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CHAPTER ONE
INTRODUCTION

Ulcer is an open sore or lesion usually found on the skin or mucosal membranes of the body tissue. A peptic ulcer is a sore in the lining of the stomach or duodenum the first part of the small intestine. If peptic ulcer is found in the stomach it is called gastric ulcer. If it occurs in the duodenum, it is called duodenal ulcer. The gastro-duodenal mucosa constantly secretes mucin which acts as a barrier that prevents self digestion by enzymes and secretions such as acids (Konturek, and Konturek, 1995) and in ulcer this protective cover is compromised. It may be due to imbalance between impaired mucosal defensive factors (Mucus, bicarbonate, prostaglandins, nitric oxide, and growth factors) and aggressive factors (acid, pepsin, Helicobacter pylori and bile salts) in the mucosal membrane of stomach and duodenum (Bigheti et al., 2005). Reactive oxygen species (ROS) has been implicated in the pathogenesis of gastric ulceration as well as several other degenerative disorders such as cancer, diabetes mellitus, atherosclerosis, arthritis, hepatic injury, tumor promotion etc. due to their damaging effects on cellular macro-molecule (Lee et al., 2002) Gastric ulceration has been attributed to various causes such as stress, hormones, drugs, alcohol, smoking and ingestion of certain foods. Recently, Helicobacter pylori have been implicated in the antral gastritis, peptic ulcer and gastric malignancy (Ramanathan, 2000). Non steroidal anti-inflammatory drugs (NSAIDs) ingestion is associated with erosions, ulcerations, interference with ulcer healing, ulcer complication and injury to small and large intestine. With the increasing use of NSAIDS such as aspirin, ibuprofen, diclofenac etc. and the possibility for co-infection with Helicobacter pylori. The prevalence of gastric ulcer is estimated to be as high as four percent (4%) with a ten percent (10%) lifetime risk. Gastric ulcers and duodenal ulcers may be acute or chronic (Palmer and Penman, 1999). There are different types of ulcers but the most common types are gastric ulcers and duodenal ulcers.

Gastric Ulcers (GU) occurs when there are lesions in the gastric mucosal membrane of the stomach due to imbalance between impaired mucosal defensive and aggressive factors in the mucosal membrane of the stomach and duodenum. Though gastric ulcers are not contagious or cancerous, gastric ulcers may become malignant.

Duodenal Ulcers (DU) is located in the first 12 inches of small intestine beyond the stomach. This is mostly benign. Duodenal ulcers are more frequent in patients with alcoholic cirrhosis, chronic obstructive pulmonary diseases, chronic renal failure and hyperparathyroidism (James and Vinay, 2003).
Peptic ulcer associated with hyperacid secretion occurs mainly in patients with hypergastrinemia due to gastrinoma (McArthur et al., 1985). This condition occurs in Zollinger-Ellison syndrome. This syndrome is associated with abnormal increase in basal acid secretion, which partly leads to the development of peptic ulcer disease (PUD). In this syndrome, the acid hypersecretion accounts for large load of acid in the small intestine which causes diarrhea, malabsorption of fat and dilatation of small intestine. Another factor that can cause increase in basal acid secretion is hyperhistaminemia (McArthur et al., 1985). This occurs when there is systemic mast cell disease and basophilic leukemia. This condition is rare and can be managed with histamine-H₂ antagonists.

There may occur basal acid hyper secretion without hyper gastrinemia or other obvious cause. This condition is more common than Zollinger-Ellison syndrome (Soll et al., 1991). The acid hyper secretion can be ameliorated by the use of low dose anticholinergics (Soll et al., 1991).

1.1. Epidemiology of Peptic Ulcer Disease

Peptic ulcer was considered to be a disease of the young and middle aged adults but it affects all age groups even children. The incidence of peptic ulcer is approximately 10% worldwide (Willemijntje and Pankaj, 2006) and it is also estimated that 50% of healthy individuals experience symptoms of peptic ulcer on daily basis (Willemijntje and Pankaj, 2006).

Incidence of PUD peaks after the fifth decade of life. Despite the advances in the diagnosis and treatment of the PUD, hospitalization for PUD has not declined. However, there is apparent decrease in DU in men (John, 1989). There is also marginal decrease in the complications like perforation and bleeding. The major complications of DU are bleeding, perforation, gastric outlet obstruction and penetration into the neighboring organs like the pancreas. Bleeding is the common cause of death from PUD especially in geriatric patients. This is because this group of patients may have other associated disease condition. Mortality rate has decreased in both sexes (John, 1989).

The major environmental factors that are important in the pathogenesis of peptic ulcer are smoking and use of non-steroidal anti-inflammatory drugs (NSAIDs). Smokers have higher incidence of duodenal ulcer and higher rate of relapse due to slow rate of healing (Mc Guigan, 1991).

Mortality rate for smokers with ulcers is higher than in non-smokers (Mc Guigan, 1991). Bleeding complication is higher in patients on NSAIDs than those not on NSAID (Soll...
The incidence is also higher in those at risk of exposure to free radicals (Feldman et al., 1980). PUD is common in patients with some disease conditions such as chronic obstructive pulmonary disease and cirrhosis (John, 1989). The association of peptic ulcer and chronic obstructive pulmonary disease is not only due to smoking and chronic hypercapnia but also due to deficiency of alpha antitrypsin in both patients (John, 1989).

1.2 The Pathogenesis of Peptic Ulcer Disease (PUD)

The pathophysiology of PUD is best viewed as an imbalance between the aggressive factors (acid, pepsin, Helicobacter pylori (HP), and bile salts) and defensive factors (mucin secretion, cellular mucus, bicarbonate secretion, mucosal blood flow and cell turnover) (Goel and Bhatteharya, 1991; Akah et al., 1998; Robert, 1981).

Ulcers are as a result of the inability of the gastric or duodenal mucosal linings to resist the corrosive effect of acid on its surface. Hence, the abnormality in acid secretion may not be the primary disorder but impairment of the defensive mechanisms (Bigheti et al., 2005). Studies have shown that acid secretion is either normal or below normal in gastric ulcer patients and that 40-70% cases of duodenal ulcer patients show acidity within normal range (Gupta et al., 1980) suggesting that other factors are also involved in ulcerogenesis. External factors, which may contribute to the formation of ulcers include infection with Helicobacter pylori, (Bigheti et al., 2005), NSAIDs, free radicals, alcohols, smoking and of less important, stress. (Bigheti et al., 2005). The periodic formation and healing of ulcers can be explained by the periodic changes in both the aggressive and defensive factors.

1.2.1 Acid Secretion

The stomach produces hydrochloric acid (HCl), pepsin (enzyme), intrinsic factors, bicarbonate and mucus. These secretions are required for the digestion of protein, absorption of vitamin B12 (Cobalamin) and iron from the intestine and protection of the gastric mucosa from injury.

The gastric acid acts also as a disinfectant against ingested organisms as well as aid in iron absorption. The two major functional parts of the stomach are the proximal and the distal regions. The proximal parts constitute the oxyntic (parietal) cells and the peptic cells. These parts have secretory activities (John, 1989). The oxyntic cells are responsible for the secretion of acid and intrinsic factors while the peptic cells secrete pepsinogen (Arthur and John, 2000). The distal or pyloric region secretes hormones; gastrin and somatostatin. Somatostatin inhibits acid and pepsin secretion in the stomach by both endocrine and paracrine routes (Rodger,
In a negative feedback mechanism, gastrin secretion is inhibited by gastric acid in the antrum acting locally through somatostatin (Willemijntije and Pankaj, 2006).

The mucus neck cells, which are located in the entire regions of the stomach, are involved in the secretion of mucus and pepsinogen. The surface epithelial cells secrete bicarbonate, mucus, and pepsinogen. Some factors influence gastric secretion such as time of day, food, and psychological states as well as other metabolic activities of the body (Russel and Norman, 1993). The rate of secretion is also increased during the cephalic phase (thought and sight of food) (Bigheti et al., 2005). Gastric phase of secretion increases with the presence of protein in the gastric lumen (Arthur and John, 2000). The enteric nervous system of the stomach produces some neuro-peptides that regulate gastric acid secretion, blood flow and motility. These peptides are gastrin releasing peptide, vasoactive intestinal peptide (VIP), leucine and methionine, enkephalins, substance P, calcitonin gene-related peptide, neuropeptide Y, and galanin (Ibu et al., 1994; John, 1989). Autonomic nervous system also regulates gastric function through the parasympathetic and sympathetic nerves supplying the stomach (Arthur and John, 2000; John, 1989).

Gastric function is also regulated by substances that are produced by cells in the gastric walls, which influence neighboring cells by local diffusion. This type of regulation called paracrine regulation is exemplified by the stimulation of the parietal cells to secret acid by locally released histamine (Walsh, 1992). Parietal cell has secretory canaliculi lined with the enzyme, hydrogen-potassium ATPase (H⁺ K⁺ ATPase), responsible for acid secretion. Hydrolysis of ATP generates energy used by the enzyme to pump hydrogen ions into the secretory canaliculi in exchange for potassium ions (John, 1989).

Equimolar amount of chloride ions enter the canaliculi through nearby chloride channels, producing hydrochloric acid. Other channels allow potassium ions to return to the canaliculi where they are exchanged for more hydrogen ions. From the canaliculi the hydrochloric acid goes into the stomach lumen. The secretion of acid into the lumen is proportional to the alkaline tide where bicarbonate is secreted from the base of the gastric glands to the area just below the surface epithelial cells (John, 1989). This flow of bicarbonate offers a protective mechanism for the gastric surface cells against the back diffusion of acid (John, 1989).

The action of hydrogen-potassium ATPase and carbonic anhydrase in the parietal cell generate hydrogen and bicarbonate ions from water and carbon dioxide (Arthur and John, 2000). Parietal cell has three receptors, histamine H₂ receptor, gastric receptor G and muscarinic cholinergic M₁ receptors that mediate the stimulation of acid secretion by the
transmitters, histamine, gastrin and acetylcholine. The parietal cell acid secretion is stimulated by cyclic adenosine monophosphate (cAMP) and intracellular Ca\(^{2+}\). Hypercalcemia stimulates gastric production, which in turn increases acid secretion (James, 2003). Gastrin analogue, pentagastrin, has a beta-alanine substitute, acts on the cck-2 gastrin receptor (Schubert, 2000) thereby increasing acid secretion and also stimulate histamine secretion of enterochromaffin cells (Angus and Black, 1982; Anderson et al., 1996). Histamine stimulates cAMP formation while acetylcholine and gastrin stimulate the increase in intracellular calcium ions (John, 1989). The activation of the muscarinic receptors in the parietal cell initiates sequential events triggered by G-protein leading to the liberation from the membrane phospholipid of inositol triphosphate and diacylglycerol (Clapham, 1995). The diacylglycerol activates protein kinase C, which increases gastric acid secretion while inositol triphosphate increases intracellular release of calcium and chloridic ion secretion (Clapham, 1995). Anticholinergics decreases parietal cell responses to histamine but the effect of anticholinergics in human beings is not as great as that of histamine H\(_2\)-receptor antagonists.

Parietal cell also has inhibitory receptors for prostaglandins apart from the stimulatory receptors for histamine (H\(_2\)), gastrin (G) and muscarinic (MI) (Feldman et al., 1980). There is increased maximal acid secretion in duodenal ulcers unlike in gastric ulcers. Gastric ulcer patients have almost the same maximal acid secretion as normal person (Aoygi and Sommerskill, 1996). Maximal acid secretion is a function of the parietal ulcer patients (Ethrington and Taylor, 1970). Disorders of the system, which may be responsible for the development of ulcer, occur when there are hyper secretion of acid and the concomitant *Helicobacter pylori* infection (Barocelli et al., 1997; Pearson et al., 1980).

### 1.2.2 Pepsin

This is a proteolytic enzyme secreted in the stomach, it is involved in the digestion of proteins. There are two types of pepsin proenzymes. Group I and Group II pepsinogen (Ethrington and Taylor, 1970). The Group I pepsinogen is produced by chief cells closely linked to parietal cells. Group II pepsinogen is secreted by the surface epithelial cells and by mucous neck cells located throughout the stomach and in the duodenum. Secretion of pepsinogen is stimulated directly by cholecystokinin and indirectly by gastrin (John, 1989). Histamine does not affect pepsinogen secretion. Cholecystokinin secretion inhibits acid secretion. Cholinergic activation stimulates pepsin secretion more than acid secretion. Patient with PUD who has gastrinoma produces excess pepsin since pepsin secretion parallels acid secretion (John, 1989). Though pepsin activity is implicated in the development of PUD in
acidified environment, use of pepsin antagonist for the treatment of PUD has not been producing reliable result. Therefore, the exact mechanism of involvement of pepsin in the pathogenesis of PUD is yet to be elucidated.

1.2.3 Mucus

This is a glycoprotein sheet that covers the gastric mucosa. The layer consists of a semi solid gel. Mucus acts as a lubricant layer between the gastric mucosa and the gastric contents. It also acts as a barrier against acid. Mucus secretion is stimulated by cholinergic activation, prostaglandins and reflexes induced by the irritation of the gastric mucosa (Pearson et al., 1980).

1.2.4 Helicobacter pylori (HP)

The organism was first documented to cause injury to the stomach in 1983 by two researchers in Australia (Walsh and Peterson, 1995). This bacterium is implicated in gastritis and PUD. It is a spiral-shaped Gram-negative rod that can colonize epithelial cells lined in gastric mucosa-associated lymphoid tissue lymphomas and gastric adenocarcinoma (Parsonnet et al., 1994; Walsh and Peterson, 1995). It is well known that Helicobacter pylori is associated with alterations in the gastric epithelial cell cycle and apoptosis, higher levels of mononuclear and neutrophilic infiltrates, more severe atrophy and intestinal metaplasia (Antonio and Gaetano, 2004). Cell cycle alterations induce mitogenic signals and proto-oncogene expression that may trigger the development of cancer (Antonio and Gaetano, 2004). Though it does not grow in a very low pH medium, it has mechanism of protecting itself from the acid of the stomach (Palmer and Penman, 1999). It produces urease, which metabolizes urea to generate energy and ammonia (Palmer and Penman, 1999). Other toxic products from the metabolism of urea by urease include ammonium chloride and monochloramine (James, 2003).

Ammonia enables it to survive in acidic medium of the stomach. The bacterium provokes a local inflammatory response in the underlying epithelium due to release of a range of cytotoxins, vacuolating cytotoxin (VaCA), cytotoxin associated gene (CagA), adhesins phospholipases and porins (Palmer and Penman, 1999; Lee, 1998). Helicobacter pylori causes increased production of proinflammatory cytokines such as interleukin (IL-1, IL-6 and IL-8) and tumor necrosis factor (TNF) (Dundon et al., 2001).

The infection of the antrum leads to the depletion of antral somatostatin and increased gastrin release from G cells (Willemijn and Pankaj, 2006). Then the gastrin stimulates G-receptors of
the parietal cells leading to increased acid secretion. The increased acid secretion further damages the duodenal mucosa. Persistent damage of the duodenal mucosa stimulates the development of patches of gastric metaplasia in the duodenum, which in turn are colonized by *Helicobacter pylori* (Palmer and Penman, 1999; Dundon *et al*., 2001).

The colonization of the duodenum by *H. pylori* allows more damage and eventual ulceration. While some of *H. pylori* infected patients develop PUD some do not. This variation may be due to the host factors and or bacterial factors (Davies *et al*., 1994). The host factors may involve the patient whose stomach linings have problems. PUD may have something to do with the combination of *H. pylori* infection and the level of acid in the stomach (Davies *et al*., 1994).

1.2.5 Non-Steroidal Anti-Inflammatory Drugs (NSAIDs)

These are used to treat long term painful conditions like arthritis. They include such agents as ibuprofen, indomethacin, aspirin and diclofenac. These agents, when used regularly, over a long period of time, can cause ulcers (Ivey, 1988). They cause mucosal damage by inhibiting cyclooxygenase activity thereby reducing the formation of protective prostaglandins E₂ and I₂. This inhibition interferes with the protective mechanisms such as mucus and bicarbonate secretion, surface epithelial hydrophobicity and mucosal blood flow (Langman *et al*., 1991; Soll *et al*., 1991). These changes promote back diffusion of acids through the breached surface to destroy cells, capillaries and veins causing haemorrhagic ulcer. NSAIDs also enhance leukotriene synthesis resulting in damaging effects. Moreover, mucosal ATP synthesis and cell turnover processes are reduced by NSAIDs. These changes by NSAIDs can induce gastric damage through the generation of reactive oxygen species (ROS) (Vaananann *et al*., 1991; Yoshikawa *et al*., 1993).

NSAIDs in addition block gastric peroxidase enzymes and increase mucosal hydrogen peroxide and hydroxyl (OH) radical level to cause oxidative mucosal damage (Banerjee, 1990). This OH causes lipid peroxidation and increased gastric lesions induced by NSAIDs (Pihan *et al*., 1987). Lipid peroxidatlon causes decrease in the level of glutathione (GSH) in the gastric mucosa. Glutathione is a free radical scavenger.

There are some risk factors for NSAIDs-induced ulcers and they are as follows:

1. Past history of PUD
2. Past history of adverse event with NSAIDs
3. Concomitant use of corticosteroid
4. High or multiple NSAIDs

5. Individual NSAIDs. Some are higher than others in GI toxicity. Azapropazone and piroxicam are highest compared with ibuprofen.

6. Age: The risk is high at above 60 years.

### 1.2.6 Smoking

Smoking relaxes the pyloric sphincter, which permits the regurgitation of bile into the stomach, which damages the gastric and duodenal mucosa (Mc Guigan, 1991). This increases the risk of getting ulcers because the nicotine in cigarettes stimulates acid secretion in the stomach (Arthur and John, 2000). The risk is higher for gastric ulcer than duodenal ulcer. Smoking delays ulcers healing (John, 1989).

### 1.2.7 Alcohol

Overtime, alcohol wears down the linings of the stomach and intestines. Hence, it is a predisposing cause of acute and haemorrhagic gastric erosion in humans (Debashis et al., 2002). Ethanol lowers the concentration of non-protein sulphydryls especially glutathione (Szabo et al., 1981) thereby exerting ulcerogenic effect by increasing reactive oxygen species formation (Pihan et al., 1987; Szelenzyl et al., 1985).

The development or prevention of vascular injury in the gastric mucosa plays a crucial role in gastric mucosal injury and protection (Szabo et al., 1990; Cho et al., 1992). Haemorrhagic mucosal lesions due to ethanol, hydrochloric acid and sodium hydroxide (NaOH) in rats are preceded by micro vascular damage, increased vascular permeability and capillary stasis (Szabo et al., 1986). Leukorienes are one of the important causes of ulceration induced by ethanol (Goel, 2002).

### 1.2.8 Free Radicals

Free radicals and lipid peroxidation may contribute to the formation of gastric lesions (Itoh and Guth, 1985; Smith et al., 1987 and Gutteridge, 1995). Free radicals also play some roles in the development of gastric mucosal lesions induced by alcohol (Salium, 1990). These radicals through the release of lysosomal enzymes from cellular membranes worsen tissue damage (Pal, 1994). Studies have shown that oxidative stress is also involved in the pathogenesis of peptic ulcers (Bairy et al., 2002). Reactive Oxygen specie generated in the cells of aerobically respiring organisms due to many factors have been implicated in the pathogenesis of many human sufferings like Parkinson’s, Alzheimer’s, Huntington’s diseases,
liver cirrhosis, ulcers, artherosclerosis and cancer (Ajaikumar et al., 2005). Reactive oxygen species (ROS) is implicated in certain ischaemic, cardiovascular and pulmonary diseases, cataratogenesis and reproductive disorders (Halliwell and Gutteridge, 1989).

The involvement of reactive oxygen species in the pathogenesis of gastric ulceration was first evident from the studies on ischemia re-oxygenation-induced gastric mucosal injury (Yoshikawa et al., 1989; Yuda, 1993; Perry et al., 1996). There are experimental and clinical evidence, which suggest that mucosal damage by ethanol, NSAIDs, and Helicobacter pylori is mediated through reactive oxygen species (Davies et al., 1994; Vaananann et al., 1991; Yoshikawa et al., 1993; Pihan et al., 1987; Szelenzyl et al., 1985; Yoshikawa et al., 1996; Phull et al., 1995).

Reactive oxygen species may play an important role in gastric ulceration induced by several types of stress (Yoshikawa, et al., 1996; Phull et al., 1995). Reactive oxygen species also decreases the levels of endogenous antioxidants such as glutathione (GSH), alphatocopherol and ascorbate and make the mucosa more prone to oxidative damage (Phull et al., 1995).

1.2.9 Stress

Stress is also a major cause of gastric ulcer (Barocelli et al., 1997). Stress as implicated in the development of ulcer consists of physical and emotional stresses. Emotional stress may make an ulcer more painful and more difficult to heal but the stress itself does not cause an ulcer (Cho et al., 1992). Emotional stimuli frequently increase interdigestive gastric secretion to 50ml or more (and highly peptic and acidic) per hour in very much the same manner that the cephalic phase of gastric secretion at the onset of a meal (Arthur and John, 2000).

Mucins are high molecular weight glycoprotein responsible for the gel forming property of gastric mucus secretion (Allen et al., 1993). Stress reduces the quality and amount of mucus adhering to the gastric mucosa (Allen et al., 1993).

In condition of emotional stress, there are greater destruction of mucus and a decreased synthesis of its components as well as a change in quality thereby affecting the biochemical processes of translation, acylation and glycosylation of the ribosomal peptides (Tsukada et al., 1989).

1.3 Diagnosis of PUD
Peptic ulcer disease can be diagnosed by some symptoms and signs experienced by individual patients. Other ways of diagnosis include physical examination, laboratory studies and methods of visualization of ulcers like endoscopy.

1.4 Symptoms and Signs

Gastric and duodenal ulcers share common symptoms and signs, which include the following:

1.4.1 Pain

This is the most common ulcer symptom. It is a gnawing or burning pain in the upper quadrant of the abdomen or in the back. In the abdomen it is between the breastbone and the navel. The gastric ulcer pain can be distinguished from the duodenal ulcer pains by its persistence after food as well as its continuous rather than episodic in nature (Walsh, 1992). Duodenal ulcer is associated with heartburn while gastric ulcer does not produce heartburn.

1.4.2 Bleeding

Bleeding ulcers can be painless and may present such symptoms as haematemesis (vomiting), and anaemia (Walsh, 1992). Patients with gastric obstruction present with repeated vomiting leading to dehydration and chloride depletion (Walsh, 1992).

1.4.3 Dyspepsia

Dyspepsia (indigestion) as a symptom may indicate any one of several disease states such as *Helicobacter pylori* infection, peptic ulcer disease, gastroesophageal reflux disease (GERD), gastric motility disorders, non-ulcer dyspepsia and malignancy (Walsh, 1992). It is a common complaint with the estimate of 4-5% of patients naming dyspepsia as the primary reason for visiting their primary health care provider (Devauli and Castelli, 1999).

The appropriate diagnostic approach for dyspepsia is endoscopy which shows about 60% of patients with dyspepsia not having abnormal findings or definitive etiology (functional or non-ulcer dyspepsia) (Devauli and Castelli, 1999).

Dyspepsia is a relapsing condition with 50-80% rate of relapse. This requires maintenance therapy in many cases (Devauli and Castelli, 1999). Other symptoms are nausea (tendency to vomit) and weight loss.

1.5 Laboratory Studies
The laboratory studies that are required in the investigation of peptic ulcer disease are the invasive and non-invasive tests for *Helicobacter pylori* (Nomura *et al*., 1991). The non-invasive method involves serological and urea breathe test. The invasive methods include histology rapid urease test and microbiological culture (Nomura *et al*., 1991). Invasive method involves the use of an endoscopy to obtain biopsy specimen for the evaluation while non-invasive method depend on blood and breath samples (Rabeneck and Graham, 1997). Blood tests measure anti-bodies to make a diagnosis while the breath test uses radioactive or non-radioactive forms of urea, which the patients drink (Rabeneck and Graham, 1997).

### 1.6 Visualization of Ulcers
Techniques for the visualization of ulcers include:

#### 1.6.1 Endoscopy
This involves the use of an endoscope which is lowered into the throat, down to the esophagus and finally into the stomach and upper intestines. After successful introduction of the instrument, the inner lining of these organs can be observed from the camera on a television screen. During endoscopic technique, tissues can be taken and tested for *H. pylori*. This technique has high specificity and sensitivity for PUD. It also offers an opportunity for biopsy and histological examination of questionable lesions. The major limitation of this technique is the high cost, which very few can afford. It is indicated for patients older than 45 years especially with alarm symptoms (American Society for Gastrointestinal Endoscopy, 1999).

#### 1.6.2 Radiography
This method is less accurate than endoscopy for the diagnosis of small ulcers. This involves the radiological study of the stomach, duodenum and esophagus. This is carried out by administering a barium meal to the patient and obtaining the x-ray of the gastrointestinal tract. The presence of ulcer will be outlined on the x-ray.

### 1.7 Complications of PUD
Complications of peptic ulcer disease include bleeding, gastric outlet obstruction, perforation and penetration.

### 1.8 Treatment of PUD
#### 1.8.1 Aims of Treatment
The aims of treatment of PUD are the following:

i. Relief of pain
ii. Healing
iii. Prevention of relapses
iv. Prevention of complication

1.8.2 Non-Specific Therapeutic Measures

This approach plays adjunctive roles in the treatment and management of PUD. The following measures are involved in this approach. Avoidance of Smoking: Smoking delays ulcer healing and increases reoccurrence rate. Hence, avoidance of smoking will have beneficial effects on healing and reoccurrences (Palmer and Penman, 1999). Stress Management: Since stress especially emotional stress worsens ulcer, its management will facilitate healing (Cho et al., 1992). Therefore, exercise plays some roles in reducing stress. Exercise relieves and promotes weight loss, muscle gain and feeling of well-being. It also releases hormones, endorphins, which not only relieve stress but also cause a reduction in cortisol levels (Walsh, 1992).

Restriction of Alcohol Consumption: This is important in patients with alcohol cirrhosis as it affects healing as well as increases the rate of reoccurrences after initial healing (Palmer and Penman, 1999).

1.8.3 Specific Therapeutic Approaches for PUD

Acid suppressive therapy has marginal benefit over placebo as evidenced by a meta analysis of anti-secretory agent for non-ulcer dyspepsia showing a benefit of only 20% over placebo (Dobrilla et al., 1989). Agents used for the treatment of PUD have limiting side effects (Barrowwan and Pfeiffer, 1992). There is no single agent with absolute healing activity without relapse (David, 1998).

1.8.3.1 Antacids

For decades, the only relief for ulcer patients has been provided by antacids. Antacids by their alkaline nature act by neutralizing the gastric acid. This neutralization reaction weakens the corrosive effects of the acids and reduces ulcer pains. They also strengthen the mucosal defensive mechanism through their stimulation of prostaglandin production in the mucosa (Mc Quaid, 2004). Moreover, neutralization reduces the peptic activity in the gastric juice and inactivates pepsin at pH above 5 (Arthur and John, 2000).
Animal studies have demonstrated mucosal protection by antacids either through the stimulation of prostaglandins production or the binding of an unidentified injurious substance (David, 1998). Antacids affect bowel movement and secretions. Aluminum containing antacids possess constipating effect by decreasing bowel motility while magnesium-containing antacids have cathartic effects by increasing bowel motility (D’Arcy and McElnay, 1987). Antacids containing mixtures of aluminum and magnesium compounds do not significantly change bowel function (D’Arcy and McElnay 1987).

1.8.3.2 Cytoprotective agents

The therapeutic approaches aimed at reducing gastric acid secretion and therapeutic measures involving the use of a variety of cytoprotective agents are also employed in the treatment of peptic ulcer disease. These cytoprotective agents strengthen the gastric and duodenal defenses (Venkatanganna et al., 1998). These compounds include bismuth subsalicylate and colloidal bismuth subcitrate.

Another important agent is sucralfate, which is a product of the reaction between sucrose octasulfate and aluminum hydroxide. At acidic pH it undergoes polymerization and cross linking of sucralfate to form a gel that is viscid and demulcent (McCarthy, 1999).

The mechanism of cytoprotective and healing properties of sucralfate involves stimulation of prostaglandin synthesis, adsorption of pepsin and stimulation of local production of epidermal growth factor (McCarthy, 1999). Food or antacids do not affect the gel’s adherence integrity. The gel adsorbs proteins in food thereby increasing the cytoprotective layer (McCarthy, 1999).

1.8.3.3 Prostaglandin analogs

Prostaglandin analogs are useful in the treatment of peptic ulcers. Gastric mucosa synthesizes prostaglandins especially PGE$_2$ and PGI$_2$. These prostaglandins inhibit the secretion of acid and stimulate the secretion of mucus and bicarbonate as well as blood flow (Ibu et al., 1994). Misoprostol a prostaglandin E$_1$ analog is effective in the treatment of PUD (Collins, 1990). It is particularly useful for patients who require NSAIDs for the treatment of arthritis and other diseases and for the prevention of gastric ulcers induced by NSAIDs (Collins, 1990). PGE$_2$ protects the stomach against erosive actions of gastric acids, pepsin, NSAIDs and alcohol (Pennington, 1985).

1.8.3.4 Histamine H$_2$-Receptor Antagonists
The development of histamine H\(_2\)-receptor antagonists not only provided specific class of antisecretory agents but also revolutionized PUD management and treatment. These agents do not have any effect on histamine H\(_1\)-receptors. Their development in the seventies revolutionized the treatment of PUD. Drugs in this class include cimetidine, ranitidine, famotidine and nizatidine. These groups of drugs are more hydrophilic than H\(_1\) blockers thereby making them less penetrable to the CNS.

H\(_2\)-histamine receptor antagonists competitively inhibit the interaction of histamine with H\(_2\)-receptors thereby blocking gastric secretion by histamine and other H\(_2\) agonists in a dose dependent manner. This antagonistic effect of these agents is most pronounced in gastric acid secretion (Bertaccini et al., 1981). Study has shown that delay in gastric emptying will slow the evacuation of the gastric content which in turn enhances the absorption of orally administered anti ulcer agents and eventual promotion of healing (Bertaccini et al., 1981). Some H\(_2\) blockers were shown to reduce gastric emptying in rats by a mechanism totally independent of H\(_2\)-receptor blockade (Bertaccini et al., 1981).

The physiological effects of H\(_2\) blockers on H\(_2\) receptors in vascular and bronchial smooth muscles are not clinically significant (Mohammed and Hunt, 1994). They inhibit gastric and muscarinic agonist-stimulated acid secretion. These agents inhibit basal and nocturnal acid secretions. They alter the cephalic, gastric and intestinal phases of acid secretion (Russel and Norman, 1993). They also reduce the volume of gastric juice and its hydrogen (H\(^+\)) concentration as well as the output of pepsin and intrinsic factor (Binder and Donaldson, 1978). H\(_2\)-blockers and other antisecretory drugs are effective in PUD and hypersecretory states associated mastocytosis where acid hyper secretory activities are involved (Bambery et al., 1992; Mohammed and Hunt, 1994).

1.8.3.5 Eradication of Helicobacter pylori

The recognition of the role of Helicobacter pylori in causing gastritis and in the development of PUD provided the therapeutic insight that the eradication of this small, spiral-shaped, flagellated gram-negative bacterium would be a useful strategy for promoting the healing of ulcers and prevention of their recurrence.

Single agent therapy for H. pylori infections has proven relatively ineffective in vivo and has led to the emergence of resistant strains. Multiple drugs are needed in the eradication of H. pylori infection because of resistance development. Evidence has shown that probiotics, which are live microbial food supplements beneficially, affect the host by improving its microbial balance (Fuller, 1991). Studies have also shown that ingesting lactic acid bacteria
exerts a suppressive effect on *H. pylori* infection in both humans and animals while supplementing with lactobacillus and Bifidobacterium-containing yoghurt (AB-yoghurt) was shown to improve the rates of eradication of *H. pylori* in humans (Kuan-Yuan *et al*., 2004). The inhibition of *H. pylori* growth occurred as a result of in vitro production of organic acid by lactobacillus acidophilus (Midolo *et al*., 1995). Lactobacillus acidophilus supernatant decreased *H. pylori* viability in vitro and decreased urease activity and the histopathologic degree of gastric lesions in mice infected with *Helicobacter felis* (Coconnier *et al*., 1998). Elimination of *H. pylori* infection leads to a significant increase in the levels of the powerful appetite-stimulating hormone, ghrelin in the tissues of the stomach where it is produced (Tatsuguchi *et al*., 2004). This may be responsible for the increase in weight of patients undergoing treatment for the eradication of *H. pylori* (Murray *et al*., 2003; Baena *et al*., 2002). Study has shown that modest consumption of wine and beer (approximately 7 units/week) protects against *H. pylori* infection, presumably by facilitating eradication of the organism (Liam *et al*., 2002).

*Helicobacter pylori* peptic ulcers are treated with drugs to kill the bacteria, drugs to reduce stomach acid and drugs to protect the lining of the stomach (Walsh and Peterson, 1995). This triple therapy has been shown to kill the bacteria, reduce ulcer symptoms and prevent recurrence in over 90% of patients (Walsh and Peterson, 1995). The antibiotics most commonly used to kill the bacteria are amoxicillin, Claritromycin, metronidazole and tetracycline. The drugs to reduce stomach acids are the histamine (H₂) blockers (cimetidine, famotidine, nizatidine and ranitidine) and proton pump inhibitors (lansoprazole and omeprazole). The drug that is used to protect stomach lining is bismuth subsalicylate or bismuth subeitrate. The major draws back for this multiple therapy are compliance, cost and unpleasant side effects.

### 1.8.3.6 Proton pump inhibitors (PPIs)

The inhibitors of the enzyme, H⁺K⁺-ATPase, which is responsible for the final step in the acid secretion by the parietal cells, have offered effective means of selectively blocking the proton pump (Lindberg *et al*., 1990). The enzyme, H⁺K⁺-ATPase (proton pump) mediates acid secretion in parietal cells. Inhibitors of the enzyme play remarkable role in the inhibition of acid secretion (McTavish *et al*., 1991). The inhibitors include substituted benzimidazoles (Lansoprazole and Omeprazole). A prototype, Omeprazole is pH sensitive and at neutral pH it is lipid soluble, chemically stable and weak base (Barradell *et al*., 1992).

At neutral pH, the drugs are diffused into the secretory canaliculi and become protonated and trapped (McTavish *et al*., 1991). The protonation is associated with the structural rearrangement of the molecules to form active metabolite, sulfenic acid and a
sulfonamide (McTavish et al., 1991). The activated forms covalently binds to the sulfhydryl groups of the $\text{H}^+\text{K}^+\text{-ATPase}$ in such a manner that requires two molecules per molecule of enzyme. Proton pump inhibitors (PPIs) have specific activity due to their selective effect on $\text{H}^+\text{K}^+\text{-ATPase}$, their acidic requirement for the generation of active metabolite, trapping within the acidic canalculi of the protonated drugs and sulfenamide.

Due to covalent binding of the active metabolite, acid secretion can only resume with the synthesis of the $\text{H}^+\text{K}^+\text{-ATPase}$ (Barradell et al., 1992). Studies have shown that lansoprazole has similar mechanism of action but its inhibitory effect can be reversed by a mechanism requiring glutathione and that synthesis of new enzyme is not required for the resumption of acid secretion (Barradell et al., 1992). They do not affect gastric motility and have marginal changes in the amount of gastric juice, pepsin and intrinsic factor. Their actions persist even after the withdrawal of the drug. They are available as sustained release capsules. They are formulated into micro encapsulation because of ease of degradation by gastric acid.

### 1.8.4 Herbal Products Employed in the Treatment of PUD

Reports on clinical evaluation of conventional anti-ulcerogenic drugs show that there are incidences of relapses, adverse effects and danger of drug interactions during ulcer therapy (Goel and Sairam, 2002). As a result, the search for an ideal anti-ulcer drug continues and has also been extended to herbal drugs in search for new and novel molecules which afford better protection and decrease the incidence of relapse (Goel and Sairam, 2002).

Herbal medicines play an important role in health care delivery and about 70-80% of the population depends on traditional healers for most of their ailments including peptic ulcer (Akah et al., 1998). Diseases such as AIDS, herpes, malaria, tuberculosis and other emerging multi-drug resistant diseases continue to encourage research efforts into herbal medicines (Rabiu, 2002). Herbal drugs possess potentials in combating various disease conditions (Pezzuto, 1997., Amos et al.,2001). About 60% of the world’s population relies almost entirely on plants for medications and natural products, which have long been recognized as an important source of therapeutically effective medicine (Ajaikumar et al., 2005). The prevalent rate is decreasing in developed but increasing in developing countries (Shayne, 2002). Many plants have anti ulcer constituents (Akah et al., 1996). Studies have shown that *Bacopa monniera* and *Azadirachta Indica* have anti-ulcer and ulcer healing activities, which are attributed to their effects on various mucosal offensive and defensive factors (Dorabubu et al., 2004).
Since free radicals and *Helicobacter pylori* are implicated in the pathogenesis of PUD, natural product with antioxidant, antimicrobial as well as anti ulcer properties offer beneficial therapeutic outcome. As a result, research has shown that water extract of stinging nettle (*Urtica dioica* L) possesses antioxidant properties and anti ulcer activity against ethanol-induced ulcerogenesis (Ray, 2004).

There is extensive experimental evidence that indicates certain substances through scavenging of free radicals protect the gastric mucosa (Galvin and Szabo, 1992). *Shankha bhasma* provides anti-ulcer activity in rats by acting as gastric cytoprotective agent and modulation of free radicals (Pandit *et al*., 2000). *Bacopa monniera* extract exerts anti-ulcer activity through its anti *Helicobacter pylori* increase in prostanoids (PGE and PGI2), increase in mucin secretion, increase in life span of mucosal cells and gastric antioxidant effect (Ray, 2003). *Centella asiatica* water extract and its active ingredient, asiaticoside are used as anti-gastric ulcer agents due to their ability to reduce the size of ulcers and the concomitant attenuation of myeloperoxidase activity at the ulcer tissues (Sairman *et al*., 2001). The methanol extract of *Punica granatum* (Pomegranate) possesses gastroprotective activity through its antioxidant mechanism (Ajai Kumar *et al*., 2005).

Studies have also shown that “Parsely” *Petroselinum crispum* extract possesses anti-ulcerogenic activity by replenishing ethanol-induced depleted gastric wall mucus and non-protein sulfhydryl contents (Al-Howiriny *et al*., 2003). Researchers have shown that Guarana (*Paullinia cupana* Mart) has gastroprotective properties (Campos *et al*., 2003). Since urease of *Helicobacter pylori* is essential for its colonization. (Matsubara *et al*., 2003) Research has focused on foodstuffs, which inhibit the activity of this enzyme. Among some plant-derived foodstuff sample tested, some tea and rosemary extracts were found to clearly inhibit *Helicobacter pylori* urease in vitro (Matsubara *et al*., 2003). This inhibition is attributed to the hydroxyl group of 5(1)-position of the active constituent, catechins (Matsubara *et al*., 2003).

Whey protein concentrate protects gastric mucosa from ethanol damage and the protection depends on sulfhydryl compounds present in the whey protein concentrate, including its capacity to stimulate glutathione synthesis (Rosaneli *et al*., 2002). Propolis extract exhibited dose-dependent superoxide scavenging activity and antioxidant effects on absolute ethanol-induced lipid peroxidation in rat gastric mucosal homogenates thereby protecting the gastric mucosa from oxidative stress (Ray, 2002). Other plants with anti-ulcerogenic properties include extracts of unripe plantain (*Musa sapientum*), ginger (*Zingiber officinale*) and Satavari (*Asparagus racemosus*) (Goel and Sairam, 2002).
Studies have equally shown that regular intake of yoghurt containing Bifidobacterium and lactobacillus plays curative and healing roles in PUD by suppressing H. pylori infection (Kuan-Yuan, 2004). Raw cabbage juice that is high in glutamic acid is very effective in ulcer healing (Fullick, 1994).

1.9 Important Biochemical Markers

1.9.1 Lipid Peroxidation

Lipid peroxidation refers to the oxidative degradation of lipids. It is the process whereby free radicals “steal” electrons from the lipids in cell membranes, resulting in the cell damage. This process proceed by a free radical chain reaction mechanism. It most often affects polyunsaturated fatty acids because they contain multiple double bonds which lie in between the methylene -CH2- groups that possess especially reactive hydrogens. As with any radical reaction, the reaction consists of three major steps; initiation, propagation and termination (Sies, 1997).

1.9.1.1 Mechanism of Lipid Peroxidation

1.9.1.1.1 Initiation

Initiation is the step whereby a fatty acid radical is produced. The initiators in living cells are most notably reactive oxygen species [ROS], such as OH-, which combines with a hydrogen atom to make water and fatty acid radical (Aruoma, 1993).

1.9.1.1.2 Propagation

The fatty acid radical is not a very stable molecule. So it reacts readily with molecular oxygen thereby creating a peroxyl-fatty acid radical. This, too, is an unstable species that reacts with another free fatty acid producing a different fatty acid radical and a hydrogen peroxide or a cyclic peroxide if it had reacted with itself. This cycle continues as the new fatty acid radical reacts in the same way.

1.9.1.1.3 Termination

When a radical reacts, it always produces another radical, that is why the process is called a “chain reaction mechanism”. The radical reaction stops when two radicals react and produce a non radical species. This happens only when the concentration of radical species is high enough for there to be a high probability of two radicals actually colliding. Living
organisms have evolved different molecules that speed up termination by quenching free radicals and therefore protect the cell membrane. One such important antioxidant is alpha-tocopheral, also known as vitamin E. Other antioxidants made within the body include the enzymes superoxides dismutase, catalase and peroxidase (Sies, 1997).

1.9.1.4 Hazard

Lipid peroxidation if not terminated fast enough will damage the cell membrane which consists mainly of lipids. Phototherapy may cause haemolysis by rupturing red blood cell membranes in this way (Sies, 1997). In addition, end products of lipid peroxidation may be mutagenic and carcinogenic. For instance, the end product malondialdehyde reacts with deoxyadenosin and deoxyguanosin DNA, forming DNA adducts to them (Sies, 1997).

1.9.1.2 Assay for Lipid Peroxidation

Free radicals have a very short half-life, which makes them very hard to measure in the laboratory. Multiple methods of measurement are available today, each with their own benefits and limits. Radicals can be measured using electron spin resonance spin trapping methods. Exogenous compounds with a high affinity for free radicals (i.e. xenobiotics) are utilized in the spin techniques. The compound and radical together form a stable entity that can be easily measured. This indirect approach has been termed “fingerprints” (Karlsson, 1997). However, this method is not 100% accurate. Spin trapping collection techniques have poor sensitivity, which can skew results (Acworth and Bailey, 1997). Commonly used alternate approach measures markers of free radicals rather than the actual radical. These markers of oxidative stress are measured using a variety of different assays. These assays are described below. When a fatty acid is peroxidized, it is broken down into aldehydes which are excreted. Aldehydes such as thiobarbituric acid reacting substances (TBARS) have been widely accepted as a general marker of free radical production (Clarkson, 1995). The most commonly measured TBARS is malondialdehyde (MDA) (Karlsson, 1997). The TBA test has been challenged because of its lack of specificity, sensitivity and reproducibility. The use of liquid chromatography instead of spectrophotometer techniques help reduce these errors (Wong et al., 1987). In addition, the test seems to work best when applied to membrane system such as microsomes (Halliwell and Chirico, 1993). Gases such as pentane and ethane are also created as lipid peroxidation occurs. These gases are expired and commonly measured during free radical research (Karlsson, 1997; Dillard et al., 1978) was one of the first to determine that expired pentane increased as Vmax increased (Kanter et al., 1988) has reported that serum
MDA levels correlated closely with blood levels of creatine kinase an indicator of muscle damage. Lastly, conjugated dienes (CD) are often measured as indicators of free radical production. Oxidation of unsaturated fatty acids results in the formation of CD. The CD formed is measured and provided a marker of the early stages of lipid peroxidation (Halliwell and Gutterridge, 1985). A newly developed technique for measuring free radical production shows promise in producing more valid results. The technique uses monoclonal antibodies and may prove to be the most accurate measurement of free radicals. However, until further more reliable techniques are established it is generally accepted that two or more assays be utilized whenever possible to enhance validity (Halliwel and Gutteridge, 1985).

1.9.2 Catalase

Catalase is a common enzyme found in nearly all living organism which are exposed to oxygen where it functions to catalyse the decomposition of hydrogen peroxide to water and oxygen (Chelikani et al., 2004) catalase has one of the highest turnover number of all enzymes; one molecule of catalase can convert millions of molecules of hydrogen peroxide to water and oxygen. (Goodsell, 2004) catalase is a tetramer of four polypeptide porphyrin heme, it contains four (iron) groups that allow the enzyme to react with the hydrogen peroxide. The optimum pH for catalase is approximately 7 and has a fairly broad maximum (the rate of reaction does not change appreciably at pH between 6.8 and 7.5) (Boon et al., 2007) the pH optimum for other catalase varies between 4 and 11 depending on the specie, the optimum temperature also varies by species (Toner et al., 2007).

1.9.2.1 Action of Catalase

The reaction of catalase in the decomposition of hydrogen peroxide is $2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{O}_2$. Catalase can oxidise different toxins such as formaldehyde, formic acid, phenols, and alcohols. In doing so, it uses hydrogen peroxide according to the following reaction.

$2\text{H}_2\text{O}_2 \rightarrow 2\text{H}_2\text{O} + \text{R}$ The exact mechanism of this reaction is not known. Any heavy metal ion (such as copper, cations in copper 11 sulphate) will act as a non competitive inhibitor on catalase. Also the poison cyanide is a competitive inhibitor of catalase, strongly binding to the heme of catalase and stopping the enzymes action. Three dimensional protein structures of the peroxidated catalase intermediates are available at the protein data bank. This enzyme is commonly used in laboratories as a tool for learning the effect of enzymes upon reaction rates.
1.9.2.2 Cellular Roles of Catalase

Hydrogen peroxide is a harmful byproduct of many normal metabolic processes and to prevent damage, must be quickly converted into other less dangerous substance. To this end, catalase is frequently used by cells to rapidly catalyse the decomposition of hydrogen peroxide into less reactive gaseous oxygen and water (Gaetani et al., 1996). The true biological significance of catalase is not always straightforward to access. Mice genetically engineered to lack catalase are phenotypically normal, indicating that this enzyme is dispensable in animals under some condition (Ho et al., 2004). Some human beings have very low levels of catalase (acatalasia), yet show few ill effects. It is likely that the predominant scavenger of H₂O₂ in normal mammalian cells is peroxiredoxins rather than catalase (Ho et al., 2004). Catalase is usually located in a cellular organelle called the peroxisome (Alberts et al., 2002) peroxisomes in plant cells are involved in photorespiration (the use of oxygen and production of carbon dioxide) and symbiotic nitrogen fixation.

Hydrogen peroxide is used as a potent antimicrobial agent when cells are infected with a pathogen. Pathogen that are catalase positive, such as Mycobacterium tuberculosis, Legionella pneumophila, and Campylobacter jejuni, make catalase in order to deactivate the peroxide radicals, thus allowing them to survive unharmed within the host (Srinivasa-Rao et al., 2003).

1.9.2.3 Distribution of Catalase Among Organism

All known animals use catalase in every organ, with particularly high concentration occurring in the liver, one unique use of catalase occurs in bombadier beetle. The beetle has two sets of chemicals ordinarily stored separately in its paired glands. The larger of the pair, the storage chamber or reservoir contains hydroquinones and hydrogen peroxide, whereas the smallest of the pair, the reaction chamber contains catalase and peroxidases. To activate the spray, the beetle mixes the content of the two compartment, causing oxygen to be liberated from hydrogen peroxide. The oxygen oxidizes the hydroquinones and also act as the propellant (Eisner and Aneshansley, 1999). Catalase is also universal among plant and many fungi are also high producers of the enzyme (Isobe et al., 2006). Very few aerobic microorganisms are known that do not use catalase. Streptococcus species are an example of aerobic bacteria that do not use catalase. Catalase has been observed in some anaerobic microorganism, such as Methanosarcina barkeri (Brioukhanov et al., 2006).

1.9.2.4 Catalase Test
The catalase test is also one of the main three tests used by microbiologists to identify species of bacteria. The presence of catalase enzymes in the test isolate is detected by using \( \text{H}_2\text{O}_2 \). If the bacteria possess catalase (i.e., catalase positive), when a small amount of bacterial isolate is added to hydrogen peroxide, bubbles of oxygen are observed. In microbiology, the catalase test is used to differentiate between bacterial species in the laboratory. The test is done by placing a drop of hydrogen peroxide on a microscope slide using an applicator stick; a scientist touches the colony and then smears a sample into the hydrogen peroxide drop. If bubble or froth forms, the organism is said to be catalase positive, staphylococci (Rollin, 2000) and micrococci (Johnson, 2009) are catalase positive.

If not, the organism is catalase negative. Streptococci (Fox, 2009) and enterococci are catalase negative. While the catalase test alone cannot identify a particular organism, combined with other tests such as antibiotic resistance, it can aid diagnosis. The presence of catalase in bacterial cells depends on both the growth condition and the medium used to grow the cells.

### 1.9.3 Alkaline Phosphatase

Alkaline phosphatases are a group of enzymes found primarily in the liver (isoenzyme ALP-1) and bone (isoenzyme ALP-2). There are also small amounts produced by cells lining the intestines (isoenzymes ALP-3). The placenta and the kidney (in the proximal convoluted tubules) what is measured in the blood is total amount of alkaline phosphatases released from these tissues into the blood. As the name implies, these enzymes work best at an alkaline pH and thus the enzyme itself is inactive in the blood. Alkaline phosphatase acts by splitting off phosphorus (an acid mineral) creating an alkaline pH (pH of 10). The primary importance of measuring alkaline phosphatase is to check the possibility of bone disease or liver disease. Since the mucosal cells that line the bile system of the liver are the source of alkaline phosphatase, the free flow of bile through the liver, down into the biliary tract and gallbladder are responsible for maintaining the proper level of this enzyme in the blood. When the liver, bile ducts or gallbladder system are not functioning properly or are blocked, this enzyme is not excreted through the bile and alkaline phosphatase is released into the blood stream. Thus, the serum alkaline phosphatase is a measure of the integrity of the hepatobiliary system and the flow of bile into the small intestine.

In addition to liver, bile duct, or gallbladder dysfunction, an elevated serum alkaline phosphatase can be due to rapid growth of bone since it is produced by bone-forming cells called osteoblasts. One would expect that growing children have higher levels than full-grown adults. The relationship of alkalinity to bone development warrants further discussion because
it plays a major role in the prevention and reversal of osteoporosis. Just as calcium builds up around faucets, so is calcium laid down into bone. The reason the calcium deposits on your faucet is because the water is alkaline and calcium comes out of solution and crystallizes in an alkaline environment. The reverse is also true, "Lime-Away", vinegar, or any other acidic solution dissolve the calcium deposits because they are acidic. It makes sense those osteoblasts by creating a local environment of alkalinity via alkaline phosphatase helps build bone. It also implies that in order to slow bone loss, one can not be in an acidic state. Studies have shown that giving bicarbonate of potassium is just as effective as calcium in correcting osteoporosis! One would expect then that in an acidic state, the body will compensate for this by increasing the bone alkaline phosphatase levels because, acid-alkaline is influenced by many other glands, the implications of serum alkaline phosphatase levels must consider more than just bone and liver function. Associated organs/glands include adrenals, uterus, prostate, and intestine.

The consequences of impaired bile flow are pervasive since bile is critical to the body's ability to process fats. As a result, fats remain undigested in the digestive tract and can cause bloating, cramps, light coloured stools, gaseousness, etc. especially after a rich food. Many patients report pressure or pain in the right upper area of their abdomen where the liver and gallbladder are located. You may have discomfort in the right shoulder or between your shoulder blades anywhere from your mid-back to the base of your neck. Many people say they "carry my stress in the upper back and neck." This may due to gallbladder dysfunction. Unfortunately, a normal alkaline phosphatase does not exclude hepatobiliary dysfunction. In many cases, even the ultrasound shows no gallstones, etc. Rather the problem is that the bile does not flow freely throughout the system, which may result in insufficient bile action.

The consequences of impaired bile function involve the endocrine system in a major way because all of the steroid hormones are metabolized in part by the liver. These include the sex hormones (androgens and estrogens). As a result the menstrual cycle, sexual functions and sex characteristics can be affected.

The optimal range for alkaline phosphatase depends on your age. A growing adolescent will have a much higher alkaline phosphatase than a full grown adult because his/her osteoblasts are laying down bone very rapidly. For an adult, 50-75 mg/dl is considered a reasonable optimal range.

An increased serum alkaline phosphatase may be due to: congestion or obstruction of the biliary tract, which may occur within the liver, the ducts leading from the liver to the gallbladder, or the duct leading from the gallbladder through the pancreas that empty into the
duodenum (small intestine). Any of these organs (liver, gallbladder, pancreas, or duodenum) may be involved.

An elevated alkaline phosphatase almost always requires other tests to determine the origin of the condition. For example, liver enzyme tests to check the integrity of the liver, x-rays or other bone images if a bone abnormality is evident. Although not used often, the isoenzyme profile of alkaline phosphatases can be determined to see if the elevation of alkaline phosphatase came primarily from liver (ALP-1), bone (ALP-2), or elsewhere. Most often, however there is a modest elevation from ideal but the actual value is within the laboratories reference range and the origin is inferred from the symptoms, exam, or existing lab results.

A decreased serum alkaline phosphatase may be due to:

- Zinc deficiency.
- Hypothyroidism.
- Vitamin C deficiency/Scurvy.
- Folic acid deficiency.
- Excess Vitamin D intake.
- Low phosphorus levels (hypophosphatasia)
- Celiac disease.
- Malnutrition with low protein assimilation (including low stomach acid production/hypochlorhydria).
- Insufficient Parathyroid gland function.
- Pernicious anemia
- Vitamin B₆ insufficiency

1.10 The Plant *Carica papaya* (Pawpaw)
1.10.1 Botanical Classification

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1.10.2 Plant Description
*Carica papaya* (Caricaceae) known as pawpaw is common in tropical and subtropical countries. It is an evergreen tree that originated in Central America. It is herbaceous, dicotyledonous plant that may produce fruits for more than 20 years i.e. it is perennial. It is a soft wooded single stemmed perennial tree, with a crown of large palmate leaves emerging from the apex of the trunk, but trees may become multi stemmed when damaged. It grows 2-10cm in height, and the soft hollow, cylindrical trunks ranges from 30 cm in diameter at the base to about 5cm in diameter at the crown. The fruits are fleshy berries 7-10 cm long with thin smooth green skin, which turns yellow or orange when ripe. The seeds are many, parietally attached in five rows to the interior wall of the ovary, spherical about 5mm in diameter, black or grayish, wrinkled, and enclosed in gelatinous sarcotesta formed from the outer integument. Latex abound in all parts of the plant but is obtained mainly from the fruits, seeds and leaves, ripe papaya fruits contains no latex or papain. The latex contains cysteine lysozymes, proteinases (papain, chymopapain, caricain, and glycyl endopeptidase), which are proteolytic enzymes. Other enzymes from papaya latex include glycosyl hydrolases such as b-1, 3-glucanases, chitinases, and protease inhibitors such as cystatin, and glutaminyl cyclotransferases and lipases (Ezike et al., 2009). Other phytoconstituents from papaya are an alkaloid carpaine (seeds, leaves, and unripe fruits), carbohydrates, fixed oil glycosides, and saponins. The ripe fresh fruit is eaten throughout the tropics for breakfast and desert and in fruit salad. Nutritionally, papaya is a good source of calcium and vitamins A and C. It is used for making soft drinks, jam, ice cream, flavouring, and crystallized fruit and is canned in syrup. The unripe fruits are cooked as a substitute for marrow and for applesauce. The fresh fruits are carminative and used as a diuretic, stomachic, and antiseptic (Ezike et al., 2009). In southeast Nigeria the unripe whole fruit is used in the management of ulcer (Ezike et al., 2009). The whole unripe fruit (including seeds), cut into cubes and macerated in water 3-4 days, after which it is sieved. Half a glass of the extract is usually taken three times daily and is claimed to be highly effective. In south-south Nigeria also the seeds are macerated in lime water, sieved, and a glass is taken in the morning before meal for seven days and it is also claimed to effect a permanent cure of peptic ulcer.

1.11 Aim and Objectives of the Study
This study is aimed at ascertaining the anti-ulcerogenic activity of chloroform-ethanol extracts of Carica papaya fruit (pulp), seed and skin (peel) on diclofenac-induced gastric ulcers in rats.

The specific objectives of the study are as follows:

1. To ascertain the part of the C. papaya fruit that has the highest activity.
2. To provide the scientific validity for its use as an anti-ulcer remedy in traditional Nigerian medicine.
MATERIALS AND METHODS

2.1 Materials

2.1.1 Animals

Thirty six (36) apparently healthy male albino rats (Wistar strain) weighing 156-291g which were used for this study were obtained from the Animal Unit of the Faculty of Veterinary Medicine, University of Nigeria, Nsukka, Enugu State of Nigeria. The Animals were maintained at 12-hour light and 12-hour dark conditions in the Animal House for three weeks before the commencement of the experiment. Food pellets and water provided ad libitum.

2.1.2 Instruments/Equipment

The instruments used in this study include waterbath and chemical balance (Gallenkamp, England), centrifuge (3500rpm, pic, England), adjustable micro pipette (Perfect, USA), pH meter (Pye unicam 293, England), refrigerator (Kelvinator, Germany), spectrophotometer (Janeway 6405 uv/vis.), weighing balance (brand scientific and instrument company), measuring cylinder (pyrex), syringe (Alvera Industries Limited), No 1 Filter paper (Whatman), rotatory evaporator, and sterile surgical blade (Gamma Radiation).

2.1.3 Chemicals and Reagents

The chemicals and reagents used for this study were of analytical grade obtained from Merck (Germany), BDH Chemical Limited (England), May and Baker Ltd, (England) Rie-De-Haen Hannow (Germany), Hopkins and Wiliams Essex (England).

2.2 Methods

2.2.1 Collection of Plant Materials.

Fresh unripe matured fruits of Carica papaya used for this work were obtained from Orba village near Nsukka in June 2010 and were identified at the herbarium, Department of Botany, University of Nigeria, Nsukka by Mr. Paulinus O. Ugwuozor.

2.2.2 Preparation of Extract

Fresh unripe matured Carica papaya fruits (pulp) after removal of the skin (peels) and seeds were pulverized using a commercial grinder. The same treatment was applied to the seeds and skin respectively to obtain the following.

Weight of wet sample (pulp) =2.53kg
Weight of wet sample (seed) = 1.42kg
Weight of wet sample (seed) = 929g

Extraction was done using chloroform and ethanol in the ratio of 2:1, hence three liters (3) of chloroform and 1.5 litres of ethanol was used to soak the pulp sample for 24hrs, filtered with whatman No 1 filter paper and the filtrate concentrated with rotary evaporator to obtain 3.7g chloroform pulp and 3.5g ethanol pulp extracts. Two litres of chloroform and one liter of ethanol was used to soak the skin sample for 24hrs, filtered with whatman No 1 filter paper and the filtrate concentrated with rotary evaporator to obtain 3.1g chloroform skin and 3.2g ethanol skin extract and finally two litres of chloroform and one litre of ethanol was used to soak the seed sample for 24hours, filtered with whatman No 1 filter paper and the filtrate concentrated with rotary evaporator to obtain 9.8g chloroform seed and 3.9g ethanol seed extracts.

2.2.3 Treatment of Animals

The animals were randomly divided into nine groups. Groups I, 2 and 3 rats were pretreated with chloroform pulp, seed and skin extracts respectively for five days before treatment with the non-steroidal anti-inflammatory drug (NSAID) diclofenac. Groups 4, 5, and 6 rats were pretreated with ethanol pulp, seed and skin extracts respectively for five days before treatment with diclofenac. Group 7 rats (negative control) represented the vehicle group treated with diclofenac only. Group 8 rats (positive control) were pretreated with cimetidine and then diclofenac. Group 9 rats (standard control) were not exposed to any treatment but fed with normal rat feed with water.

2.2.4 Dose Selection and Mode of Administration

All the animals were fed orally with the aid of feeding tube. A single predetermined dose of the extract (300 mg/kg b.w.) suspended in 5 ml/kg 3% tween 20 which was the lowest dose with anti-ulcer activity was used to dissolve the extracts. Then the non-steroidal anti-inflammatory drug, Diclofenac sodium, used as the ulcerogenic agent was given at a dose of 100 mg/kg b.w.

2.2.5 Pharmacological Studies

2.2.5.1 Diclofenac Induced Gastric Lesions
All the groups of animals after the five days pretreatment period were kept for overnight fasting but with free access to tap water. The animals in groups 1 to 6 were treated with the extract (300 mg/kg b.w.). Group 7 rats (negative control) were administered the vehicle (5ml/kg 3% tween 20); Group 8 rats (positive control) were pretreated with the anti-ulcer drug, cimetidine (100mg/kg) while Group 9 rats (normal control) did not receive any form of treatment. After 30 minutes of the last administration of the extract, diclofenac was administered to the animals in Groups 1 to 8. After 8 hours of treatment with diclofenac, the animals were sacrificed by a blow on the head.

2.2.5.2 Collection and Preparation of Stomach Samples

The abdominal region was opened and the stomach carefully removed from the body. The stomach was fixed by inflating with 10% formaldehyde in saline, opened along the greater curvature, pinned flat on a cockboard and observed with hand lens (x10). The gastric lesions were counted. The glandular portion of the stomach was scrapped and 1g of the portion was suspended in 4ml of ice cold physiological saline and homogenized. The homogenate was then centrifuged at 3500 rpm for 10 minutes and the supernatant was used for biochemical analyses.

2.2.5.3 Ulcer Index

The ulcer index (UI) of the control group and the UI and preventive ratio (PR) of the treated groups were calculated using the following relations: UI = (A*B)/100, where A = degree of ulceration and B = percentage of group ulcerated, and PR = [(UI_U/ UI_p) /UI_U]*100, where UI_U = Ulcer index of ulcerated group and UI_p = Ulcer index of the protected group. Degree of ulceration (DU) was calculated using the relation DU = (total ulcer score / number of ulcerated animals) (Ezike et al., 2009).

2.2.6 Biochemical Estimations

The glandular portion of the stomach was scrapped and 1g of the portion was suspended in 4ml of ice cold physiological saline and homogenized. The homogenate was then centrifuged at 3500 rpm for 10 minutes and the supernatant was used for biochemical analysis:

2.2.6.1 Lipid Peroxidation

Lipid peroxidation was estimated spectrophotometrically by thiobarbituric acid reactive substances (TBARS) in this case malondialdehyde (MDA) method as described by (Buege and Aust, 1978). 2ml of thiobarbituric acid reagent (15%w/v TCA, 0.375%w/v TBA and 0.5N HCl)
were added to 1 ml of the extract. The solution was heated for 15 mins in a boiling water and allowed to cool, centrifuged at 5000rpm for 10 mins and the absorbance of the clear supernatant was read at 532nm and finally the concentration of MDA was calculated based on absorbance coefficient of MDA-TBA adduct (1.56×10^{-5}CM^{-1}M^{-1}).

### 2.2.6.2 Catalase

Catalase activity was measured based on the fact that the purple colour of permanganate is bleached by hydrogen peroxide by following the method described by (Cohen et al., 1970). When excess potassium permanganate is added to the reaction mixture, its residual colour depends on the amount of peroxide present in the sample after reaction with catalase. To 5 ml prepared stomach tissue homogenate was added 5 ml cold 6 mM hydrogen peroxide. The reaction was stopped after 3 minutes by adding 1 ml 6 N tetraoxosulphate (vi) acid rapidly and mixing. Excess potassium permanganate (7 ml) was added to the tubes, shaken quickly and the absorbance taken at 480 nm within 30 seconds. A spectrophotometric standard was prepared by adding 7 ml of 0.01 N KMNO₄ to a mixture of 5.5 phosphate buffer and 1.0 ml of 6 N tetraoxosulphate (vi) acid. Absorbance of the spectrophotometric standards and the reaction blanks were also read at 480 nm.

### 2.2.6.3 Alkaline Phosphatase Activity

Alkaline phosphatase activity was determined by the method of (Klein et al., 1960) according to the procedures outlined in the test kit of Quimica Clinica Applicada (QCA).

**Principle**

Serum alkaline phosphatase hydrolyzes a colourless substrate of phenolphthalein monophosphate giving rise to phosphoric acid and phenolphthalein which at alkaline pH values, turns to pink colour that can be photometrically determined.

The concentrations in the reagent solution are:

<table>
<thead>
<tr>
<th>Component</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-Amino-2-methy-1-propanol pH 11</td>
<td>7.9</td>
</tr>
<tr>
<td>phenolphthalein monophosphate</td>
<td>63 mM</td>
</tr>
<tr>
<td>Na₂PO₄</td>
<td>80 mM</td>
</tr>
</tbody>
</table>

**Stabilizers and preservatives**

**Procedure**

Distilled water (1 ml) was pipetted into 2 sets of test tubes labeled SA (sample) and ST (standard) respectively. Then one drop of each of the chromogenic substrates was added to the
distilled water in the two sets of test tubes. Their contents were mixed and incubated at 37°C for 5min.

A standard solution of 0.1M (alkaline phosphate) was added to the standard test tube (ST) only, followed by the addition of 0.1ml of the serum sample to the sample test tube (SA). The contents of the test tubes were mixed and incubated at 37°C for 20 min in a water bath. A colour developer (phenolphthalein monosulphate) (5.0ml each) was added to both sets of test tubes.

Absorbance of the sample against the blank (water) was read at a wave length of 550nm.

The activity of alkaline phosphatase in the serum was obtained from the formula below.

\[
\text{SA O.D} \times 30 = \text{U/L of Alkaline phosphatase}
\]

\[
\text{ST O.D}
\]

\[
\text{SA O.D} = \text{Sample Optical Density}
\]

\[
\text{ST O.D} = \text{Standard Optical Density}
\]

Normal values

- Adults = 9 – 35 U/L
- Children = 35 – 100 U/L

2.3 **Statistical Analysis**

Data were expressed as mean ± SEM. Significance was determined using one way analysis of variance (ANOVA) and results were considered significant at p<0.05.

**CHAPTER THREE**

**RESULTS**

3.1 **Extract Yield**
The different fraction of *Carica papaya* extract yielded the following after concentration in a rotary evaporator.

Chloroform pulp extract = 0.15%
Chloroform seed extract = 0.69%
Chloroform skin extract = 0.33%
Ethanol pulp extract = 0.13%
Ethanol seed extract = 0.27%
Ethanol skin extract = 0.34%

### 3.2 Effect of *C.papaya* on Diclofenac sodium induced Gastric Ulcer (group 7, negative control)

The observed gastric ulceration shown in Fig. 2a is an indication that ulcer has been induced with sub mucosal edema. However pre-treatment with *C. papaya* significantly reduced the rate of ulceration (Fig. 2b).
Fig. 2a Stomach sample of the untreated vehicle control (group 7) showing gastric lesions.

Fig 2b; Stomach sample of *C. papaya* pre-treated rat showing only a single lesion as indicated by the arrow

3.3 Effect of Chloroform-Ethanol *Carica papaya* Fruit Extracts (Pulp, Seed and Skin) on Diclofenac Induced Gastric Lesions
The result in Table 1 showed that the different parts of the *Carica papaya* fruit chloroform extracts (pulp, seeds and skin) significantly (p<0.05) inhibited the ulcerogenic effect of diclofenac induced gastric lesions as shown by the lower values of ulcer index (1.33±0.3, 2.33±0.33 and 3.00±0.58 for groups 1, 2 and 3 respectively) and higher percentage protection (92.15%, 86.27% and 82.35%) values respectively relative to the vehicle control (group 7) which had a higher ulcer index (17.00±1.15) and without protection. The different parts of the *C. papaya* fruit ethanol extract (pulp, seeds and skin) groups 4 to 6 also inhibited the ulcerogenic effects of diclofenac induced gastric lesions as revealed by the lower values of the ulcer index (11.33±0.33, 5.00±0.58, and 6.00±0.88) and higher percentage protection (40.47%, 70.58% and 64.70) values respectively relative to vehicle control group 7 (17 ± 1.15). The inhibition was also statistically significant (p<0.05).

Pretreatment with the standard anti-ulcer drug cimetidine also protected the gastric mucosa as revealed by the reduced ulcer index (9.33±0.88) and a percentage protection value (45.09%) against the ulceration induced by diclofenac compared with the vehicle control (group 7). Although the percentage protection value was lower, it was also found to be statistically significant (p<0.05).

**Table 1: Effect of Chloroform-Ethanol Extract of *Carica papaya* Fruit (Pulp, Seeds and Skin) on Diclofenac Induced Gastric Ulcers in Rats**

<table>
<thead>
<tr>
<th>Treatment Ratio (%)</th>
<th>Dose (mg/kg b.w)</th>
<th>Ulcer Index</th>
<th>Protection (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>300</td>
<td>1.33 ± 0.33</td>
<td>92.15</td>
</tr>
<tr>
<td>Group 2</td>
<td>300</td>
<td>2.33 ± 0.33</td>
<td>86.27</td>
</tr>
<tr>
<td>Group 3</td>
<td>300</td>
<td>3.00 ± 0.58</td>
<td>82.35</td>
</tr>
<tr>
<td>Group 4</td>
<td>300</td>
<td>11.33 ± 0.33</td>
<td>40.47</td>
</tr>
<tr>
<td>Group 5</td>
<td>300</td>
<td>5.00 ± 0.58</td>
<td>70.58</td>
</tr>
<tr>
<td>Group 6</td>
<td>300</td>
<td>6.00 ± 0.58</td>
<td>54.72</td>
</tr>
<tr>
<td>Group 7</td>
<td>-</td>
<td>17.00 ± 1.15</td>
<td>-</td>
</tr>
<tr>
<td>Group 8</td>
<td>100</td>
<td>9.33 ± 0.88</td>
<td>45.09</td>
</tr>
</tbody>
</table>

*Group 1: chloroform pulp extract (300mg/kg); Group 2: chloroform seed extract (300mg/kg)*

*Group 3: chloroform skin extract (300kg/kg); Group 4: Ethanol pulp extract (300mg/kg)*

*Group 5: Ethanol seed extract (300mg/kg); Group 6: Ethanol skin extract (300 mg/kg)*

*Group 7: Vehicle control (5 ml/kg 3% tween 80) (Negative control)*

*Group 8: Cimetidine (100 mg/kg) (positive control)*

**3.4 Effect of Chloroform-Ethanol Extracts of *Carica papaya* Fruit on the Malondialdehyde Concentration in Rats**
It was observed that the different parts of the chloroform-ethanol extracts of *Carica papaya* fruit significantly (*p*<0.05) inhibited lipid peroxidation estimated by measuring malondialdehyde (MDA) concentration when compared to diclofenac treated rats (group 7). The animals in group 6 treated with ethanol skin extract showed non-significant (*p*>0.05) inhibition of MDA concentration relative to the animals in groups (1 to 5) and group 7. There was significantly (*p*<0.05) increased MDA concentration in the diclofenac treated group 7 rats relative to groups 1 to 5 and 8 to 9 rats. Animals in group 8 which received the standard anti-ulcer drug cimetidine showed significant (*p*<0.05) inhibition of MDA concentration relative to the vehicle control group 7. There was significant (*p*<0.05) increase in MDA concentration when group 8 animals were compared to groups 1, 4 and 5. There was significant (*p*<0.05) decrease in MDA concentration when group 9 animals were compared to group 6 and 7 respectively.
Fig. 3: Effect of different fractions of *Carica papaya* fruit extracts on the malondialdehyde concentration of rats

<table>
<thead>
<tr>
<th>Group</th>
<th>Description</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>Chloroform pulp extract</td>
</tr>
<tr>
<td>Group 2</td>
<td>Chloroform seed extract</td>
</tr>
<tr>
<td>Group 3</td>
<td>Chloroform skin extract</td>
</tr>
<tr>
<td>Group 4</td>
<td>Ethanol pulp extract</td>
</tr>
<tr>
<td>Group 5</td>
<td>Ethanol seed extract</td>
</tr>
<tr>
<td>Group 6</td>
<td>Ethanol skin extract</td>
</tr>
<tr>
<td>Group 7</td>
<td>Vehicle (5ml 3% Tween 80)</td>
</tr>
<tr>
<td>Group 8</td>
<td>Cimetidine (100mg/ml)</td>
</tr>
<tr>
<td>Group 9</td>
<td>Food + Water</td>
</tr>
</tbody>
</table>

Fig. 3: Effect of different fractions of *Carica papaya* fruit extracts on the malondialdehyde concentration of rats
3.5 Effect of Different Fractions of Carica papaya Fruit Chloroform-Ethanol Extracts on the Catalase Activity of Diclofenac Induced Gastric Lesions in Rats

Fig. 4 shows non-significant (p>0.05) effect on the catalase activities of groups 1 to 6 rats administered various fractions of the chloroform-ethanol Carica papaya fruit extract compared with the catalase activity of the normal control rats (group 9). The vehicle control group 7 had significantly (p<0.05) reduced catalase activity compared with the rats in normal control group. Rats in group 8 which were treated with the anti-ulcer drug cimetidine showed significant increase (p<0.05) in the catalase activity compared with group 2; however, significantly (p<0.05) reduced catalase activity compared to group 9 (normal control) was observed as shown in Fig. 4.
Fig. 4: Effect of different fractions of *Carica papaya* fruit extracts on the catalase activities of rats.

Group 1=Chloroform pulp extract  
Group 2=Chloroform seed extract  
Group 3=Chloroform skin extract  
Group 4=Ethanol pulp extract  
Group 5=Ethanol seed extract  
Group 6=Ethanol skin extract  
Group 7=Vehicle (5ml 3% Tween 80)  
Group 8=Cimetidine (100mg/ml)  
Group 9=Food + Water
3.6 Effect of Chloroform-Ethanol Extracts of *Carica papaya* Fruit on the Alkaline Phosphatase (ALP) Activity of Diclofenac Induced Gastric Lesions in Rats

There was significant decrease (p<0.05), (as shown in Fig. 5) in the alkaline phosphatase activity of the chloroform pulp extract pretreated rats in group 1 compared with the rats in groups 2 to 9. There was non-significant difference (p>0.05) in ALP activity of group 2 rats compared with group 3. There was significant increase (p<0.05) in the ALP activity of the negative untreated control rats (group 7) compared with the ALP activities of all rats in other groups. Group 8 rats showed significantly (p<0.05) reduced ALP activity compared with the ALP activities of rats in groups 2 to 5 and 7 respectively. There was also non-significant difference (p>0.05) in ALP activity of rats in groups 6, 8 and 9 respectively. Normal control (group 9) showed significantly (p<0.05) reduced ALP activity compared with the ALP activities of rats in groups 2 to 5 and group 7 respectively (Fig. 5).
Fig. 5: Effect of different fractions of *Carica papaya* fruit extracts on the alkaline phosphatase activities of rats

![Graph showing the effect of different fractions of *Carica papaya* fruit extracts on the alkaline phosphatase activities of rats.]

**Groups**

- Group 1 = Chloroform pulp extract
- Group 2 = Chloroform seed extract
- Group 3 = Chloroform skin extract
- Group 4 = Ethanol pulp extract
- Group 5 = Ethanol seed extract
- Group 6 = Ethanol skin extract
- Group 7 = Vehicle (5ml 3% Tween 80)
- Group 8 = Cimetidine (100mg/ml)
- Group 9 = Food + Water

**CHAPTER FOUR**
DISCUSSION
Gastric mucosa is normally well protected against aggressive and corrosive activities of gastric acid and pepsin by defensive cytoprotective factors such as mucus, bicarbonate, prostaglandin secretions and blood flow. The mucosal concentration of prostaglandin has been found to be directly related to the gastric mucus, bicarbonate secretions and blood flow (Komoike et al., 2003) The observed increase in mean area of ulceration (gastric) in group 7 animals treated with (100mg/kg body weight) of diclofenac could be attributed to the reduction in mucosal prostaglandins biosynthesis accompanied by the inactivation of the cyclooxygenase system (COX) which mediates the synthesis of prostaglandins in the mucosa via the arachidonic acid pathway (Maroney et al., 1988). However, the different fruit parts of the chloroform and ethanol extracts of C. papaya fruit administered to the animals in groups 1 to 6 before treatment with diclofenac has significant anti-ulcerogenic activity as suggested by the result of this study. The different fractions of the chloroform and ethanol Carica papaya extracts significantly (p<0.05) reduced the ulcer index in pretreated rats relative to vehicle control (group 7). The extract significantly protected the gastric mucosa against injury suggesting cytoprotective activity and enhancement of mucosal defensive factors. The extracts produced a more potent activity than the standard reference drug cimetidine, the H₂ receptor antagonist used as positive control (group 8). This observation is consistent with previous findings (Ezike et al., 2009) since cimetidine is anti-secretory rather than enhance mucosal defensive factors. We may not be able to precisely describe the effect of these extracts on Prostaglandin (PG) synthesis at this stage; however, the chemical profile of C. papaya fruit (Ezike et al., 2009) showed the presence of mainly polar constituents, some of which have been shown to inhibit some aspects of ulcerogenesis (Das, 1986.), for example hyoscine have been used to suppress acid secretion (Akah, et al, 1998) and as such the alkaloidal content of these extracts might have been partly responsible for its anti-ulcerogenic activity. The extract also contains flavonoids known to inhibit acid secretion, increased capillary resistance and improved microcirculation, which renders the cells less liable to injury by precipitating factors (Parma, et al., 1976). Most of these effects have been attributed to the effect of flavonoids on arachidonic acid (AA) (Maroney et al., 1988).

Rats pretreated with the Carica papaya fruit extracts showed significant (p<0.05) inhibition of lipid peroxidation estimated by measuring malondialdehyde (MDA) concentration in the rats compared to the rats treated with diclofenac (group 7). Diclofenac treatment significantly (p<0.05) increased the concentration of MDA in the vehicle control (group 7); thereby suggesting oxidative damage that may be due to the accumulation of toxic free radicals
in the mucosal cells (Omotuyi et al., 2008). Lipid peroxidation is one of the molecular mechanism for cell injury and is associated with a decrease of cellular anti-oxidants such as glutathione, superoxide dismutase and catalase (Hijiora et al., 2005). Malondialdehyde is a decomposition product of peroxidised polyunsaturated fatty acids that is widely preferred for the detection of free oxygen radical in various pathological condition (Hijiora et al., 2005).

Pretreatment of rats with Carica papaya fruit extracts provided protection against the action of diclofenac by blocking lipid peroxidation which was revealed by the significantly reduced MDA concentration in the pretreated rats. This is a reflection of the anti-oxidant bioactivity of the extract.

Anti-oxidant enzymes such as catalase, superoxide dismutase, glutathione s-transferase and glutathione are present in oxygen handling cells which are the first line of cellular defense against oxidative injury (Ologundudu et al., 2008). Superoxide ions and H$_2$O$_2$ are decomposed before they interact to form more reactive radicals. Catalase is highly specific in its catalytic mode of action as it decreases the gastric mucosal damaging effect of NSAIDs. C. papaya extracts pretreatment of rats in groups 1 to 6 showed non-significant (p>0.05) effect on catalase activity. The increase in catalase activity of normal control rats (group 9) relative to vehicle control (group 7) and rats pretreated with extracts are necessary for effective anti-oxidant activity.

The different parts of the chloroform-ethanol extract with the exception of chloroform pulp extract (group 1) which had a significantly reduced ALP activity showed a significant (p<0.05) increase in ALP activity suggesting little or no effect on the ALP activity of the animals by the extracts, although ALP is not a reliable marker of the integrity of some specific tissues and organs. The vehicle control group 7 showed significantly (p<0.05) increased alkaline phosphatase activity compared to those of all other groups. The release of alkaline phosphatase has been suggested to play a role in tissue necrosis associated with various models of gastrointestinal ulceration (Ologundudu et al., 2008). The increased activity of this enzyme found in (group 7) treated with diclofenac only is in agreement with the above statement. It appears that diclofenac induces ulceration by reducing intracellular concentration of arachidonic acid (AA) in leukocytes perhaps by altering its release or uptake; this deprives the cyclooxygenase (COX) system that mediates the synthesis of prostaglandins in the mucosa of its important substrate (AA), hence a reduction in mucosal prostaglandins biosynthesis (Vane., 1971). It is therefore reasonable to assume that the observed gastric mucosal lesion induced by diclofenac is due to a deficiency of mucosal prostaglandins. The standard reference rats treated
with cimetidine used as positive control also had increased alkaline phosphatase activity comparable to those of the normal control (group 9).

In conclusion, results of this study showed that whatever the bioactive constituent is, either chloroform or ethanol can effectively extract the active constituents responsible for anti-ulcerogenic properties, however the chloroform extracts produced a greater activity than the ethanol extract. This result has therefore justified the use of extracts of unripe matured fruits of *C. papaya* in the traditional management and treatment of gastric ulcers in Nigeria.

4.1 **Suggestions for Further Studies**

The detailed phytochemical studies followed by pharmacological investigation and toxicological evaluation of *Carica papaya* fruit are still required to isolate the pure bioactive constituent in order to present new therapeutic options for the treatment of gastric ailments like ulcers.

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