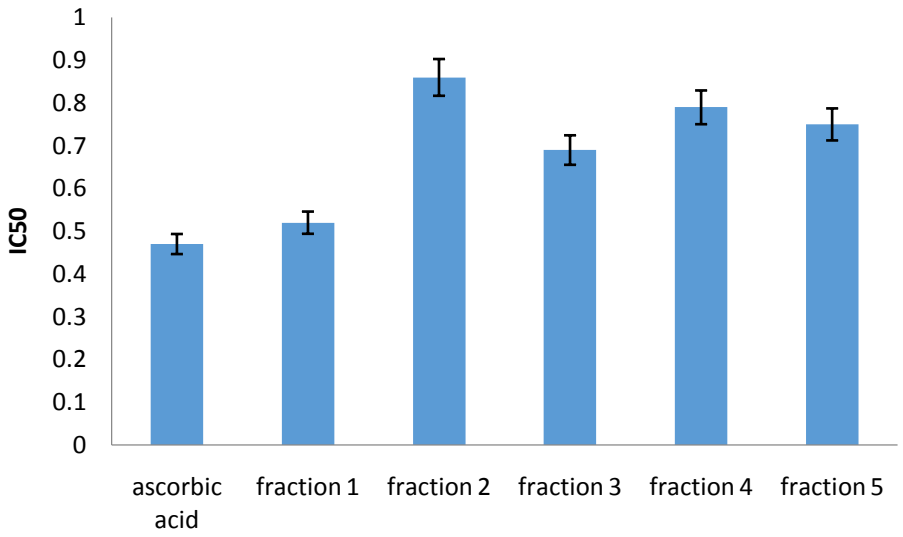

Figure 4.2: DPPH Scavenging activity of various fraction of methanolic extract of *Vitex doniana*.

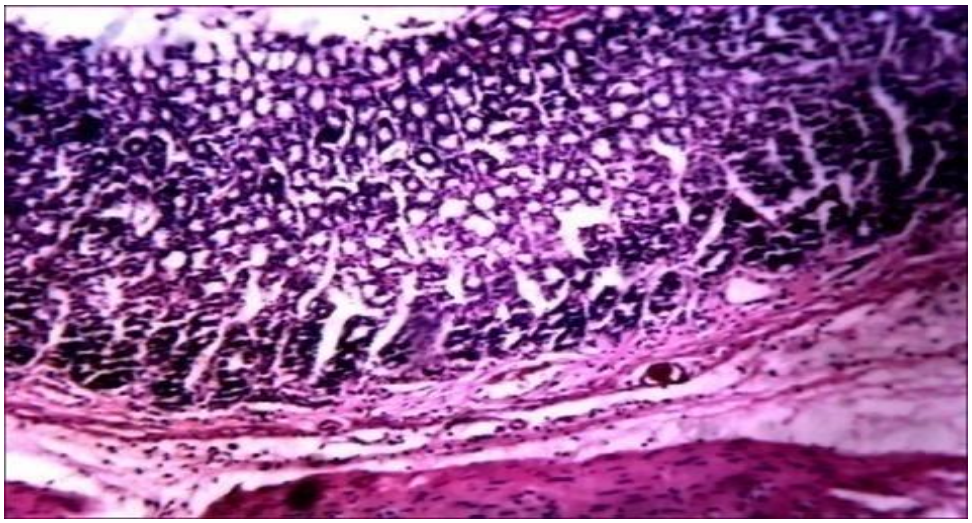

Plate 4.1A: photomicrograph of stomach tissue of normal control showing normal mucosa with intact architecture of the lining. (x100) H & E Stain

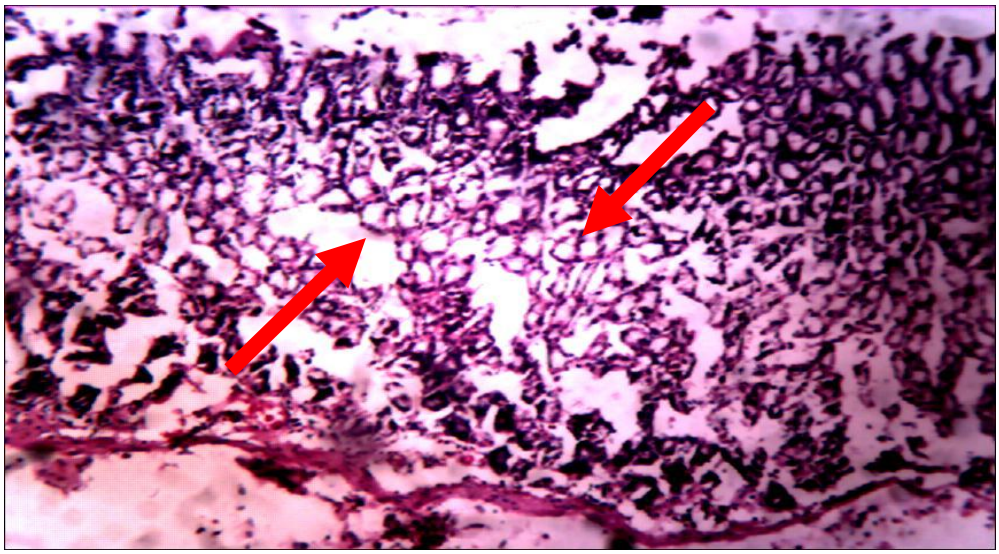

Plate 4.1B: photomicrograph of stomach tissue of aspirin induced damage (400 mg/kg) showing necrosis and severe disruption of the entire mucosal lining. (x100) H & E Stain

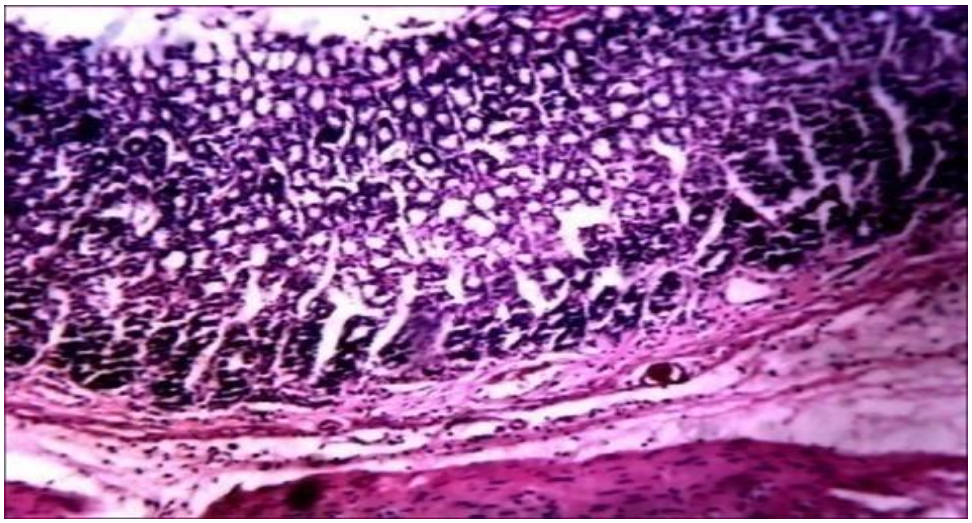

Plate 4.2A: photomicrograph of stomach tissue of normal control showing normal mucosa with intact architecture of the lining. (x100) H & E Stain

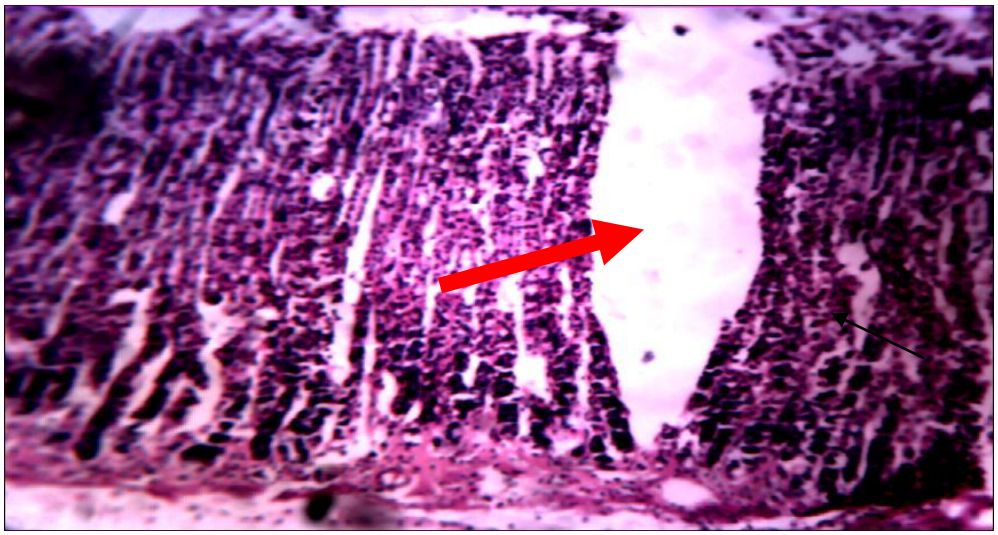

Plate 4.2B: photomicrograph of stomach tissue with treatment of aqueous extract 250 mg/kg showing protection but a deep ulceration on some areas of the mucosal lining without necrosis. (x100) H & E Stain

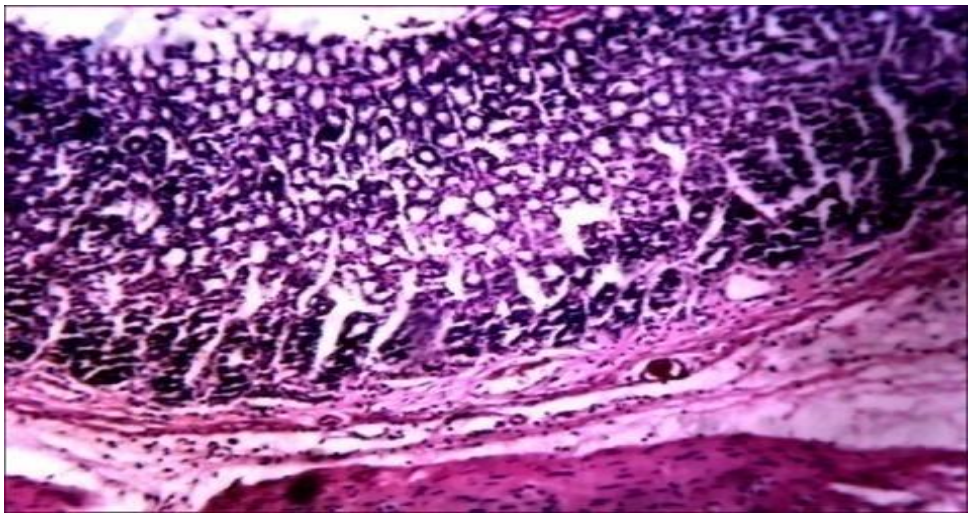

Plate 4.3A: photomicrograph of stomach tissue of normal control showing normal mucosa with intact architecture of the lining. (x100) H & E Stain

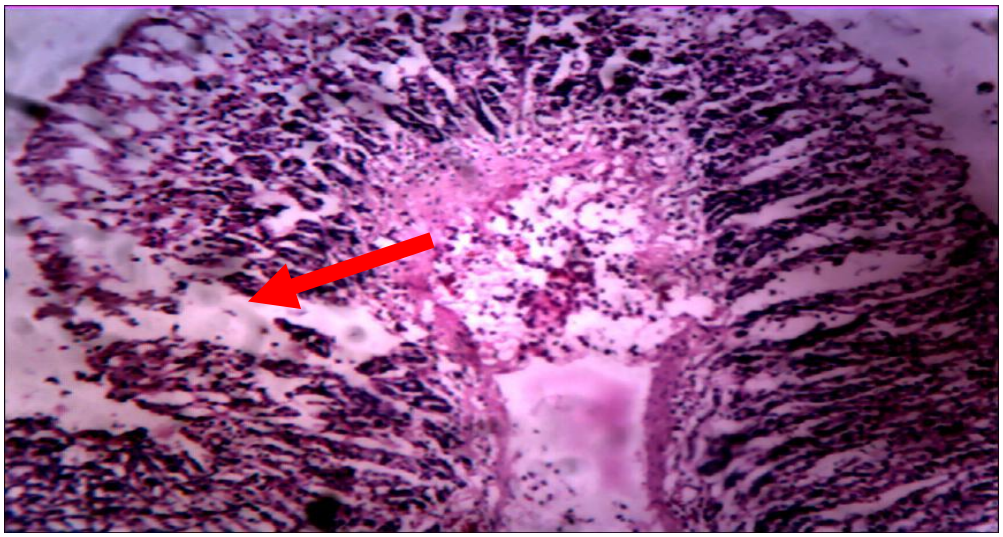

Plate 4.3B: photomicrograph of stomach tissue with treatment of aqueous extract 500 mg/kg showing partial protection but with slight necrosis of the mucosa lining. (x100) H & E Stain

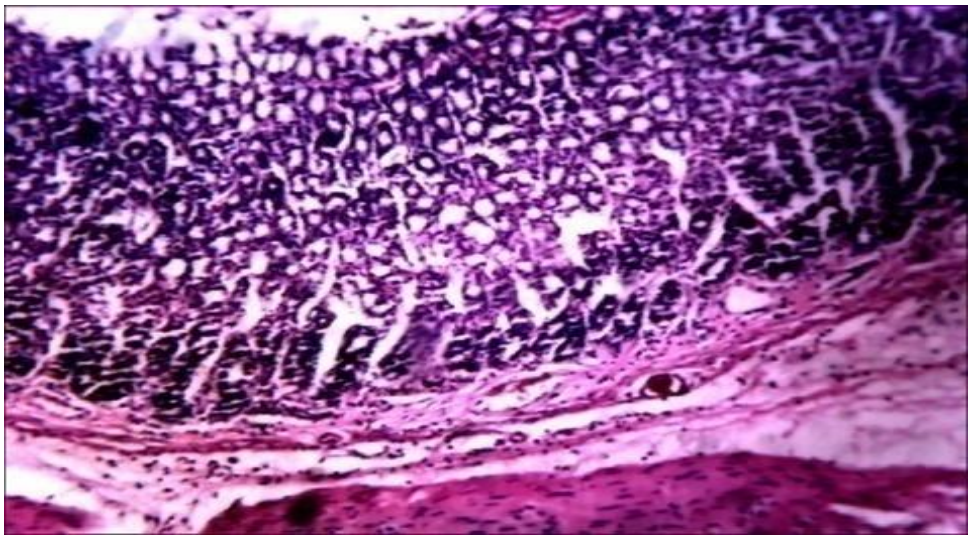

Plate 4.4A: photomicrograph of stomach tissue of normal control showing normal mucosa with intact architecture of the lining. (x100) H & E Stain

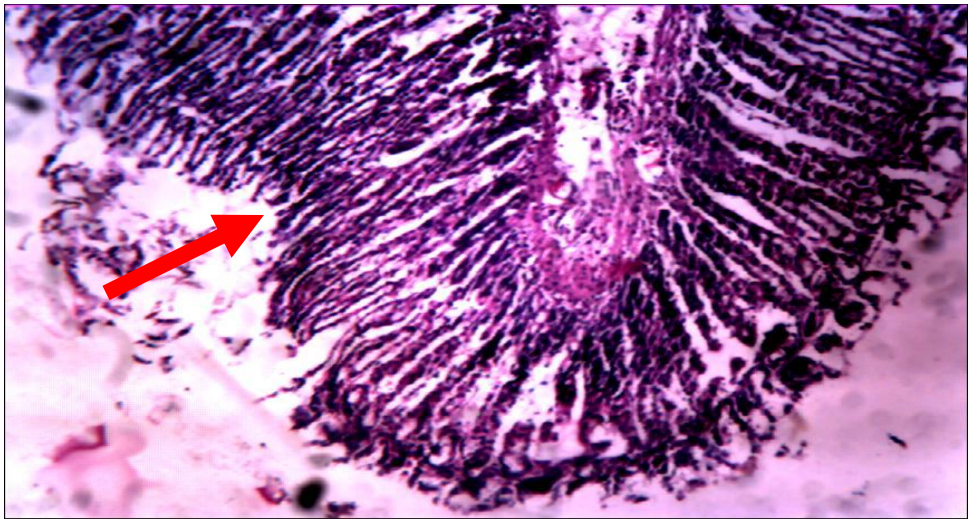

Plate 4.4B: photomicrograph of stomach tissue with treatment of methanolic extract 250 mg/kg, showing slight damage of the mucosal lining. (x100) H & E Stain

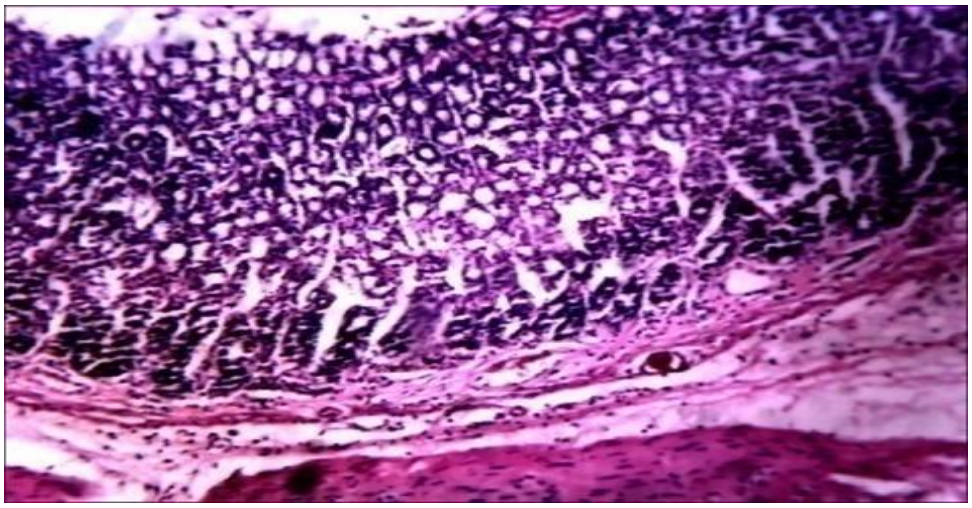

Plate 4.5A: photomicrograph of stomach tissue of normal control showing normal mucosa with intact architecture of the lining. (x100) H & E Stain.

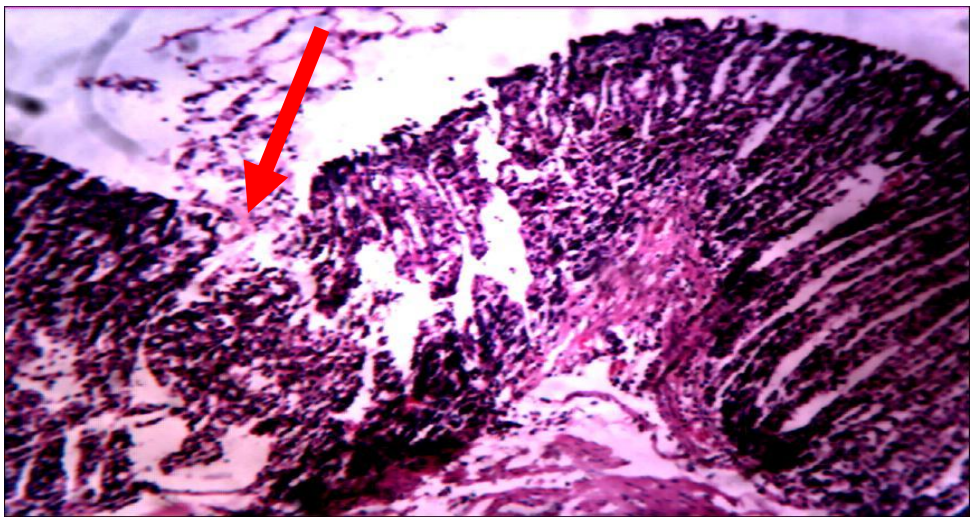

Plate 4.5B: photomicrograph of stomach tissue of treatment with methanolic extract 500 mg/kg, showing moderate ulceration and damage on the mucosa lining. (x100) H & E Stain

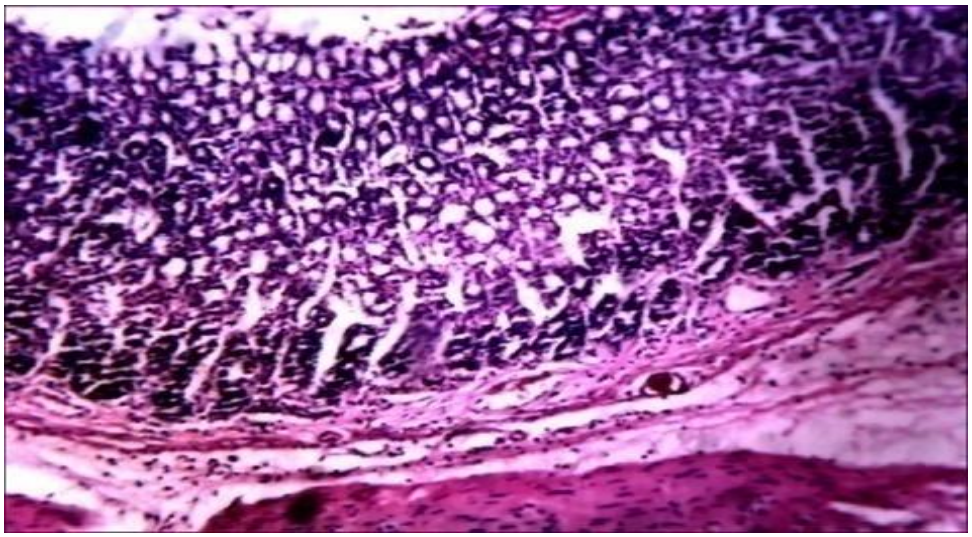

Plate 4.6A: photomicrograph of stomach tissue of normal control showing normal mucosa with intact architecture of the lining. (x100) H & E Stain

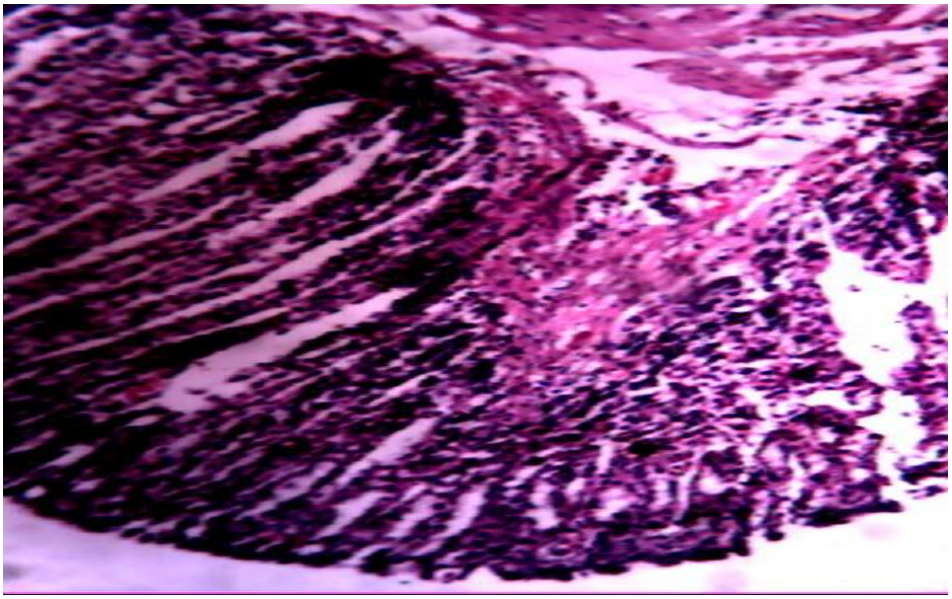

Plate 4.6B: photomicrograph of stomach tissue of treatment with standard drug cimetidine showing highly reduced ulcer area on the mucosal lining. (x100) H & E Stain

Table 4.7: Fourier Transform Infrared Spectrophotometer (FTIR) Output of Fraction 1 of methanolic extract of *Vitex doniana*.

Peak no	Absorbance	Class of compound	Functional group	Intensity
1	556.48	Alkyl halides	C-Br stretch	strong
2	1026.16	Alkyl halides	C-F Stretch	strong
		Ethers	=C-O-C	strong
3	1095.6	Alkyl halides	C-F Stretch	strong
		Ethers	C-O-C Stretch	strong
4	1240.27	Alcohols	C-O stretch	medium,strong
		Ethers	=C-O-C stretch	medium,strong
5	1442.8	-	-	-
6	1639.55	Alkenes	C=C	medium,weak
7	2076.44	Ketenes	X=C=Y	medium,strong
8	2346.48	-	-	-
9	2929.97	Alkanes	C-H stretch	strong
		Carboxylic acid	O-H stretch	strong,broad
10	3443.05	Amides	N-H stretch	medium
		Alcohols, phenols	O-H	medium
		Carboxylic acids	O-H stretch	strong,broad
11	3821.11	-	-	-

CHAPTER FIVE

5.0 Discussion

The use of non-steroidal anti-inflammatory drugs (NSAIDs) is considered to be the major risk factor in gastric ulcers. The mechanisms suggested for the gastric damage caused by NSAIDs are inhibition of prostaglandin synthesis and inhibition of epithelial cell proliferation in the ulcer margin, which is critical for the reepithelization of the ulcer crater (Levi *et al.,* 1990). There has been a considerable interest in finding natural antioxidants from plant materials to replace synthetic ones for effective management of therapeutic drug toxicity such as peptic ulcer (Pratt, 1992).

The non-lethal effects produced with the high dose of this extract are an indication that the leaf extracts of *V. doniana* is relatively safe on acute oral exposure. It can therefore be concluded that *V. doniana* leaf extracts is non-toxic which is in agreement with Bruce (1987), Nwogo *et al.,* (2013) and ASTME, (1987), that any chemical substance with LD_{50} estimate greater than 3000-5000 mg kg^{-1} (oral route) could be considered of low toxicity and safe, this is also in agreement with OECD (2001).

The phytochemical studies on the leaves of *Vitex doniana* have shown the presence of tannins, flavonoids, glycosides and saponins in the aqueous extract (James *et al*, 2013) and presence of alkakoid, carbohydrates and proteins in methanolic extract (Iwueke, 2006) among these secondary compounds, saponins and flavonoids are referred to as antiulcer compounds (Lewis and Hanson, 1991).

The observed protective effect and ability to reduce ulcer index in both extract treated group of the *V. doniana* is an indication of its vasoconstricting effect due to some phytochemicals (tannins) present in the plants. These results compared favourably with the gastric ulcer lowering effects of *Exoecaria aggallocha* (Thirunavukkarasu and Ramanathan, 2009) and the astringent action of tannins (Enechi and Nwodo., 2014). Free radical scavenging ability of flavonoids has been reported to protect the gastrointestinal tract from ulcerative and erosion lesion (Borelli and Izzo, 2000). Also the reduction in the ulcer index in the extracts pretreated group could be due to the antioxidant activity of the plant; this is in agreement with report of (Havsteen, 2002; Repetto and Llesuy, 2002).

The volume of acid present in gastric secretion which encompasses HCl, pepsinogen, mucus, bicarbonates, intrinsic factor and protein reflects acid volume. Exposure of unprotected lumen of the stomach to accumulating acid could facilitate ulceration (Olsen, 1988). Another major aggressive factor responsible for ulcers is the content of acid present in gastric juice. Over secretion of histamine contributes to increased secretion of gastric juice (Grossman, 1978). When the concentration of hydrogen ions in gastric juice decreases, it is reflective of high pH. The genesis of ulcer and gastric damage is facilitated by hydrogen ions which serve as another aggressive factor (Lullmann *et al.,* 2000). Decreased prostaglandin level impairs almost all aspects of gastro protection and increases acid secretions which, in turn, aggravate the ulcer (Miller, 1983). Histamine (H2) receptor activity stimulates adenylate cyclase system and in turn causes increases in calcium ion concentrations (Enechi and Nwodo., 2014), which ultimately leads to activation of proton pump and consequently leads to hyperacidity and ulcer (Al-Mofleh *et al.,* 2006). It is likely that protection by the extract against aspirin-induced gastric ulceration is

achieved by the suppression of acid secretion. Similarly, the extract blocked histamine-induced contractile responses in a similar fashion as the standard drug, cimetidine. The antagonism of histamine-induced contractile response by the extract seemed to suggest that the extract may possess inhibitory activity at histamine receptor sites. The anti-histamine effect of the extract could be due to one or several phytochemicals present in the plant extract. Flavonoids have been demonstrated to antagonize the effects of histamine which is a major mediator in ulcerogenesis (Sharma *et al.,* 1996). The result from this present studies is in line with earlier research by (Enechi and Nwodo., 2014), that the ethanol extract of *Buchholzia coriacea* seed was found to reduce the gastric secretion and acidity due to its ability to suppress the effect of histamine which is likely to play a role in the observed activity. The protective effect of the plant extract could also be due to its antisecretory potential and antioxidant activity this is in accordance with (Magaji *et al,* 2007).

Gastric mucus is an important protective factor for the gastric mucosa and consists of a viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoprotein that cover the entire gastrointestinal mucosa. Moreover, mucus is capable of acting as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals (Repetto and Llesuy, 2002). The protective properties of the mucus barrier depend not only on the gel structure but also on the amount or thickness of the layer covering the mucosal surface (Penissi and Piezzi, 1999). In this study, the decreased mucus secretion in the aspirin-administered rats indicated reduced ability of the mucosal membrane to protect the mucosa from physical damage and back diffusion of hydrogen ions. Mucosal damage can be easily produced by the generation of exogenous and endogenous active oxygen and free radicals (Naito *et al.*, 1995). An increase in mucus

production usually assists the healing process by protecting the ulcer crater against irritant stomach secretions (Hcl and pepsin) thereby enhancing the rate of the local healing process. Treatment with both extract protected the gastric mucosa from damage by increasing the mucus content this could be due to the free radicals scavenging property, some phytochemicals (flavanoids and the anti-secretory ability of the plant, once histamine production is reduced or impaired gastric volume reduces and gastric mucus increases which contribute in protecting the gastric mucosa from oxidative damage. this result is in accordance with earlier reports by Metowogo *et al* (2009), that hydroalcohololic extract *Aloe buettneri* possess antiulcer activity, increase the production of gastric mucus and significantly reduced ulcers induced by ethanol which may be attributed to the plants antisecretory and antioxidant properties, also reports by Ofem *et al* (2012) showed that the aqueous leaf extract of *Ocimum gratissimum* decrease gastric acid secretion and ulceration and also produced an increase in the gastric mucus secretion which might possibly be due to some phytochemical constituents like flavonoids ,alkaloids,oligosaccharides, phytates and tannins present in the leaf extract which tend to exhibit some anti-inflammatory and anti-oxidant properties.

The antioxidant enzyme system plays an important role in the defense of cells against oxidative damage. It has been reported that antioxidant properties of flavonoides from several plant extracts posses stimulatory action and exert a stimulatory action on transcription and gene expression of certain antioxidative enzymes (Sreelatha *et al.,* 2009).Lipid peroxidation can be used as an index for measuring the damage that occurs in membranes of tissue as a result of free radical generation. This might lead to aggravated tissue damage during stomach ulceration (El-Missiry *et al.,* 2001). These present study are in line with these previous data. Enhanced lipid

peroxidation (LPO) is a measure of membrane damage as well as alteration in structure and function of cellular membranes (Halliwell, 1995). In this present study, treatment with both extract significantly reversed the aspirin-induced changes in Malondialdehyde (MDA), these significant reduction in MDA level suggest decreased lipid peroxidation which might be due to the antioxidant properties of the plant against free radical generation and thus its antiulcerogenic activity. Similar observation was reported by Sathish *et al.,* (2011), that the ethanolic extract of *Passiflora foetida showed a* reduction in lipid peroxidation due to its antioxidant activity which may be one of the important defensive factors involved in its antiulcer effect, this is also line with research done by Tan *et al.,* (2013).

Catalase traps the harmful hydrogen peroxide and converts into water and oxygen. Catalase is a haemoprotein containing four heme groups, that catalyses the decomposition of H_2O_2 to water and O_2 and thus, protects the cell from oxidative damage by H_2O_2 and OH (Gupta *et al.,* 2004). The activity of CAT was found to be decreased in aspirin treated rats. The inhibition of CAT activity during aspirin induced ulcer may be due to the increased generation of reactive free radicals, which can create an oxidative stress in the cells. There was no significant difference between the aqueous pretreated group and the aspirin group. The administration of methanolic extract of *V.doniana* increased CAT activity showing excellent antioxidant properties when compared with the standard drug cimetidine.

Histopathological studies on the gastric mucosa revealed that aspirin administration induced a mucosal ulceration, associated with significant increase in lipid peroxidation. This was manifested as mucosa epithelial necrosis, and leukocytic infiltration. This effect on mucosal

oxidative stress and histological derangement was in accordance with the reports of (Valcheva-Kuzmanova *et al*. 2007 and El-Moselhy *et al*. 2009). *Vitex doniana* leaves extract had some protective effect against aspirin-induced inflammatory infiltration and congestion at the ulcer sites this may be due to its flavonoid content. Flavonoids could scavenge free radicals, inhibit lipid peroxidation, and increase prostaglandins and mucosal content of the gastric mucosa; showing cytoprotective effects (Alanko *et al.,* 1999), this is in accordance with works done by Sathish *et al* (2011).

2,2-diphenyl 1-picryl hydrazyl (DPPH) radical scavenging activity is one of the effective methods for evaluating the concentration of radical scavenging materials actively by a chain breaking mechanism (Maisuthisakul *et al.,* 2005). DPPH is a stable free radical at room temperature and accepts an electron or hydrogen radical to become a stable diamagnetic molecule (Soares *et al*.; 1997). The reduction capability of DPPH was determined by the decrease in its absorbance at 520 nm, which is induced by antioxidants. Results were reported as IC_{50}, which is defined as the amount of antioxidant required to inhibit 50% of DPPH free radicals under the experimental conditions (Antolovich *et al.,* 2002; Nanasombat and Teckchuen, 2009).

The fraction that required the lowest concentration to promote 50% of inhibition from the present study was fraction 1 of methanolic extract (Fig 4.2) when compared with the standard ascorbic acid this might be due to some phytochemicals and antioxidant property present in the plant. Similar report by Agbafor and Nwachukwu (2011), have shown that the aqueous and ethanol extract of *Vitex doniana* posses some phytochemicals, and has the ability to inhibit DPPH radical

and thus was used to evaluate their free radical scavenging activity, this is also in line with reports by Metowogo *et al* (2009) that a lower IC_{50} means better scavenger of free radicals. Earlier reports showed that the phytochemicals which might be responsible for the scavenging activity in this species are phenolic and flavonoid constituents (Rohman *et al.,* 2010; Masoumeh *et al.,* 2011). Phenols may not be solely responsible for the antioxidant activity (Kumbhare *et al.,* 2012). Polyphenols scavenging potential and metal chelating ability are dependent upon their unique structures, the number and position of the hydroxyl groups (Pazos *et al* 2005). It may be that the flavonoids in the methanolic leaves extract have the required structure which attributes to this result.

The Fourier Transform Infrared Spectrophotometer (FT-IR) analyses of fraction 1 demonstrated an intense peak at 3443.05 cm which is the characteristic absorption of hydroxyl groups, this might be responsible for the extracts hydrogen or electron release, hence their radical scavenging activity, this is in accordance with work done by Li *et al.,* (2007).

CHAPTER SIX

6.0 Summary, Conclusion and Recommendation

6.1 Summary

The lethal dose determination of *V.doniana* in albino rat showed no mortality within 48 hours after oral administration, the LD_{50} value was estimated to be > 5000mg/kg body weight.

The results of the study showed that administration of both extract significantly ($p<0.05$) reduced ulcer index when compared to the aspirin untreated group.

Ulcer inhibitory effect of both extract was not dose dependent as lower dose of methanolic extract showed a higher percentage inhibition when compared with other doses of both extract.

Total acidity was significantly ($p<0.05$) reduced in all the extract treated group but 250mg/kg b.w of methanolic extract was most effective in reducing the total acidity when compared to the normal control and therefore provide the best gastro-protective activity.

Pretreatment with both extracts significantly ($p<0.05$) reduced the gastric juice volume when compared to the aspirin untreated group.

Difference in pH of gastric juice in all groups was not significant ($p>0.05$)

The effect of both extracts of *vitex doniana,* significantly ($P<0.05$) reduced MDA, both doses of methanolic extract significantly ($P<0.05$) increased CAT in all the treated groups compared to the aspirin untreated group, but there was no significant difference in CAT activity between the aqueous extract and the untreated group.

The effect of both extract, significantly ($p<0.05$) increased gastric mucus in all the treated group compared to the aspirin untreated group, but lower doses of both extract was most effective. This reinforces the gastro-protective activity of the extract

Histological examination carried out on the gastric tissue revealed that gastro protective action of the methanolic extract was best at 250mg/kg body weight.

Fractionation of methanolic extract which showed best gastric protection was carried out and DPPH scavenging activity was carried out on the various pooled fraction, where fraction 1 had the highest antioxidant activity and was further characterized using FTIR and found to contain functional groups (alkyl halide, carboxylic acid, amide, alkenes, alcohols and ethers)

6.2 Conclusion

i. The result from this study showed that extracts of *vitex doniana* is of low toxicity and therefore safe for consumption.

ii. The aqueous and methanolic leaf extract of vitex doniana was found to posses gastro-protective effect against gastric mucosal damage.

iii. The plant extract showed antioxidant activity both in-vivo and in-vitro with methanolic extract showing a better a better gastro protective effect and also improved endogenous antioxidant activity in aspirin induced model of gastric ulceration.

6.3 Recommendations

i. The various fractions of the methanolic extract of *vitex doniana* should be tested on animals so as to be able ascertain which of the fractions is most effective *in vivo.*

ii. Further studies should be carried out on the characterization, isolation and identification of the bioactive component of the leaves extract of *V. doniana* responsible for the anti-ulcerative effect.

iii. Further investigations are needed to elucidate the exact mechanism(s) of action of the leaves of *V. doniana.*

6. 4 Contribution to Knowledge

i. From the results obtained in this present studies, we can say that the leaves of *Vitex doniana* can be used as a supplement singly or with other anti-ulcer drugs in the prevention, treatment and management of gastric ulcer in Nigeria and other parts of the world.

ii. The results of this research will also help in Nigeria to reduce the incidence of gastric ulcer to a minimal level as this plant is less expensive to obtain especially to those in the rural area as well as helping reduce some of the effects and pitfalls associated with some synthetic drugs used in the treatment and management of gastric ulcer.

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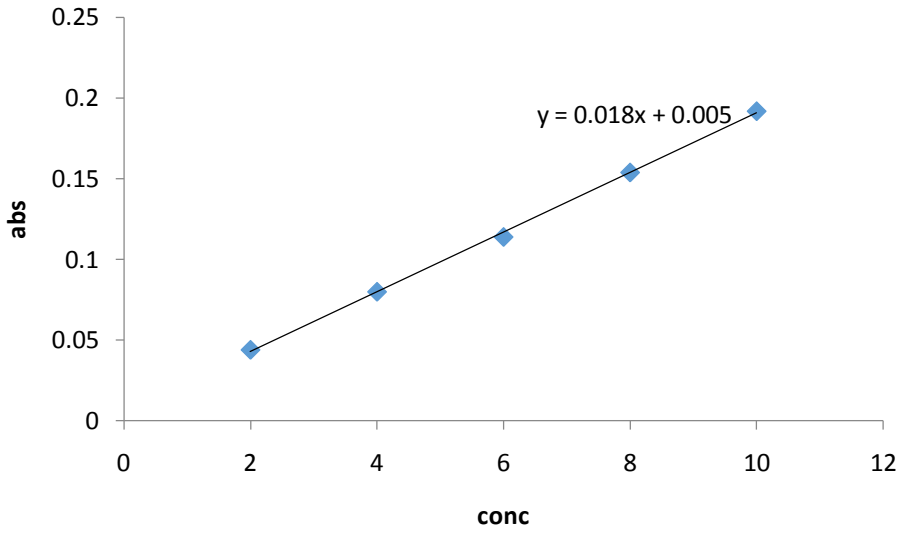
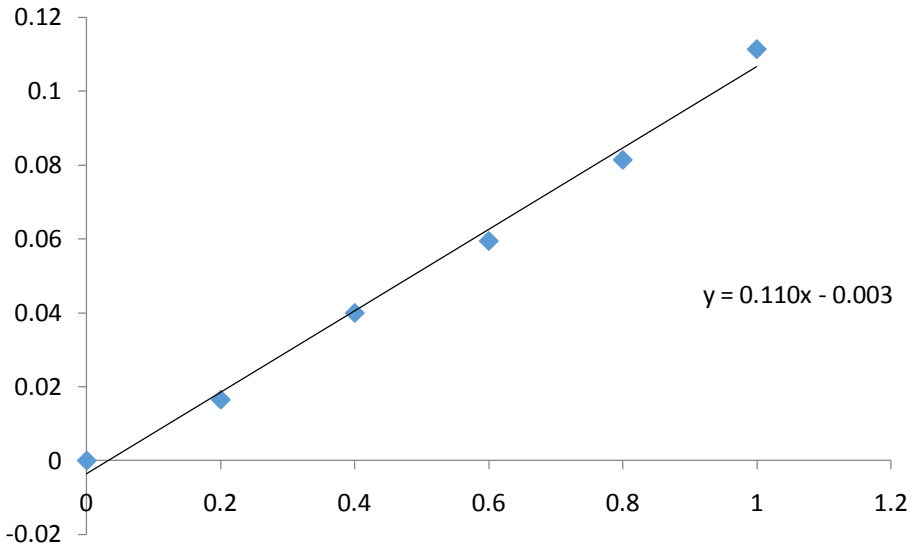
APPENDICES

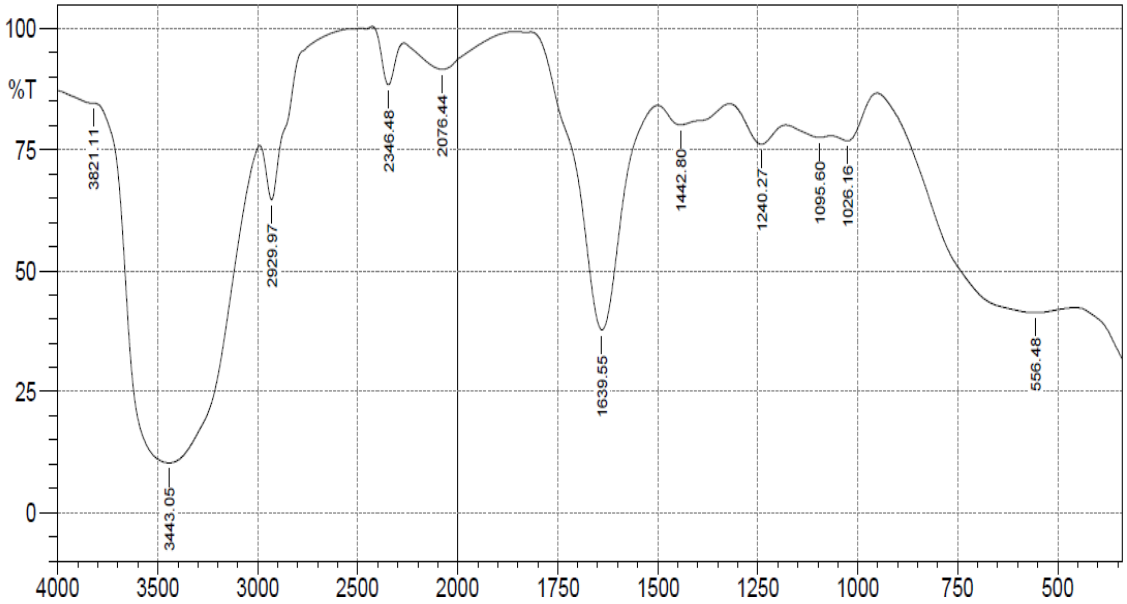
Appendices I: Standard Curve for Protein



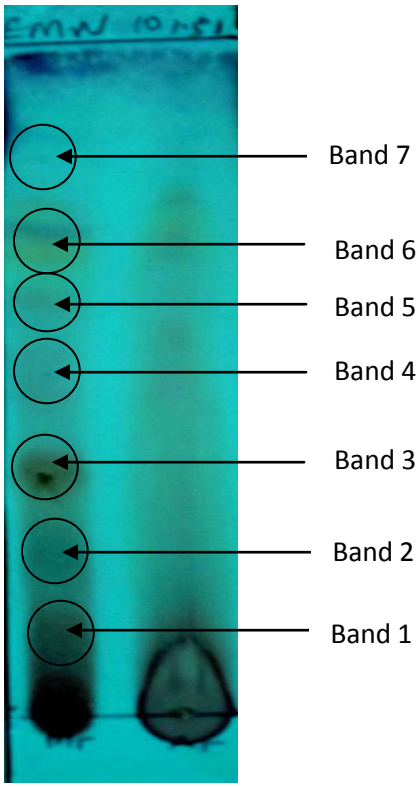
Appendices II: Standard Curve for Gastric Wall Mucus



Appendices III: Fourier Transform Infrared Spectra



Appendices IV: Thin layer chromatography of methanolic extract showing different bands



Methanolic extract