EFFECT OF AQUEOUS EXTRACT OF *BRASSICA OLERACEA* LEAVES (CRUCIFERAE) ON ETHANOL-INDUCED GASTRIC ULCER IN ALBINO RATS

BY

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DEPARTMENT OF HUMAN PHYSIOLOGY
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APRIL, 2008.

DECLARATION

I declare that the work in the thesis entitled (the effect of aqueous extract of *Brassica oleracea* leaves (cruciferae) on ethanol-induced gastric ulcer in albino rats) was performed by

me in the Department of Human Physiology under supervision of Prof. M. Mabrouk and Prof. N.D.G. Ibrahim. The thesis was not previously presented in whatever form to any institute or organization other than Ahmadu Bello University, Zaria, Nigeria for the award of any degree. The information derived from the literature has been duly acknowledged in the text and a list of references provided.

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Fatimah A. Dawud **Date**

CERTIFICATION

I certify that this thesis entitled: THE EFFECT OF AQUEOUS EXTRACT OF *BRASSICA OLERACEA* LEAVES (CRUCIFERAE) ON ETHANOL-INDUCED GASTRIC ULCER IN ALBINO RATS carried out by Fatimah A. Dawud, meets the regulation governing the award

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DEDICATION

To the loving memory of my mum Hauwa Kulu.

To my greatest love Abubakar, you are the most wonderful gift ever.

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ABSTRACT

Brassica oleracea (cruciferea) is among the rich unexploited world flora with folkloric medicinal uses (Alonso, 1998). The plant juice has been reported by clinical research to be used in the treatment of gastric ulcers and prevention of ulcers induced by drugs (Cheney, 1952). Gastric lesions result from an imbalance between aggressive and defensive factors. Both endogenous and exogenous irritants (aggressive factors) include: ethanol, gastric acid, reactive oxygen species, non-steroidal anti-inflammatory drugs (NSAIDs) etc, damage the mucosal cells inducing cell death, which leads to the formation of gastric lesions (Holzer, 1998). In this study, the effect of extract *Brassica oleracea* (EBO) on ethanol- induced gastric

ulcers in albino rats was evaluated. An acute toxicity study of EBO was carried out using the method of Lorke (1983). Gastric ulcers were induced by oral administration of 70% ethanol (EtOH) 1ml/200g of rat. After 1hr, ulcer parameters were determined from mean ulcer score ($P<0.05$). Pre-treatment with oral administration of EBO at various concentrations 100, 200 and 400 mg/kg b.w. for 7days, dose-dependently protected the gastric mucosa against ethanol-induced ulcers, with preventive indices of 15, 41 and 83% and significantly decreased the percentage of ulceration by 100, 75 and 37.5% respectively. The severity of the reaction to ethanol on the gastric mucosa and cytoprotection conferred by EBO were apparent by histological assessment of the gastric mucosa. Acid concentration, total acid output and pepsin concentration of gastric secretion was determined from gastric juice collected, after 4hrs of pyloric ligation. In the normal rats, acid concentration, total acid output and pepsin concentration was found to be 1.88 ± 0.24, 6.78 ± 0.69 and 608 ± 0.23 respectively; these was significantly decreased in the control rats (70% EtOH). Pretreatment with EBO at concentrations of 100, 200 and 400mg/kg b.w showed no statistical difference when compared with the control group, while cimetidine at 50mg/kg b.w afforded a significant increase in pepsin concentration and acid concentration. Preliminary phytochemical screening of *Brassica oleracea* gave the positive test for proteins, carbohydrate and flavonoids. These results indicate that the plant *Brassica oleracea* possesses a potent antiulcer activity, which justifies the ethnomedical, claim.

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ABBREVIATIONS

AMP	Adenosine monophosphate
AO	Aldehyde oxidase
Asc^-	Ascobate radical
ATP	Adenosine triphosphate
Cag A	Cytotoxin-associated gene protein-A
CCK	Cholecystokinin
COX	Cyclooxygenase
DU	Duodenal ulcer
EBR	Extract *Brassica oleracea*
ECL	Enterochromaffin like cells
EGF	Epidermal growth factor
ENS	Enteric nervous system
EtOH	Ethanol
FGF	Fibroblast growth factor
GIT	Gastrointestinal tract
GRO	Growth regulated oncogene
GU	Gastric ulcer
H.Pylori	Helicobacter pylori
HCL	Hydrochloric acid
HCO_3^-	Hydrogen carbonate
IFN	Interferon

KCL	potassium chloride
NIDDK	National Institute of Diabetes and Digestive and Kidney Diseases
NSAID	Nonsteroidal anti-inflammatory drugs
PG	Pepsinogen
PGE_2	Prostaglandin E_2
Pic	platelets induced cytotoxin
PKA	Protein kinase A
PUD	Peptic ulcer disease
RA	Rheumatoid arthritis
RNS	Reactive nitrogen species
ROS	Reactive oxygen species
TGF	Transforming growth factor
$TNF_{\propto}$	Tumor necrosis factor alpha
TRH	Thyroid releasing hormone
UI	Ulcer index
Vac	Vacuolating cytotoxin
VEGF	Vascular endothelial growth factor
XO	Xanthine oxidase

CHAPTER ONE

INTRODUCTION

Peptic ulcer (PU) is a lesion of gastric or duodenal mucosa occurring at a site where the mucosal epithelium is exposed to aggressive factors (Govindarajan et al., 2006). Ulcers are defined as a break (lesion) in the mucosal surface ≥5mm in size, with depth to the submucosa (Del Valle, 2001). Regardless of location and etiology, chronic peptic ulcers are similar pathologically, but several factors distinguish them from one another. Duodenal ulcer (DU) is the most common accounting for three quarters of all cases, occurs most often in the first portion of the duodenum (>95%), with ~90% located within 3cm of the pylorus, they are usually ≤ 1cm in diameter but can occasionally reach 3 to 6cm (giant ulcers), while gastric ulcer (GU) tent to occur later in life with more than half of GU cases occurring in males (Del Valle, 2001).

Peptic ulcer is one of the most common diseases affecting mankind and the incidence has been estimated to range from 3 to 10% (Akimoto et al., 1998). PU represents a serious medical problem largely due to its relatively high frequency and high economic costs. In the U.S., prevalence of peptic ulcer disease (PUD) in patients seen by physicians and those indulging in self-medication is about 14 million per year and the total costs amount to more than 7 billion U.S. dollars. The annual mortality rate due to peptic ulcer is not low and deaths are due, in large part to ulcer complications such as hemorrhage and perforation (Isenberg and Soll, 1996). In Africa, PUD has worldwide distribution but its incidence varies geographically and even in the same country. It is common among West Africa, Burundi. Rwanda, eastern Zaire, the highlands of Ethiopia, and east Africa especially around Mount Kilimanjaro (Badoe et al., 1994). However in Nigeria there is no record on the incidence of peptic ulcer but seroprevalence of Helicobacter pylori in patient with peptic ulcer was carried

out in western part of the country, of 92 patient screened, 59% presented with gastritis and 41% with peptic ulcer (Mendal et al., 1992).

In spite of the vast amount of research on peptic ulcer, the etiology of peptic ulcer in most cases is unknown. However it is generally accepted that they result from an imbalance between aggressive factors and the maintenance of mucosal integrity through endogenous defense mechanisms (Piper and Stiel, 1986). Major aggressive factors are gastric acid, pepsin, ethanol, reactive oxygen species, non-steroidal anti-inflammatory drugs (NSAIDs) etc, these damage the mucosal cells inducing cell death, which leads to the formation of gastric lesions (Holzer, 1998). Defensive factors involve mucus-bicarbonate secretion and prostaglandins (Hoogerwerf and Pasricha, 2001).

The modern approach to control gastric ulceration is to inhibit gastric acid secretion, to promote gastroprotection, to block apoptosis and to stimulate epithelial cell proliferation for effective healing (Bandhopadhyay et al., 2002). However the discovery of *Helicobacter pylori* as a cause of peptic ulcer, have revolutionized the etiology and treatment of the disease (Marshall, 1995). Most drugs used are antisecretory drugs such as: Proton pump inhibitors (Omeprazole, Lansoprazole, Etc.) and Histamine H_2-receptor blocker (cimetidine, Ranitidine etc.), which extensively control increased acid secretion and acid related disorders (Martelli et al., 1998; Wolfe and Sachs, 2000). However there are reports of different adverse effects of these drugs besides high rate of recurrence of the disease (Radwan et al., 2003).

Qualitative and quantitative studies have reported that, complimentary medicine have long lasting relief for which conventional medicine has not provided a satisfactory solution either because it is insufficiently effective or because it causes many adverse effect. Besides, over the years researchers have shown that crude Plant extracts, herbal mixtures, pure active compounds isolated from the crude extract and their derivatives are some of the most

attractive sources of new drugs and have been shown to produce promising results for the treatment of PUD (Alkofahi and Atta, 1998; Hiruma-Lima et al., 2000a, 2000b, 2001). Plant extract with antiulcerogenic effect tends to reduce the offensive factors and proved to be clinically effective, better patient tolerance, relatively less expensive and globally competitive (Goel and Sairam, 2002).

Several unexploited world flora have folkloric medicinal uses and one such plant is *Brassica oleracea* (Alonso, 1998). Epidemiological studies have shown that *Brassica* vegetables, including cabbages, kale, Brussels sprouts and broccoli can play protective roles against cancers and ulcers (Van Poppel et al., 1999). Gastroprotective substances not only effectively prevent gastric mucosa from the development of erosions and ulcerations, but also accelerate ulcer healing (Zayachkivska et al., 2005), these could play a potential role in the prevention and treatment of peptic ulcer disease.

1.1 Justification

Owing to the persistent problem of recurring ulcers after treatment and the occurrence of several adverse reactions with the anti ulcer agent, new approach are constantly being pursued. Different therapeutic agents especially plant extracts are currently undertaken in various studies primarily to evaluate its antiulcerogenic potential. Juice from the plant *Brassica oleracea* has been used in folk medicine for the treatment of gastric ulcers (Gomez, 2004). The anti-ulcer effect of the plant is associated with a dose-dependent activity and reduced acid output (Repetto and Liesuy, 2002). The plant juice was also claimed by clinical researchers in 1950 for the relief of symptoms related to gastric ulcers or gastritis and also for the prevention of drugs induced ulcers (Cheney, 1952). This study lies on the bases of this information. To investigate the potential antiulcer property of this plant through basic scientific research, experimental animal model can be used.

Additionally various studies have shown that anti-ulcerogenic active Plant extract in an animal experimental model decreased acid output in the parietal cells and pepsin output in the chief cells of the stomach (Singh, 2002). These investigations also justify the study, to determine the effect of *Brassica oleracea* extract on gastric mucosal secretion, which might elucidate the possible mechanism of action of the plant.

1.2 Hypothesis

Brassica Oleracea produces a dose-dependent antiulcer activity.

1.3 Aim and objectives

The aim of this study is to investigate the effect of aqueous extract of *Brassica oleracea* on ethanol- induced gastric ulcers in albino rats.

The study is designed with the following objectives.

(a) The gross and microscopic effect of *Brassica Oleracea* extract on the gastric mucosal membrane.

(b) The effect of *Brassica Oleracea* extract on gastric mucosal secretion.

(c) To compare the effect of *Brassica Oleracea* extract and cimetidine on the gastric mucosal membrane and gastric secretion.

CHAPTER TWO

LITERATURE REVIEW

The digestive and absorptive functions of the gastrointestinal system depend upon a variety of mechanism, which involve gastric acid secretion and various digestive enzymes (Boron and Boulpaep, 2005). Ulceration occurs when the stomachs or duodenums mucosal lining cannot withstand the corrosive action of gastric juice. Gastric juice consisting of hydrochloric acid and enzyme pepsin that breaks down protein can digest any living tissue, including the stomach and duodenum. However protective mechanisms prevent the stomach from digesting itself (Del Valle, 2001).

Pathophysiology of peptic ulcer is due to an imbalance between aggressive factors and mucosal defensive factors (Hoogerwerf and Pasricha, 2001). Hydrochloric acid and pepsin are the primary aggressive factors, while defensive factors involve all elements that constitute the gastric mucosal barrier that counters the effect of aggressive factors. These elements include; mucous secretion, bicarbonate secretion, blood flow, cellular regeneration, and cytoprotection by endogenous and exogenous protective agents such as Prostaglandins and epidermic growth factors (Repetto and Liesuy, 2002). Hypersecretion of gastric acid is a pathological condition, which occurs due to uncontrolled secretion of hydrochloric acid (Sachs et al., 1995). However, even the normal rate of acid secretion may cause ulceration in the breached mucosa when some gastroprotective factors are lost (Jainu and Devi, 2006). Studies have shown that reactive oxygen species, especially the hydroxyl radical, plays a major role in oxidative damage of gastric mucosa (Das et al., 1997; Phull et al., 1995).

The mucosal defense system is highly a complex biological system that provide defense from mucosal injury and repair any injury that may occur (Del Valle, 2001). The gastric mucosa is

covered by continuous layer of mucous that protects against acids and enzymes produced by the mucosa itself (Singh, 2002). Flemstronm and turnsberg (1984) suggest that mucous cannot protect the parietal cell and zymogen cells lining the gastric glands. While, Penissi and Piezz, (1999) stated that mucous acts as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals. Prostaglandins have been shown to increase the amount of luminal mucous and the resistance of the gastro intestinal tract to injury on several experimental models. They are believed to exert their cytoprotective action through stimulation of mucous and bicarbonate secretion, the maintenance of mucosal blood flow, and by enhancing the resistance of epithelial cells to injury by cytotoxins (Repetto and Liesuy, 2002).

Tissue damage to the gastroduodenal mucosa can be induced by acute /chronic ethanol toxicity which may be associated with the generation of toxic reactive species, an unbalanced oxidant/ antioxidant cellular process (Repetto and Liesuy, 2002). Terano et al., (1989) and Hernandez et al., (2000) in their studies on Ethanol induced mucosal damage revealed that ethanol is involved in the formation of free radicals generated intracellular and extracellular. The relationship between the gastric mucosal damage and antioxidant protection is still under investigation. Several studies of cytoprotective agents especially, herbs and plants extract for peptic ulcers have intensified in an attempt to find a solution to problems of recurring ulcers and to prevent occurrence of the disease, in view of the fact that the etiology is multifactorial.

This review discuses component of aggressive and defensive factors of the gastro duodenal mucosa. The role of ethanol, reactive oxygen species, NSAID and *H. Pylori* in the pathogenesis of peptic ulcer. It also covers the medicinal properties and benefit of *Brassica oleracea*.

2.1 Physiological Anatomy of the stomach

The gastrointestinal tract refers to the entire hollow tubular system extending from the mouth to the esophagus and through the stomach, small and large intestine to end at the anus.The stomach is a muscular bag forming the widest and most distensible part of the digestive tube. It is connected above to the lower end of the esophagus and below to the duodenum. It begins at the distal end of the esophagus, enters the abdomen by piercing the diaphragm opposite the 10th thoracic vertebra (T10), to the left of the midline. The stomach is relatively fixed at its esophageal end and its pyloric end, between these positions it's quite mobile. It may be j-shape or steer horn-shaped (Guyton and Hall, 2000).

The stomach lies obliquely in the upper and left part of the abdomen, most of it lies under cover of the costal margin. It is distensible and about 25cm long. The stomach has two orifices: the cardiac orifice which lies behind the left 7^{th} costal cartilage at the level of T11 vertebra and the pyloric orifice that open into the duodenum. Its position when the stomach is empty and supine is at the level of the lower border of L1 vertebra. Two curvatures mark the surface of the stomach: the lesser curvature is concave and forms the right border of the stomach; a sharp angular notch at the lesser curvature indicates the junction of the body and the pyloric part of the stomach. The greater curvature is convex and forms the left border of the stomach; it's about five times longer than the lesser curvature (Moore and Dalley, 2006).

Anatomically the stomach is divided into four regions: cardia, fundus, body and pylorus (pyloric antrum). The cardiac part or cardia is an indefinite region around cardiac orifice which receives the opening of the abdominal part of the esophagus. The fundus is a dome – shape dilated portion to the left and superior to cardiac orifice, while the body forms the major portion of the stomach, it lies between the fundus and the pyloric antrum. The pyloric

part consist of a wide portion the antrum and a narrow canal; the pyloric antrum is separated from the pyloric canal by an inconstant sulcus, sulcus intermedius that is present on the greater curvature, it is about 7.5cm long. The pyloric canal is narrow and tubular about 25cm long; at its right end it terminates at the pylorus. A distal sphincter region that guards the pyloric orifice separates the stomach from the duodenum (Fig 2.1) (Moore and Dalley, 2006).

2.1.1 Blood supply

The stomach has a rich arterial supply arising from the celiac trunk and its branches. Most blood is supplied by anastomoses formed along the lesser curvature by the right and left gastro-omental arteries. The fundus and upper body receive blood from the short and posterior gastric arteries. The gastric veins parallel the arteries in position and course, the right and left gastric veins drain into the portal vein. While the short gastric veins and left gastro-omental veins empties in the superior mesenteric vein. A prepyloric vein ascends over the pylorus to the gastric vein (Moore and Dalley, 2006).

2.1.2 Nerve supply

The gastrointestinal tract has a nervous system of its own called enteric nervous system (ENS). The ENS plays a crucial part in regulation of gastrointestinal function such as ion transport (secretion and absorption) motility and mucosal blood flow. The ENS lies entirely in the wall of the gut, it's composed mainly of two plexuses; the myenteric or Auerback's plexus which lies between the longitudinal and circular muscle that mainly controls gastrointestinal movement. The submucosa or meissner's plexus that lies in the submucosa, controls secretion and blood flow (Guyton and Hall, 2000). There are numerous connections between these neurons which means that they act as assemblies with both in-parallel and in-series connection (Kunz and furners, 1999).

Enteric neurons contain combination of transmitter related molecules and other regulatory molecules, such as calcium-binding proteins. The combination forms a code that identifies functional sub-groups of neurons (Costa et al., 1996). Sympathetic and parasympathetic fibers connect with the ENS, although it can function on its own independent of extrinsic nerve supply stimulation of the parasympathetic or sympathetic can further activate or inhibit gastrointestinal functions (Guyton and Hall 2000).

The parasympathetic nerve fibers arise from the anterior and posterior vagal trunks and their branches. Anterior vagal trunk derived mainly from the left vagus (CN X), supplies the lesser curvature of the stomach and gives rise to anterior gastric branches. While the posterior vagal trunk derived from right vagus nerve supplies the anterior and posterior surface of the stomach. It gives off a celiac branch to the celiac plexus and continues along the lesser curvature as posterior gastric branches. Stimulation of parasympathetic nerve leads to; contraction of the stomach wall, relaxation of the pyloric sphincter, secretion rich in HCl and pepsinogen, and vasodilatation of the gastric blood vessel. The sympathetic nerve supply arises from T6-T9 segment of the spinal cord and is distributed through the plexuses around the gastric and gastro-omental arteries, its stimulation are the reverse case of parasympathetic nerve (Guyton and Hall, 2000). In the stomach these neurons can be conveniently activated by stimulation of vagus, which connect the gastrointestinal tract with the medulla. Visceral sensation is carried by sympathetic pathway, which enters the spinal cord through dorsal nerve root. The motor fibers in the vagi originate in the dorsal motor nucleus of the vagus and synapse with ganglion in cells in the myenteric and Submucosal plexuses or in exocrine glands. Apart from the enteric and autonomic neurons, the gastric mucosa and submucosa is innervated by intrinsic primary afferent neurons, which arise from cell bodies in either the dorsal root (spinal afferent) or ganglia (vagal afferent) (Kunz and furners, 1999).

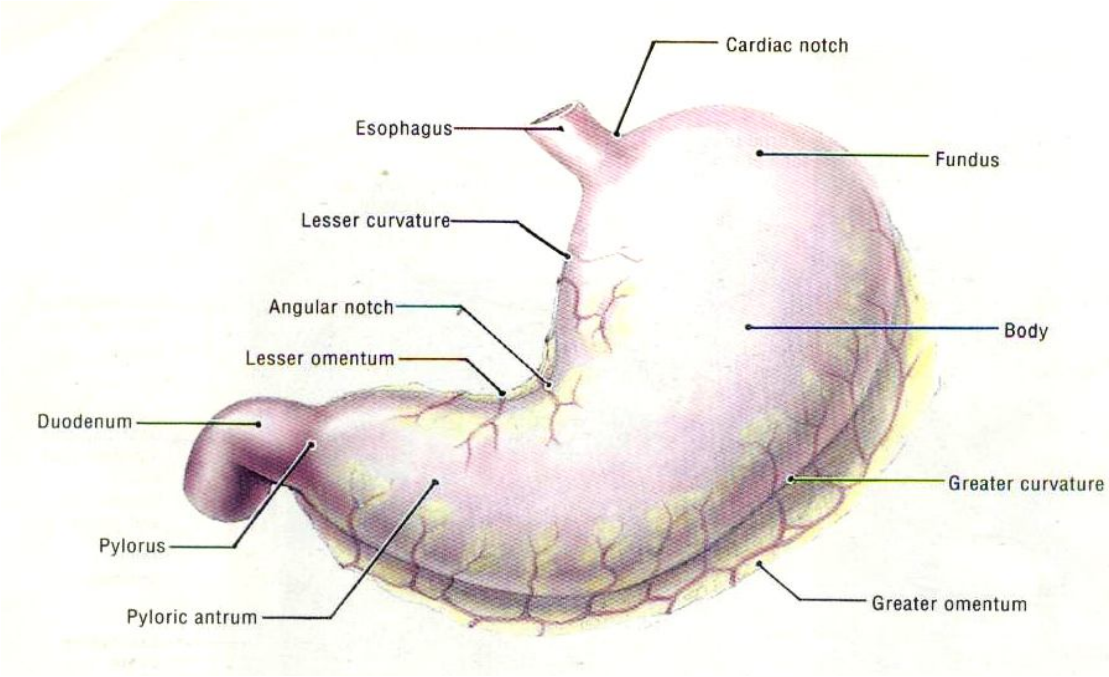


Fig 2.1: Anatomical regions of the stomach (Moore, 1991)

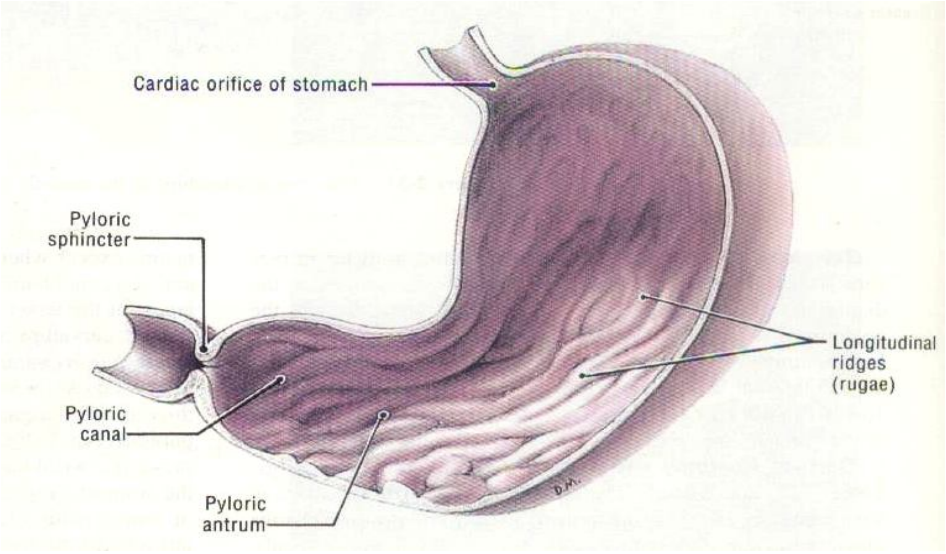


Fig 2.2: Interior of the stomach showing mucosal membrane (Moore, 1991)

2.2 Histology of the stomach

The structures that make up the wall of the GIT are the same throughout its entire length, although some regional variations exist. The common histological feature of the entire gastrointestinal tract is essentially a muscular layer lined by a mucous membrane or mucosa (Fig, 2.2). The tract has four distinct concentric layers from inside outward: mucosa, submucosa, muscularis and serosa (Wheater, 1993).

2.2.1 Mucosa

The mucosa is the innermost layer, also called the mucous membrane and generally divided into three layers: lining epithelium, a supporting lamina propria and a thin smooth muscle layer called the muscularis mucosae which form the deepest part of the mucosa; it produces local movement and folding of the mucosa and separates it from the adjacent submucosa (Singh, 2002). Ulcerations sometimes get to this layer and even beyond, in such case are accompanied by hemorrhage (Kumar and Clark, 1990).

The lining epithelium is columnar all over the gut; except in upper half of the esophagus, and the lower part of the anal canal, where it is stratified squamous epithelium. The stratified squamous epithelium has a protective function while the columnal epithelium serves either absorptive or secretory function. In the stomach the epical part of the lining epithelia cells are filled by mucin , these cells secretes mucous that protect the gastric mucosa against acids and enzymes produced by the mucosal itself. The lamina propria is made up of collagen and reticular fibers embedded in a glycosaminoglycan matrix. Prominent aggregations of lymphoid tissues as well as lymphocytes' are present in the lamina propria. At numerous places the lining epithelium dips into the lamina propria to form the walls of depression called gastric pits, which extend into the thickness of the mucosa. The muscularis mucosae

separate the connective tissue of the lamina propria from the submucosa and extent into mucosal folds but not into the villi (Singh, 2002).

2.2.2 Submucosa

The submucosa layer consists of loose areola tissue interlaced with plexus of the enteric nervous system; it connects the mucosa to the muscular layer. Its looseness permits some mobility of mucosa over the muscle. Numerous blood vessels, lymphatics and nerve fibers traverse the submucosa and smaller branches arising from them enter the mucous membrane (Singh, 2002).

2.2.3 Muscular layer

The muscular layer is also divided into three: the longitudinal, circular and oblique. The longitudinal layer is separated into two categories; a layer which is common to the esophagus and ends in the corpus, and that which originate in the corpus and spread into the duodenum. The circular layer of the muscularis continues with the circular layer of the esophagus, but absent in the fundus. The thickness of the circular layer increases in the antrum and pyloric sphincter. It does not continue into the duodenum (Wheater, 1993).

2.2.4 Serosa

Serosa is the outermost layer, its merely the visceral peritoneum that covers most part of the GIT. In places where this covering is absent, the muscle coat is covered by an adventitia made up of connective tissue (Singh, 2002).

2.3 Gastro duodenal mucosa

The arrangement of the muscular component is relatively constant throughout the tract where as the mucosa shows marked variations in four different regions: oesophago-gasric junction, the gastro-duodenal junction, ileo-caecal junction and the recto-anal junction

(Wheater, 1993). The gastro duodenal mucosa consists of surface epithelial cells which are columnar in shape. Deep to the gastric pits of the mucous membrane is packed with numerous gastric glands that open into the gastric pits (Singh, 2002).

2.3.1 Gastro duodenal mucosal glands

The glands are; gastric, cardiac and pyloric glands designated, based on their anatomic positions on the regions of the stomach. These glands may be simple tubular or coiled tubular in shape (Junqurira et al, 1998).

The gastric glands are present over the body of the stomach. They are simple or branched tubular glands (Singh, 2002). The gastric glands have characteristic parts: isthmus (middle portion), neck and base. The isthmus contains surface epithelial cells and acidophilic parietal cells; the neck has mucous neck cells and parietal cells, while the base is composed of basophilic zymogen cells or chief cells with some undifferentiated cells (Fig 2.3). In the cardiac and pyloric glands, however, the different parts of the gland contain only one type of cell, they are tall columnar with mucigenic granules. The major secretion from the pyloric gland cells is mucous and the hormone gastrin produced by triangular cells with clear cytoplasm (G cells) which are located in the upper one-third of pyloric gland (Del Valle, 2001).

2.3.2 Parietal or oxyntic cell

These cells are large, ovoid or polyhedral, with a large central nucleus.. The unstimulated parietal cells have prominent cytoplasmic tubulovesicles and intracellular canaliculi containing short microvilli along its apical surface (Fig 2.4). Upon stimulation of cells this membrane along its apical surface transform into a dense network of apical intracellular canaliculi containing long microvilli. An extensive eosinophilic (oxyntic) cytoplasm filled with mitochondria (30 to 40% of total cell volume); generate energy required for secretion.

H^+, K^+ - ATpase is expressed in the tubulovesicle membrane while acid secretion, occurs at the apical canalicular surface (Fig 2.4). The enzyme H^+, K^+ - ATpase is responsible for generating large concentration of H^+. It's a membrane-bound protein that consist of α and β subunit. The active catalytic site is found within α-subunit. This enzyme uses the chemical energy of ATP to transfer H^+ from parietal cell cytoplasm to secretory canaliculi in exchange for K^+. Parietal cells are the source of gastric HCL and in man intrinsic factor is also produced by the parietal cells. Intrinsic factor is a glucoprotein that binds ingested vitamin B_{12} and enables it to be absorbed by the intestine. A deficiency of intrinsic factor as a result of an autoimmune attack against parietal cells causes pernicious anemia (Del Valle, 2001).

The parietal cell expresses receptors for several stimulant of acid secretion including histamine (H_2), gastrin (gastrin-cholecystokinin B receptor) and acetylcholine (muscarinic, M_3).Each of these are G-protein linked, seven transmembrane-spanning receptors (Del Valle, 2001).

2.3.3 Chief Cells

Chief, peptic, or zymogen cells usually predominate in the lower portion of the fundal glands and towards the base of gastric glands. They are cuboidal or low columnar in shape with basally located nuclei (Fig 2.2). The upper two third of the cells are filled with basophilic zymogene granules. The chief cells synthesize and secrete pepsinogen, the precursor to the proteolytic enzyme pepsin. Pepsin cleaves peptide bonds, favoring those on the C-terminal side of tyrosine, phenylalanine, and tryptophan residues. Its action breaks long polypeptide chains into shorter lengths. Secretion by the gastric glands is stimulated by the hormone gastrin. Gastrin is released by endocrine cells in the stomach in response to the arrival of food. Interspersed among the parietal and chief cells are entero-chromafin-like (ECL) and mass cells, which can stimulate acid and pepsinogen secretion by releasing histamine and peptides close to the exocrine cells (Del Valle, 2001).

2.3.4 Mucous neck cells

The mucous neck cells are found at the bottom of the gastric pits, they are regenerative cells which divide and replace the surface mucous cells as they move upward to the surface where they exfoliate (Fig 2.3). This process is rapid and averages five days in normal man, the rate probably increases when there is an injury to be repaired (Hotta, 2000). The population of cells at the fundal and pyloric gland areas is very stable and there replacement is probably accelerated only after injury. The mucigenic cells, the mucous neck cells and the surface epithelium cells all secrete a highly alkaline secretion which is extruded to the surface forming a three dimensional jelly coat for the surface epithelial cells that are exposed to digestive juices (Del Valle, 2001).

2.3.5 Hormone-secreting (endocrine) cells

Near the basal part of gastric gland are endocrine cells that contain membrane bound neurosecretory granules. The endocrine cells include; G-cells and D-cells which secretes gastrin and somastostatin respectively. These two peptide hormones function as both endocrine and paracrine regulators of acid secretion. Gastrin stimulates gastric acid secretion and also a major trophic or growth factor for GI epithelial cell proliferation, while somatostatin inhibits gastric acid secretion (Boron and Boulpaep, 2005).

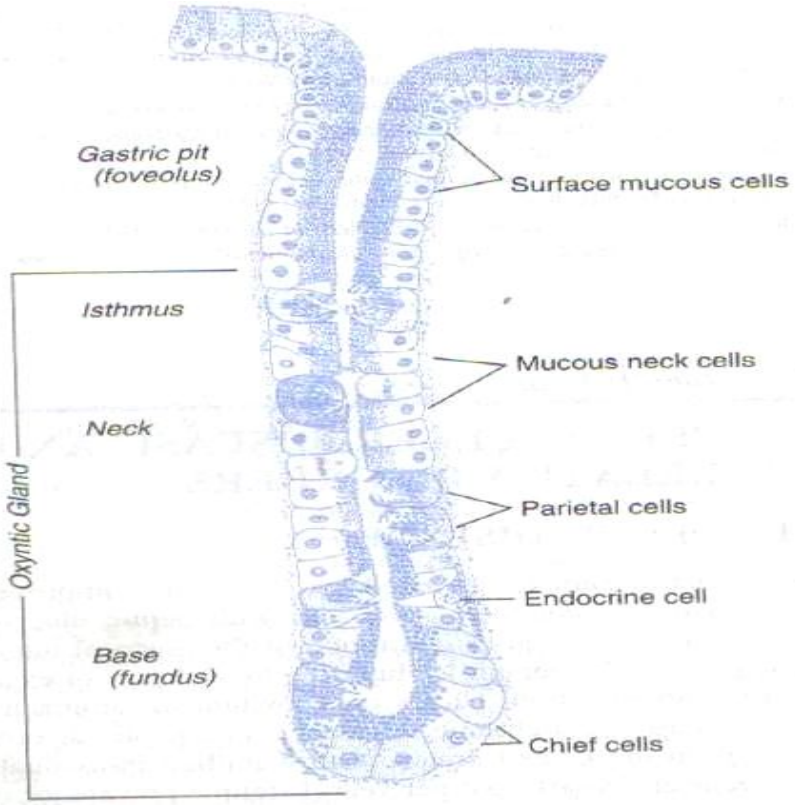


Fig 2.3: Gastric gland. (Adapted from S. Ito and R.J. Winchester: Cell Biology 16:541, 1963) (Del Valle, 2001)

2.3.6 Histology of an ulcerated mucosa

An ulcerated mucosa has an entire breakdown of epithelium and its membrane. Acute lesions are almost multiple superficial but usually heals without scarring or other sequel. Chronic lesions are deep and may penetrate to or beyond the level of the muscularis mucosae. They are usually simple and persistent, the muscular coat of the organ does not regenerate, and in such case healing is accompanied by scar or fibrosis marking the site and the mucosa may heal completely (Anderson et al, 1994).

Regardless of location and etiology, chronic peptic ulcers are similar pathologically. In addition to the focal loss of mucosal epithelial cells, these ulcers have four characteristic layers at their base: fibrinoid necrosis, exudate, granulation tissue and a fibrous scar (the

deepest layer). A layer of granulation tissue and fibrosis may be absent in acute ulcers that occur in settings of serious trauma or severe surgical or medical illnesses (Feldman, 2002).

2.4 Gastric secretion

The fluid secreted into the stomach is called gastric juice. It is a mixture of secretions of the epithelial cells and gastric glands situated in the mucosal lining of the stomach. Gastric secretory products include: salt, water, HCL, pepsinogen, intrinsic factor and electrolytes (Guyton and Hall 2000). The best-known component of gastric juice is hydrochloric acid, the secretory product of the parietal or oxyntic cell (Yao and Forte, 2003). However, hydrochloric acid and pepsinogen are the main products capable of inducing mucosal damage (Guyton and Hall 2000).

Gastric acid secretion occurs under basal and stimulated conditions. The basal acid secretion occurs in a circadian pattern, with highest levels occurring during the night and lowest level during the morning hours. Stimulated gastric acid secretion occurring by cholinergic input via the vagus nerve and histaminergic input from local gastric sources are the principal contributors to basal acid secretion (Del Valle, 2001). It is known that the capacity of the stomach to secrete HCl is almost linearly related to parietal cell numbers (Yao and Forte, 2003). Adeniyi, (1991) demonstrated that male sex hormone significantly increases gastric acid secretion in rats via its effect on parietal cell mass and male rats have a significantly greater parietal cell mass than females rats. Meanwhile Oluwale and Bolariunwa, (1990) suggested that exogenous estrogen have an inhibitory effect on gastric acid secretion and the lower incidence of peptic ulcer in females has been attributed to that. However studies by Aguwa, (1984) showed that progesterone and estrogen where found to have significant antiulcer activity in almost all the ulcer models, without any effect in acidity or volume of gastric juice in Shay's pyloric ligation model. Although, estrogen receptors have not been

located on the parietal cells of the stomach (winborn et al., 1987), but it has been shown to reduce sensitivity of tissues to histamin, it is possible that it act via histamine receptors (Csakvari et al., 1980).

2.4.1 Regulation of gastric acid secretion

This occurs primarily in three phases: Cephalic phase stimulates gastric secretion via the vagus nerve, impulses results from the sight, smell and taste of food. The gastric phase is activated once food enters the stomach. This component of secretion is driven by mechanical distention of the stomach and nutrients (amino acids and amines) that directly stimulate G-cells to release gastrin, which in turns activates the parietal cell via direct and indirect mechanisms. The third phase of gastric acid secretion is initiated as food enters the intestine and is mediated by luminal distention and nutrient assimilation. Additional neural (central or peripheral) and direct hormonal (secretin, cholecystokinin etc.) factors, play a role in counterbalancing acid secretion (Del Valle, 2001). All signals converge on the gastric parietal cell located on oxyntic gland, on several stimulant of acid secretion. The cephalic-vagal or gastric-vagal afferents directly stimulate the parietal cell via the muscarinic (M_3) type of receptors for acetylcholine while gastrin apparently activates a gastrin -cholecystokinin B receptor (CCK_B) and an oxyntic cell designated the enterochromaffin-like cells plays a central role. Gastrin and vagal afferents induce the release of histamine from the enterochromaffin-like cell, thereby stimulating the histamine (H_2) receptor on parietal cells (Fig 2.5). Binding of histamine to H_2 receptor leads to activation of adenylate cyclase and an increase in cyclic AMP. Activation of the gastrin and muscarinic receptors results in activation of the protein kinase C/phosphoinositide signaling pathway. Each of these signaling pathways in turn regulates a series of kinase cascades, which control the acid – secreting pump, H^+, K^{+-} ATpase. The discovery that different ligands and their corresponding receptors lead to activation of different signaling pathways explains the

potentiation of acid secretion that occurs when histamine and gastrin or acetylcholine are combined. Parietal cells also secrete intrinsic factor and express receptors for ligands that inhibit acid production (prostaglandins, somatostatin and EGF) (Del Valle, 2001). Somastostatin, released by gastric D cells in the antral and corpus of the stomach, is the central mechanism of inhibition of acid secretion, however several enteric hormones (Enterogastrone) and prostaglandins inhibit gastric-acid secretion through a negative-feedback mechanism (Boron and Boulpaep, 2005).

2.4.2 Mechanism of hydrochloric acid secretion

Hydrochloric acid is an important-known component of gastric juice, secreted from parietal, or oxyntic cell. It is known that the capacity of the stomach to secrete HCl is almost linearly related to parietal cell numbers. When stimulated, parietal cells secrete HCl at a concentration of roughly 160 mM (equivalent to a pH of 0.8). The acid is secreted into large canaliculi, deep invaginations of the plasma membrane which are continuous with the lumen of the stomach. Upon stimulation of acid secretion, dramatic changes exist in the morphology of the membranes of the parietal cell (Fig, 2.4). Cytoplasmic tubulovesicular membranes which are abundant in the resting cell virtually disappear in concert with a large increase in the canalicular membrane. It appears that the proton pump as well as potassium and chloride conductance channels initially reside on intracellular membranes and are transported to and fused into the canalicular membrane just prior to acid secretion. The hydrogen ion concentration in parietal cell secretions is roughly 3 million fold higher than in blood, and chloride is secreted against both a concentration and electric gradient. Thus, the ability of the parietal cell to secrete acid is dependent on active transport and the key player in acid secretion is an H+/K+ ATPase or "proton pump" located in the canalicular membrane. K^+ -ATpase are enzymes responsible for generating the large concentration of H^+. The enzyme uses the chemical energy of ATP to transfer H+ ions from parietal cell cytoplasm to

the secretory canaliculi in exchange for K^+; this ATPase is magnesium-dependent, and not inhibitable by ouabain (Yao and forte, 2003). H, K^+ - ATpase is located within the secretory canaliculus and in nonsecretory cytoplasmic tubulovesicles. The tubulovesicles are impermeable to K^+ which leads to an inactive pump in this location. The distribution of pumps between the nonsecretory vesicles and secretory canaliculus varies with parietal cell activity. Under resting conditions, only 5% of the pumps are within the secretory canaliculus, where as upon parietal cell stimulation, tubulovesicles are immediately transferred to secretory canalicular membrane, where 60 to 70% of the pumps are activated (Del Valle, 2001).

The current model for explaining acid secretion:

- Hydrogen ions are generated within the parietal cell from dissociation of water. Hydroxyl ions are formed in this process and rapidly combine with carbon dioxide to form bicarbonate ion, a reaction catalyzed by carbonic anhydrase.
- Bicarbonate is transported out of the basolateral membrane in exchange for chloride. The outflow of bicarbonate into blood results in a slight elevation of blood pH known as the "alkaline tide". This process serves to maintain intracellular pH in the parietal cell.
- Chloride and potassium ions are transported into the lumen of the cannaliculus by conductance channels, and such is necessary for secretion of acid.
- Hydrogen ion is pumped out of the cell, into the lumen, in exchange for potassium through the action of the proton pump, potassium is thus effectively recycled.
- Accumulation of osmotically-active hydrogen ion in the canaliculus generates an osmotic gradient across the membrane that results in outward diffusion of water - the resulting gastric juice is 155 mM HCl and 15 mM KCl with a small amount of NaCl (Yao and forte, 2003).

2.4.3 Control of Acid Secretion

Parietal cells have receptors for three stimulators of acid secretion, reflecting a neural, paracrine and endocrine control: Acetylcholine (muscarinic type receptor), Gastrin and Histamine (H_2 type receptor) (Fig 2.5) (Del Valle, 2001).

Histamine from enterochromaffin-like cells is considered the primary modulator, but the magnitude of the stimulus appears to result from a complex additive or multiplicative interaction of signals of each type. For example, the low amounts of histamine released Constantly from mast cells in the gastric mucosa only weakly stimulate acid secretion, and similarly for low levels of gastrin or acetylcholine. However, when low levels of each are present, acid secretion is strongly forced. Additionally, pharmacologic antagonists of each of these molecules can block acid secretion (Del Valle, 2001).

Histamine's effect on the parietal cell is to activate adenylate cyclase, leading to elevation of intracellular cyclic AMP concentrations and activation of protein kinase A (PKA). One effect of PKA activation is phosphorylation of cytoskeletal proteins involved in transport of the H^+ K^+ ATPase from cytoplasm to plasma membrane. Binding of acetylcholine and gastrin both result in elevation of intracellular calcium concentrations. Several additional mediators have been shown to result in gastric acid secretion when infused into animals and people, including calcium, enkephalin and bombesin. Calcium and bombesin both simulate gastrin release, while opiate receptors have been identified on parietal cells (Samuelson and Hinkle, 2003).

A variety of substances are capable of reducing gastric acid secretion when infused intravenously, including PGE_2 and several peptides hormones, including secretin, gastric inhibitory peptide, glucagon and somatostatin. PGE_2, secretin and somatostatin may be

physiologic regulators. Somatostatin inhibits secretion of gastrin and histamine, and appears to have a direct inhibitory effect on the parietal cell (Fig 2.5) (Del Valle, 2001).

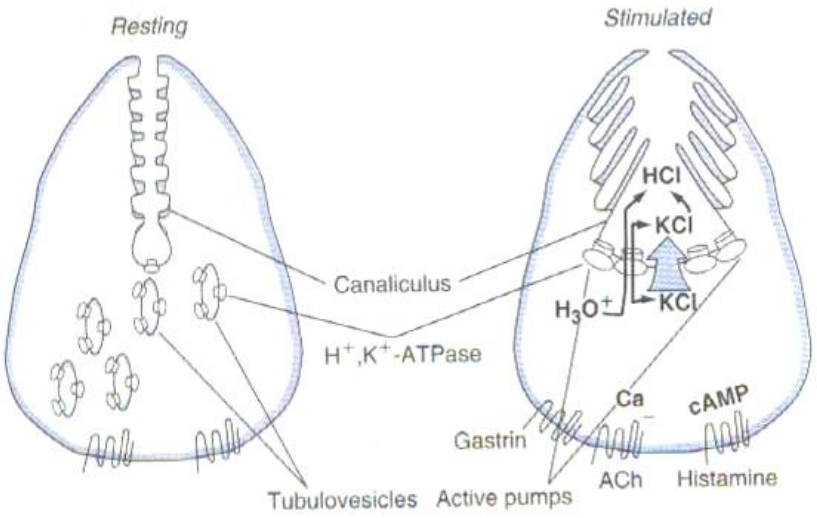


Fig 2.4: Gastric parietal cell undergoing transformation after secretagogue-mediated stimulation (Adapted from SJ Hersey, G Sachs: Physiology Review 75:155, 1995). (Del Valle, 2001)

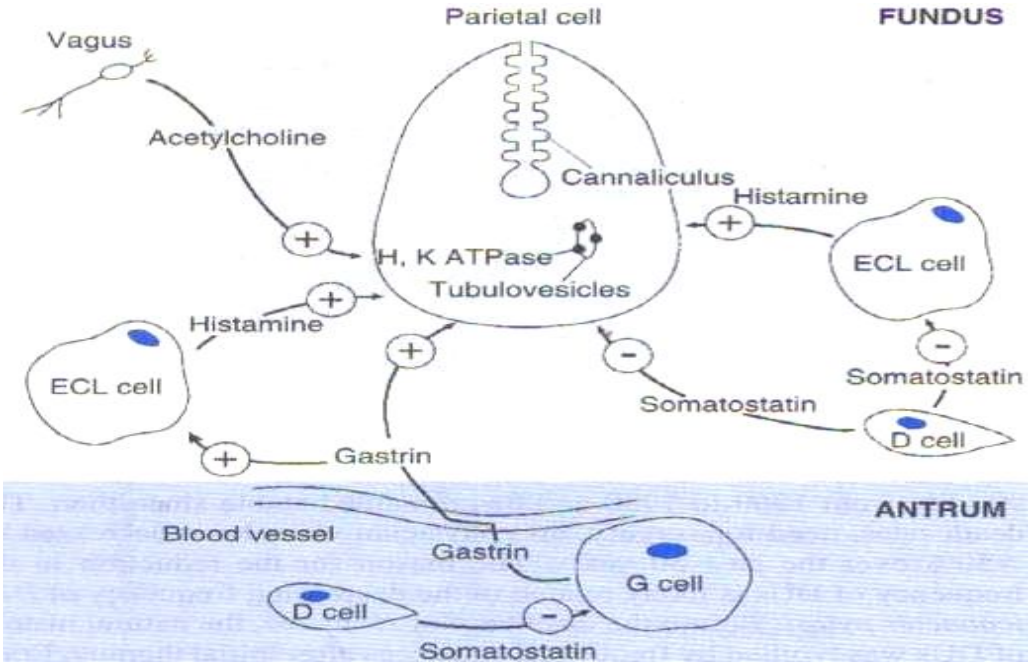


Fig 2.5: Regulation of gastric acid secretion at cellular level, enterochromafin like cell (ECL) (Del Valle, 2001).

2.4.4 Pepsinogen secretion

The chief cells in gastric glands as well as mucous cells secrete pepsinogens, a group of proteolytic proenzymes (zymogens or inactive enzyme precursors) that belong to the general class of aspartic proteinases. Pepsinogen becomes activated to form active pepsin in the presence of hydrochloric acid. Pepsinogen secretion in the basal state is approximately 20% of its maximal secretion after stimulation. Although pepsinogen secretion generally parallel that of acid secretion, however the cellular mechanism of pepsinogen secretion is quite distinct that of H^+ secretion by parietal cells (Boron and Boulpaep, 2005).

The regulation of pepsinogen secretion occurs in response to two types of signals: stimulation of the peptic cells by acetylcholine released from the vagus nerve or from gastric enteric nervous plexus and stimulation of peptic secretion in response to acid in the stomach. The acid may not stimulate the peptic cells directly but elicits enteric reflexes, thus supporting the original nervous signals to the peptic cells (Del Valle, 2001).

Release of pepsinogen across the apical membrane results from a process of compound exocytosis. This process permits rapid and sustained secretion of pepsinogen. After stimulation the initial peak in pepsinogen secretion is followed by a persistent lower rate of secretion. Two groups of agonist stimulate chief cells to secrete pepsinogen: One group acts through adenylyl cyclase and cAMP, and the other acts through increase in Ca^+. Chief cells have receptors for secretin/ VIP, β_2-adrenergic receptor, and receptor for PGE_2 which activate adenylyl cyclase. While M_3 receptor for acetylcholine, as well as receptors for gastrin/ CCK family of peptides acts through increases in Ca^+. Unlike gastric acid secretion which is stimulated via CCK_B receptor pepsinogen secretion is stimulated via the CCK_A receptor. However, the most important agonist for pepsinogen secretion is ACH release in response to vagal stimulation (Boron and Boulpaep, 2005).

2.4.5 Pepsin activity

Pepsinogen is inactive and requires activation to a protease pepsin, to initiate protein digestion. This activation occurs by spontaneous cleavage of the activation peptides but only at a pH that is less than 5.0 (Boron and Boulpaep, 2005). Moreover, Pepsin at acid pH initiates hydrolysis of any available susceptible substrates, and most importantly glycoprotein in gastric mucous (Del Valle, 2001).

Once pepsin is formed, its activity is also pH dependent. Pepsin is an endopeptidase that initiates the process of protein digestion in the stomach. Pepsin action results in the release of small peptides and amino acids (peptones) that stimulate the release of gastrin from antral G cells, and also stimulate CCK release from duodenal I cells (Boron and Boulpaep, 2005). The hydrolysis of dietary proteins by pepsin is the major factor in gastric phase of gastric secretion. Pepsins preferentially cleave peptide bonds formed by aromatic amino acids, there by exposing the particular amino acids that are the most potent stimulator of gastrin release (Taylor et al., 1982).

Peptic activity is a major indicator for proteolytic activity of gastric juice. The activity is derived from a remarkable diversity of enzymes, all of which belong to the aspartic proteinase family of enzymes. These include two types of pepsinogen; pepsinogen I (PGI), pesinogen II, (PGII) and two types of cathepsins; Cathepsin D and Cathepsin E (Samlof, 1998). Members of the aspartic proteinase enzymes are so classified because of a conserved sequence that includes an aspartic acid residue at their active sites (Pearl et al., 1987). In humans PG I are produced in the fundic gland by the chief and mucous neck cells, while PG II are also produced by chief, mucous neck cells, pyloric glands in the gastric antrum and Brunner's gland in the proximal duodenum. Zymogen granules in chief and mucus neck cells contains both PG I and PG II (Samloff, 1998). The gastric mucosa of rats does not contain

PG I (Lai et al, 1988), while PG II has the same cellular origin as in humans. Changes in their concentration in serum correlate with certain histological abnormalities of the gastric mucosa (Samloff, 1998). In human and rat gastric mucosa, cathepsin E is found mainly in the gastric surface and alveolar epithelial cells while cathepsin D is found in several cell types in gastric mucosa, including mucous neck cells, parietal cells, pyloric gland cells and macrophages (Samloff, 1998).

2.5 Gastro duodenal mucosal defense mechanisms

Protection of the gastric mucosal surface against acid and peptic ulceration involves a balance between the aggressive and defensive factors. Various researches have shown that ulcer disease does occur when this balance is lost (Hoogerwerf and Pasricha, 2001). This balance most be regulated to ensure continual mucosal protection. The defense mechanism of the gastrointestinal mucosa against aggressive factors mainly consists of functional, humoral and neuronal factors. Alkaline mucous secretion, mucosal microcirculation, and motility act as functional factors, while prostaglandin and nitric oxide act as humoral factors. All of these factors are known to contribute to mucosal protection (Mogy et al., 1990). However, recent studies implicated heat shock proteins (HSP) as additional factor utilized by the defense mechanisms of the gastric mucosa at the cellular level (Tukumi and Okabe, 2001). The HSP70 family, functions as molecular chaperone, reduces stress-induced denaturation, aggregation of intracellular proteins, and exerts its cytoprotective action by protecting mitochondria and by interfering with the stress-induced apoptotic programme (Rotukan, 2000; Saika et al., 2000). Furthermore intercellular antioxidant defense system such as glutathione and α tocopherol (vitamin E) protect the gastric mucosa from oxidative damage induced by stress and alcohol (Repetto and Liesuy, 2002). Several in-vitro studies have demonstrated that antioxidant activity inhibit oxidant enzyme and production of free radical by regeneration of α-tocopherol from α tocopheroxyl radicals (La casa et al ., 2000).

The mucosal defense system can be seen as a three-level barrier, composed of pre-epithelial, epithelial and sub-epithelial elements (Fig 2.6) (Del Valle, 2001).

The first line of defense is a mucus-bicarbonate layer, which serves as a physiochemical barrier to multiple molecules including hydrogen ions. Gastric mucosa is covered by a continuous layer of mucus. Mucus is a complex substance which is present in the mucus granules stored in the epithelial cells within the intracellular vesicles, the epithelial surface and mobile luminal mucus mixed with luminal contents (Allen et al, 1990; Hotta, 2000). 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 covers the entire gastrointestinal mucosa (Penissi and Piezz, 1999). The mucus secreted by the surface epithelial cells serves two functions; first its acts as a diffusion barrier for H^+ and also pepsins. Secondly the mucus traps a relatively alkaline solution of HCO_3^- , this HCO_3^- titrate any H^+ that diffuses into the gel layer from the stomach lumen (Boron and Boulpaep, 2005). Evidences have been accumulating which suggest that the hydrophobic lining of the stomach may be attributable to an adsorbed layer of surface active phospholipids which are key component of mucus gel that lines the gastric epithelium (Scheiman, et al, 1992). The mucus gel function in impending diffusion of ions and is considered to protect the mucosal cells against sheer forces during digestion, it also acts as a barrier to large molecules such as luminal pepsin. The adherent mucus layer of an undamaged gastroduodenal mucosa is of variable thickness but is continuous (Allen et al., 1990). Moreover, for the adherent gastroduodenal mucus to provide an effective barrier between the lumen and the mucosa, it should be continuous (Allen et al., 1990). However 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 Piezz, 1999). A decrease in gastric mucus renders the

mucosa susceptible to injuries induced by acid, aspirin, alcohol or stress (Repetto and Liesuy, 2002).

Mucin is a high-molecular-weight glycoprotein that contributes to the formation of protective layer over the gastric mucosa. It forms a hydrophobic surface with fatty acids that extends into the lumen from the cell membrane. Gastric mucosal glycoprotein plays one of the most important roles as a defensive factor of the gastric mucosa (Boron and Boulpaep, 2005).

Bicarbonate is secreted by surface epithelial cells of gastroduodenal mucosa into the mucus gel. It forms a pH gradient ranging from 1 to 3 at the gastric luminal surface (pre-epithelial barrier) and an approximation of 7.0 along the epithelial cell surface. The present model on regulation of HCO_3^- secretion suggests that vagal stimulation mediated by Ach leads to increase in Ca^{2+} which in turn stimulate HCO_3^- secretion (Boron and Boulpaep, 2005). However, bicarbonate secretion is stimulated by calcium, prostaglandins and luminal acidification (Del Valle, 2001).

The second line of defense is being provided by surface epithelial cells through several factors including mucous production, epithelial cell ionic transporters that maintain intracellular pH and bicarbonate production and intracellular tight junctions. Breaching of the pre-epithelial barrier leads to migration of gastric epithelial cells bordering a site of injury to restore the damaged region (restitution); this process occur independent of cell division and requires uninterrupted blood flow and an alkaline pH in the surrounding environment. Several growth factors including epithelial growth factor (EGF), transforming growth factor (TGF)α, and basic fibroblast growth factor (FGF) modulate the process of restitution. Larger defects that are not effectively repaired by restitution require cell proliferation. Epithelial cell regeneration is regulated by prostaglandins and growth factors such as EGF and TGF-α. Epithelial cell renewal with formation of new vessels (angiogenesis) within the injured

microvascular bed in gastric mucosa requires both FGF and vascular endothelial growth factor (VEGF) (Del Valle, 2001). If the insult is more severe, the inflammatory response leads to a decrease in blood flow and thus cell injury (Boron and Boulpaep, 2005).

The third line of defense system is composed of an elaborate microvascular system within the gastric submucosal layer. It provides HCO^-_3, which neutralizes the acid generated by parietal cell secretion of HCL. Also it provides adequate supply of micronutrients and oxygen while removing toxic metabolic by-products (Del Valle, 2001).

Prostaglandins play a central role in gastric epithelial defense/repair. Principal prostaglandin synthesized by the gastric mucosa include: PGI_2, PGE_2, PGF_2 and PGD_2. Prostaglandins have been shown to increase the amount of luminal mucus and the resistance of the gastro intestinal tract to injury on several experimental models. They are believed to exert their cytoprotective action through stimulation of mucus and bicarbonate secretion, the maintenance of mucosal blood flow, and by enhancing the resistance of epithelial cells to injury by cytotoxins (Repetto and Liesuy, 2002). Araki et al., (2000) showed that ethanol induced lesions depend on the dose of ethanol and can be prevented by PGE_2 which inhibit gastric motility and increases the mucus secretion. PGE_2 appears to bind to an EP3 receptor on the basolateral membrane of parietal cells, it inhibit acid secretion probably by inhibiting histamine's activation of parietal-cell function (Boron and Boulpaep, 2005).

Prostaglandins are derived from esterified arachidonic acid which is formed from phospholipids of cell membrane by the action of phospholipase A_2. A key enzyme that controls the rate – limiting step in prostaglandin synthesis is cyclooxygenase (Cox), which is present in two isoforms (Cox-1, Cox-2), each having distinct characteristic regarding structure, tissue distribution and expression. Cox-1 is expressed in a host of tissue including stomach, platelets, kidneys and endothelial tissues. It plays an important role in maintaining

the integrity of gastrointestinal mucosa; in contrast the expression of Cox-2 is inducible by inflammatory stimuli it is expressed in macrophages, leukocytes, fibroblast, and synovial cells (Del Valle, 2001). Inhibition of cyclooxygenase-2 is considered to be an important target for the prevention of gastrointestinal ulcers (Repetto and Liesuy, 2002). However, the highly Cox-2 selective NSAIDs have potential to provide the beneficial effect of reducing inflammation while minimizing toxicity in the gastrointestinal tract (Del Valle, 2001).

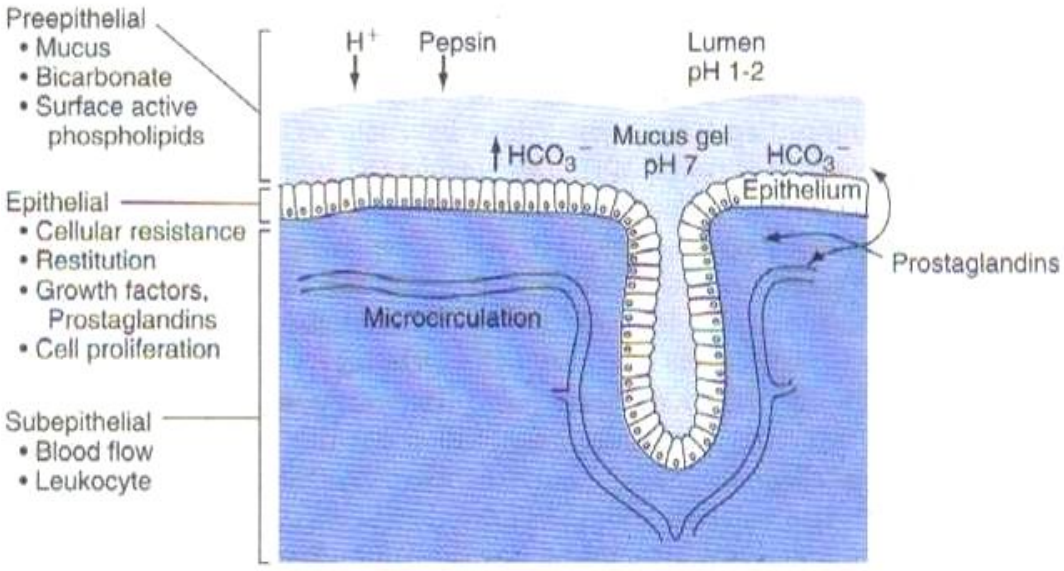


Fig 2.6: Component involving gastroduodenal defense and repair of mucosa (Del Valle, 2001).

2.5.1 Conditions that counters mucosal protection

Certain drugs that inhibits the synthesis of prostaglandins blocks the production of endoperoxide and their derivatives, these counters the mucosal protection (Guth, 1984). Alcohol produces mitochondrial permeability transition and mitochondrial depolarization, which precede cell death in gastric mucosal cells (Hirokawa et al., 1998). The loss or marked decrease in mucosal blood flow leads to ischemia and subsequent degradation of the mucosa. Hypotension usually results from severe blood loss, also from loss of fluid and electrolyte in severe burns or diarrhea that leads to dynamic ileus. A severe hypotension leads to

hypovolumic shock accompanied with marked decrease in mucosal blood flow or reperfusion, all this conditions are traumatic to mucosa and leads to disintegration of the mucosal protection covering hence bearing the underlying tissue to erosion by the corrosive gastric content, this condition thus predisposes the mucosa to ulceration (Guth,1984). Also lowering of body temperature as in cold stress induces vagally dependent acid hypersecretion mediated in part by "Thyroid releasing hormone'' (TRH) released in response to cold exposure and may be involved in the etiology of stress ulceration (Garner and O-Brien,1991)

2.6 pathogenesis of peptic ulcer

The lost of integrity of the defense mechanism and or the excessive increase in Acid-pepsin secretion combined with superimposed injury from environmental or immunologic agents forms the bases of all peptic ulcer condition. Various pathological conditions could bring about this imbalance, and also conditions that compromise the integrity of the mucosal barrier (Penissi and Piezz, 1999). Oxygen derived free radical have been implicated in the pathogenesis of a wide variety of clinical disorders and gastric damage is caused by physical, chemical and psychological factors that lead to gastric ulceration in human and experimental animals (Rao et al., 1999).

Various researches have shown that gastroduodenal ulceration occur with acute or chronic administration of ethanol (Terano et al., 1989; Repetto and Liesuy, 2002), or when the normal mucosal membrane is perturbed by infective bacteria Helicobacter pylori, associated gastroduodenitis or prolong use of non steroidal anti-inflammatory drugs (Mertz and Walsh, 1991). A decrease in gastric mucous secretion renders the mucosa susceptible to injuries induced by acid, aspirin or when the mucosal blood flow drops (Penissi and Piezz, 1999).

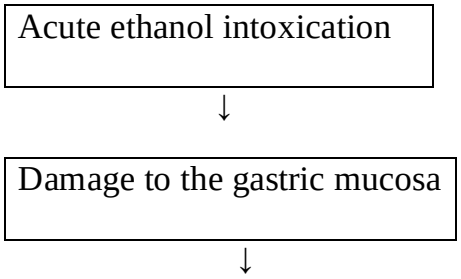
In addition oxygen derived free radicals have been implicated in the pathogenesis of a wide variety of clinical disorders and digestive system disorders such as gastrointestinal

inflammation and gastric ulcers (Repetto and Liesuy, 2002). Yoshikawa et al., (1998) showed that mucosal damage leading to gastric ulceration can be easily produced by the generation of exogenous and endogenous reactive oxygen and free radicals.

2.6.1 Pathogeneses of alcohol induced PUD

Ethanol induces gastric mucosal damage due to a direct action on the gastric epithelium causing lipid peroxidation mediated by interaction of hydroxyl radicals with the cell membrane; subsequently producing lipid-derived radicals that are extremely reactive products causing oxidative damage (Repetto and Liesuy 2002). Acute ethanol treatment induces oxidative stress, DNA damage, increased xantine oxidase activity and decreased total GSH content in the gastric mucosa cells (Gonzalez et al., 2001). In a study by Bagchi et al., (1998) reported that ethanol treatment increases superoxide anion, hydroxyl radical production and lipid peroxidation in the gastric mucosa. Kato et al., (2000) in a study examining alterations of gastric ulcer and healing responses also reported that ulcerogenic response to ethanol was prevented by inducible nitric oxide synthase and was also partially related to elevated production of prostaglandin and cyclooxigenase-2.

2.6.2 Physiological consequences of ethanol intoxication in the gastric mucosa.



- Activation of phagocytes (producing O_2^-, H_2O_2, NO and HOCl)
- Liberation of arachidonic acid, peroxide enzymatic formation for lipooxygenase and cyclooxigenase production. (Peroxide generates alcohoxyl and peroxyl radicals, which can damage other lipids and proteins)
- Liberation of ions of transition metals (Fe^{2+}, Cu^{2+}) by stimulating the change of H_2O_2 to HO. Lipoperoxides can be broken down to generate autooxidation RO_2 and RO radicals.
- Consumption of glutathione, ascorbic acid and ascorbate levels in extracellular fluids
- Convertion of xantine dehydrogenase to xanthine oxidase, the possible liberation of xanthine oxidase from the damaged cells to produce systemic damage, increasing hypoxanthine levels by rupture of the energy metabolism.

The mitochondrial damage produces an increase of electron transfer, which in turn produces O_2^-, increase in intracellular Ca^{2+} levels and triggering of nuclease activity and Ca dependent nitric oxide synthase, generating more NO and increasing the risk of $ONOO^-$ formation (Repetto and Liesuy 2002).

2.6.3 Role of *H. Pylori* infection in the pathogenesis of PUD

Researchers have long discovered that the bacterium, *Helicobacter pylori* (*H. pylori*) is a primary cause of peptic ulcers. The bacterium persistently infect the gastric mucosa of more

than half of all people worldwide causing gastritis , peptic ulcer and gastric cancers (Sela et al., 2002). The apparent role of *H. pylori* in peptic ulceration cannot be over-emphasized. *H. pylori* infection is present in virtually all patients with duodenal ulcers and about 70% of those with gastric ulcers. Early studies reported that *H. pylori* are present in 90% of people with duodenal ulcer and 80% with gastric ulcer. The relationship of *H. pylori* to duodenal ulcer is largely based upon decreased recurrence rates for duodenal ulcer disease after eradication of the bacteria (Clearfield, 1991). Several studies showed that eradication of *H. pylori* in duodenal ulcers associated with the organism will heal without acid suppression (Sharma and Ahuja, 2003).

The bacterium initially named *campylobacter pylori* is a gram-negative microaerophilic rod found most commonly in the deeper portion of the mucous gel coating the gastric epithelium. It may attach to the gastric epithelium, but under normal circumstances does not invade cells. *H. pylori* alone causes little or no effect on an intact gastric mucosa in the rat, it is strategically design to line within the aggressive environment of the stomach. The natural habitat of helicobacter pylori within the body seems to be gastric mucus and the mucus producing epithelium (Shimizu et al., 1996a; Shimizu et al., 1996b). Initially, *H. pylori* reside in the antrum, but over time, migrate towards the proximal segment of the stomach. It can survive an adverse condition by transforming into a coccoid form (dormant state).*H pylori* seeks gastric mucosa, whether is in the stomach, Barrett's oesophagus or duodenal gastric metaplasia (Dooley et al., 1988). The presence of predisposing factors leading to disruption of gastric mucosal integrity may be required for the *H. pylori* enhancement of inflammation and tissue damage in the stomach. And the continued presence of either intact *H. pylori* organisms or bacteria-free *H. pylori* filtrates in the stomachs of rats with pre-existent gastric ulcers resulted in delayed healing of the ulcers and persistence of chronic active inflammation (Ross et al., 1992). When *H .pylori* binds to cells lining the mucosa of the stomach, it uses the

same molecules that recruit immune cells to an area of inflammation, thus ensuring that the epithelium releases in response to damage from the infection (Sela et al., 2002).

2.6.4 Mechanism of H pylori induced mucosal damage

The bacterium expresses a host of factors that contribute to its ability to colonize the gastric mucosa and produce mucosal injury. *H. pylori* secretes urease, catalase, lipase, adhensins, platelet-activating factor, cytotoxin-associated gene protein (Cag A), induced cytokines (pic B), and vacuolating cytotoxin (Vac A). Multiple strains of these bacteria exist and are characterized by their ability to express several of these factors. *H.Pylori* is able to facilitate gastric residence, induce mucosal injury and avoid host defense due to its ability to express these factors. Cag A and Pic A are the virulence factors that contribute to its pathogenicity. These virulence factors in conjunction with additional bacterial constituent can cause mucosal damage. The *H.pylori* cell wall lipopolysaccharide is implicated as a virulence factor causing weakening of the mucus barrier through interaction with gastric epithelial cell receptors, glycocalyx and secreted mucus, protein of the extracellular matrix and bioactive polypeptide. Exposure to *H.pylori* lipopolysaccharide leads to the histological abnormalities of acute gastritis and increase epithelial cell apoptosis (Piotrowski, 1998). The secretion of urease allows the bacteria to reside in the acidic medium of the stomach; it generates free ammonia which can damage epithelial cells. Other research has also suggested that the action of urease in creating ammonia causes a rise in pH which could destabilize the mucus layer (Sidebotham et al., 1991). The bacteria produce surface factors which are chemotactic for neutrophils and monocytes which release myeloperoxidase producing hypochlorous acid yielding, in turn, monochloramine in the presence of ammonia. Both hypochlorous acid and monochloramine are strong free radicals that can destroy mammalian cells. It makes proteases and phospholipases that break down glycoprotein lipid complex of the mucous gel, thus reducing the efficacy of the first line of mucosal defense. *H. pylori* expresses adhensins

which facilitate attachment of the bacteria to gastric epithelial cells, and lipopolysaccharides which unlike in other gram-negative bacteria, has low immunologic activity (Del Valle, 2001).

The host respond to *H. pylori* infection by mounting an inflammatory response, contribute to gastric epithelial cell damage without actually providing to gastric immunity against the infection. Neutrophills, T lymphocytes and plasma cells are components of the chronic inflammatory response. A number of cytokines are released from both epithelial and immune modulatory cells in response to *H. pylori* infection including proinflamatory cytokines, tumor necrosis factor (TNF) α, interleukin (IL) 1α/β, and IL-6 Interferon (TFN) γ and granulocyte-macrophage colony stimulating factor. Several chemokines such as IL-8 and growth regulated oncogene (GRO) α, involved in neutrophill recruitment/activation, and rantes, which recruits mononuclear cells, have observed in *H .pylori* infected mucosa (Del Valle, 2001). Other events may act alone or in concert with *H. pylori* to promote peptic ulceration. Gastric hyperacidity, when present, may be strongly ulcerogenic. Similar to duodenal ulcer and gastritis, eradication of *H.pylori* infection is associated with gastric ulcer healing (Taylor and blaser, 1991). Moreover Jost and Marbet, (1998) observed that patients with successfully eradication of H.pylori remain largely free of symptoms and ulcer reoccurrence.

2.6.5 Non-steroidal anti-inflammatory drugs induced PUD

Non-steroidal anti-inflammatory drug and aspirin (collectively called NSAIDs) use, is the second most common etiologic factor for peptic ulcer disease and a major factor for peptic ulcer complications (Voutilainen et al., 2001). Nonsteroidal anti-inflammatory drugs (NSAIDs) represent one of the most commonly used medications worldwide and the pathogenesis of NSAIDs induced ulcers is multifactorial. Unlike *H. pylori*-related peptic ulcers, which more often occur in the duodenal bulb, NSAID induced ulcers typically occur

in the stomach. Though, NSAIDs can also cause ulcers in the jejunum, ileum, or colon, areas where there is little or no acid-pepsin. These ulcers are not actually peptic ulcers (Feldman, 2002). A gastric or duodenal ulcer associated with NSAID use is classified as a peptic ulcer, and it usually heals with potent acid antisecretory therapy like proton pump inhibitors, even if NSAID use is continued (Feldman, 2002).

The interaction between NSAIDs and Helicobacter pylori infection on ulcer development is controversial (Voutilainen et al., 2001). Rheumatoid arthritis (RA) patients using NSAIDs continuously are at a greatly increased risk of developing both *H. pylori*-negative and positive GUs; Corticosteroid use are an independent risk factor for ulcer development. Most (RA) patients have chronic gastritis, whereas reactive gastritis is rarely associated with continuous NSAID use in RA patients (Voutilainen et al., 1998). Not everyone treated with NSAIDs develops ulcers even fewer develop complication, raising the possibility that only a subpopulation of individuals with enhanced susceptibility develop ulcers with NSAIDs therapy (Soll et al., 1989). Genetic predisposition for NSAIDs induced ulcer has been proposed, Blood type O NSAID Patients have been found with greater frequency of mucosal lesions (Semble et al., 1987). The role of NSAIDs in the pathogenesis of uncomplicated peptic ulcer is less well understood (Voutilainen et al., 2001).

2.6.6 Mechanism of NSAID induced PUD

Chronic use of NSAIDs suppresses mucosal prostaglandin synthesis, a critical role in maintaining gastroduodenal mucosal integrity and repair. Prostaglandins (PGs), in particular PGE2, enhance these protective mechanisms, and are therefore thought to comprise a major gastric mucosal defensive factor (Miller, 1983). Experimental evidence has shown that incidence of ulcers in patients that take this drugs is as a result of failure of the mucosa to develop resistance to the damaging effect of these drugs (Wallace et al., 1995).

The most important mechanism appears to be inhibition of cyclooxygenase-1 (COX-1), the rate-limiting enzyme in GI prostaglandin synthesis. NSAIDs reduce pain and inflammation by blocking this enzyme, which is involved in the production of prostaglandins. An excess of PGs causes intestinal contraction and inflammation. The enzyme cyclooxygenase converts free eicosapoyenoic acid, to cyclic endoperoxides key intermediates in PGs generation. Prostaglandins normally protect the GI mucosa from damage by maintaining mucosal blood flow and increasing mucosal secretion of mucus and bicarbonate. Blockade of COX-1 activity by NSAIDs reduces prostaglandin synthesis and thus lowers GI mucosal blood flow and secretion of mucus and bicarbonate (Feldman, 2002). In addition to unwanted adverse effects in the gastrointestinal tract (Burke et al., 2006). It therefore follows that interruption of prostaglandin synthesis can impair mucosal defense and repair, thus facilitating mucosal injury via a systemic mechanism. Most NSAIDs including aspirin block both COX-1 and COX-2, unlike COX-1, COX-2 is induced and expressed at inflammatory sites but not in the normal GI tract (Del Valle, 2001). Selective COX-2 inhibitors (coxibs) such as celecoxib and valdecoxib are analgesic and anti-inflammatory but seem to cause less GI ulcer formation than currently available cyclooxygenase inhibitors when used in recommended doses (Feldman, 2002). Synthetic NSAIDs like aspirin cause mucosal damage by interfering with prostaglandin synthesis, increasing acid secretion and back diffusion of H+ ions, resulting in overproduction of leukotrienes and other products of 5-lipoxygenase pathway (Rao et al., 2000).

The extent to which the damaging effects of NSAIDs on the stomach are topical rather than systemic is unclear. Many NSAIDs, such as aspirin, are acidic and thus nonionized in the acidic stomach, where they can be absorbed and initiate gastric mucosal damage. However, NSAIDs (e.g., ketorolac) given by potential injection are ulcerogenic, as are so-called NSAID prodrugs, such as Sulindac and Nabumetone (neither drug inhibits gastric prostaglandins until

it is metabolized to its active form after GI absorption). Evidence suggests that acute mucosal damage by NSAIDs (i.e., hemorrhages and erosions, but seldom ulcers) is mainly caused by the topical damaging effects of NSAIDs. Chronic ulcer formation, often with complications such as bleeding and perforation, is mainly the result of the systemic effect of NSAIDs on prostaglandin synthesis by the GI mucosa (Feldman, 2002).

2.6.7 Role of reactive oxygen, lipid peroxidation, and antioxidants in the pathogenesis of peptic ulcer

Free radicals are atoms or molecules containing unpaired electrons (Punchard and Kelly, 1996). The unpaired electron is highly reactive molecule (causes oxidative damage) or is passed from molecule to molecule causing turning the recipient into a free radical and neutralizing the donor. In the case of lipid peroxidation, there is a chain reaction which involves both damage and passing of radicals. Cellular macromolecules are vulnerable to free radical damage: lipids, protein and nucleic acids can all be damaged. Most free radicals in biological systems are derivatives of oxygen ("Reactive Oxygen Species", **ROS**), but there are also derivatives of nitrogen ("Reactive Nitrogen Species", **RNS**). Conditions of high pro-oxidant activity due to free radicals are often described by the phrase oxidative stress, however. The most reactive and damaging free radicals are the hydroxyl radical (especially) and the peroxynitrite ($ONOO^-$) radical (Wei, 2002).

Mitochondria are the major source of free radicals, there are numerous other sources. The inflammatory cytokine, Tumor Necrosis Factor alpha (TNF−α) stimulates free radical production by mitochondria. Ultraviolet light (UV) produces free radicals. Free radicals are released from white blood cells (neutrophils) associated with inflammation. Neutrophils use oxidative free radicals (superoxide, hydrogen peroxide, hypochlorus acid and hydroxyl) to kill bacteria. The lysosomal enzyme myeloperoxidase catalyzes the production of bactericidal hypochlorite from hydrogen peroxide and chloride ions. Free radicals are generated by

eicosanoids from arachidonic acid during ischemia-reperfusion injuries. During reperfusion the endothelial enzyme xanthine oxidase converts oxygen to superoxide, which can react with nitric oxide to produce peroxynitrite (Wei, 2002).

2.6.7.1 *Lipid peroxidation*

The process of lipid peroxidation is mediated by the interaction of hydroxyl radicals with the cell membrane; subsequently producing lipid-derived free radicals such as conjugated dines and lipid hydroperoxides. These radicals are known to be extremely reactive products that cause oxidative damage (Repetto and Liesuy, 2002). Studies have shown that reactive oxygen species, especially the hydroxy radical, plays a major role in oxidative damage of gastric mucosa in almost all forms of gastric ulcers (Phull et al., 1995; Das et al., 1997). It is well established that lipid peroxidation is one of the reactions set into motion as a consequence of the formation of free radical in cells and tissues (Compoeti, 1993).

Free radicals affect lipids by initiating peroxidation. Superoxide (O_2^-), hydrogen peroxide (H_2O_2) and hydroxyl radical (OH) are important ROS causing tissue damage (Fridovich, 1986); and lipid peroxide level is an indicator for the generation of ROS in the tissue (Rao et al., 2004).

2.6.7.2 *Antioxidants*

Antioxidants are compounds that protect cells against the damaging effects of reactive oxygen species such as; singlet oxygen, superoxide, peroxyl radicals, hydroxyl radicals and peroxynitrite. An imbalance between antioxidants and reactive oxygen species results in oxidative stress, leading to cellular damage (Burler and Miranda, 2003). Antioxidants are known to inhibit lipid peroxidation and scavenge free radicals (Di Carlo et al., 1999). Antioxidants also inhibit other free radical mediated processes protecting the human body from several diseases attributed to the reaction of radicals (Czinner et al, 2001).

Antioxidants are notable for boosting the immune system because immune system cells in the bloodstream are so easily accessed by free radicals as well as by antioxidants. Cells of the immune system (like T-Cells, B-Cells and Macrophages) have membranes that are particularly rich in long-chain unsaturated fatty acids (such as arachidonic acid or EPA). For this reason (and also because of their mobility and functions) immune system cells are more vulnerable to free radical oxidation than other cells (Meydani, 1990).

Antioxidant molecules neutralize free radicals by accepting or donating an electron to eliminate the unpaired condition. Typically this means that the antioxidant molecule becomes a free radical in the process of neutralizing a free radical molecule to a non-free-radical molecule. But the antioxidant molecule will usually be a much less reactive free radical than the free radical neutralized. It may be readily neutralized by another antioxidant and/or it may have another mechanism for terminating its free radical condition. Molecules with loosely-held hydrogen atoms can use those hydrogen atoms like electrons to neutralize free radicals. The hydrogen atoms are called reducing equivalents, and the molecules having such hydrogen atoms are said to be in a reduced state (Wei, 2002).

Natural antioxidant enzymes manufactured in the body provide an important defense against free radicals. Glutathione peroxidase, glutathione reductase, catalase, thioredoxin reductase and superoxide dismutase are the most important antioxidant enzymes (Marklund, 1982). Moreover, glutathione peroxidase is found throughout the cell, whereas catalase is often restricted to peroxisomes (Schriner, 2005). The enzyme superoxide dismutase converts two superoxide radicals into one hydrogen peroxide and one oxygen. To eliminate hydrogen peroxide before the Fenton Reaction can create a hydroxyl radical, organism’s use catalase and/or glutathione peroxidase (Marklund, 1982).

The recognized dietary antioxidants are vitamin C, vitamin E, selenium, and carotenoids. However, recent studies have demonstrated that flavonoids found in fruits and vegetables may also act as antioxidants, they contain chemical structural elements that may be responsible for their antioxidant activities (Alanko, 1999). Vitamin C (ascorbate, $AscH^-$), for example, can donate a hydrogen atom to a free radical molecule (**R**·) thereby neutralizing the free radical while becoming an ascorbate radical itself (·**Asc**$^-$, or **Asc**$^{\cdot-}$, in different notation). But the ·**Asc**$^-$ free radical is very stable because of its resonance structure (shown by the dashed lines in Fig 2.7). Moreover, **AscH**$^-$ is readily regenerated from ·**Asc**$^-$ with NADH or NADPH-dependent reductases (Hossain, 1985). Although Vitamin E (tocopherol, **Toc**) is only moderately effective against singlet oxygen; it is the most effective antioxidant for terminating the chain reactions of lipid peroxidation in cell membranes. Intracellular antioxidant such as glutathione and tocopherol protect the gastric mucosa from ethanol-induced oxidative stress (Repetto and Liesuy, 2002).

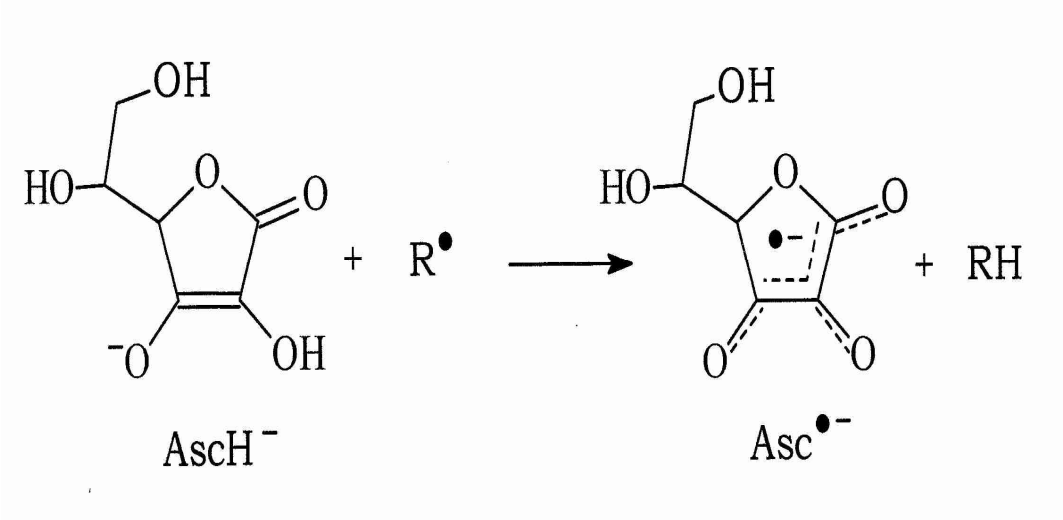


Figure 2.7: Ascobate free radical formation

2.6.7.3 ***Flavonoids***

Flavonoids are polyphenolic compounds that are ubiquitous in nature and are categorized, according to chemical structure, into flavonols, flavones, flavanones, isoflavones, catechist,

anthocyanidins and chalcones. Over 4,000 flavonoids have been identified, many of which occur in fruits, vegetables and beverages (tea, coffee and fruit drinks). The flavonoids have aroused considerable interest recently because of their potential beneficial effects on human health-they have been reported to have antiviral, anti-allergic, antiplatelet, anti-inflammatory, and antitumor and antioxidant activities (Burler and Miranda, 2003). Galati et al., (2003) describe that there is a considerable correlation between the antioxidant and the antiulcer activity of flavonoids.

Scientific research has confirmed the efficacy of several plants extract for the treatment of gastro duodenal disease. The anti-ulcergenic effect of some of these plants is related to their flavonoid content, micronutrients activity, organic and inorganic compounds, (Czinner et al., 2001). Phytochemical studies by Steinmetz and Putter, (1996) and Raloff, (2000) shows that *Brassica oleracea* is such plant with high level of glutamine, S- methionine and flavonoids among many other phytochemicals with medicinal properties. Several in vitro studies have demonstrated that flavonoids can scavenge superoxide, dihydroxyl and peroxide radicals, affecting various steps in the arachidonate cascade via cyclooxygenase-2 or lipooxygenase (Alanko, 1999). In addition to this important effect, they have membrane-stabilizing properties and also affect some processes of intermediary metabolism and inhibit lipid peroxidation in different system (Repetto and Liesuy, 2002). Some of them have shown to increase the mucosal content of prostaglandins and mucus in gastric mucosa, showing cytopprotective effects. Several of them prevent gastric mucosal lesions produced by various experimental ulcer models and protect the gastric mucosa against different necrotic agent (Alanko, 1999).

Three distinct mechanism of protection by flavonoids have been identified:

- The alteration of GSH metabolism
- Quenching of reactive oxygen species

- The inhibition of Ca^{2+} influx that signals the last step in the cell death cascade induced by glutamate (Ishige et al., 2001).

However several mechanisms may account for the antioxidant activity of flavonoids in addition to free radical scavenging, i.e., chelating of transition metal ions by preventing metal ions from producing free radical reactions, inhibition of oxidant enzyme or production of free radicals by cells, and regeneration of α-tocopherol from α-tocopherolxyl radicals. They promote gastric mucosa formation, diminish acid mucosal secretion, and inhibit the production of pepsinogen and decrease ulcerogenic lesions (La Casa et al., 2000).

2.7 Peptic ulcer management

Prior to the discovery of the H_2 receptor antagonists and the realization of the role of *H. pylori* infection in the development of peptic ulcers, therapy consisted of the intake of antacids, anticholinergics and a bland diet. The introduction of antisecretory drugs, H_2 receptor antagonists and proton pump inhibitors, along with the utilization of upper gastrointestinal gastroscopy and the development of antibiotic combination therapies for eradication of *H. pylori* have revolutionized peptic ulcer therapy and resulted not only in healing the ulcer, but in curing many cases (Sharma and Ahuja, 2003).

Management therefore include general measures that would aid the physiological mechanisms help heal the body, drug therapy and surgical therapy if needed. General measures include discontinuation of the use of agents that causes injury to the mucosa or prevent normal healing process. It is usually recommended that patients eat frequent small meals consisting of foods known to be non-irritating. For many patient fatty, highly spiced, heavy or fibrous foods are likely to provoke pain (Anderson et al., 1994). Recurrences may occur within 12 months. However, the rate of recurrences when *H. pylori* are eradicated is

much lower. To prevent the risk of relapse, maintenance therapy should be in instituted (Sharma and Ahuja, 2003).

2.7.1 Antiulcer drugs

- *Antisecretory agents*

Histamine H_2 – Receptor Antagonists, enhance healing by suppressing secretion of gastric acid as well as its hydrogen ion concentration through blockade of the H_2 – receptors. In addition, these agents reduce the gastric stimulating effects of gastrin and acetylcholine. The H2 - receptor antagonists are: cimetidine (Tagamet®), famotidine (Pepcid AC®), nizatidine (Axid®), and ranitidine (Zantac®) (ACPE, 2003).

- *Proton Pump Inhibitors*

Proton pump inhibitors act as gastric antisecretory agents by binding to hydrogen/potassium adenosine triphosphatase located in the parietal cells. Inhibition of this enzyme system, which is known as the proton pump, can blockade the final step in the pathway for the secretion of hydrochloric acid by these cells. Proton pump inhibitors usually accumulate in the parietal cell secretory canaliculi, where the drug is converted to its active metabolite, and ultimately binds with the sulfhydryl groups of H^+/K^+ exchange ATPase, thereby inactivating the proton pump and inhibiting the transport of H^+ to the gastric lumen. The duration of action of the proton pump inhibitors usually is prolonged due to the fact that it will require more time for additional enzymes to be synthesized. Might decrease the incidence of NSAID-induced peptic ulcers and can be used to help prevent peptic ulcers in chronic NSAID users at high risk (Le and fantry, 2007).

Proton pump inhibitors includes: Omeprazole (Prilosec®), Lansoprazole (Prevacid®) and Esomeprazole (Nexium®) a newer proton pump inhibitor that is being used quite often for gastroesophageal reflux disease (GERD) (ACPE, 2003). Recent studies show that

Omeprazole blocks stress-induced increased generation of OH^- and associated lipid peroxidation, and protein oxidation indicating that its antioxidant role plays a major part in preventing oxidative damage (Biswas et al., 2003).

- *Mucosal protectants*

Sucralfate (Carafate®) is a complex that consists of an anionic sulfated disaccharide and aluminum hydroxide (ACPE, 2003). It is used as an antiulcer drug due to the protection it provides against acid and pepsin. In the acidic medium of the stomach, sucralfate undergoes polymerization and cross-linking reactions, resulting in a viscid and adhesive gel that adheres and forms a complex with the fibrinogen in the ulcer lesion. This action results in the establishment of a barrier to pepsin, and bile (ACPE, 2003).

- *Antacids*

Antacids are alkaline compounds that are commonly used in the treatment of peptic ulcers. Their main function is to lower the acidity of the gastric fluid. By doing so, they tend to deactivate pepsin. Consequently, healing is promoted, and pain is relieved. Antacids have no inhibiting effect on gastric secretion. With the exception of sodium bicarbonate, antacids are poorly absorbed, and, therefore, they have no effect on systemic pH. The most widely used antacids are aluminum hydroxide, calcium carbonate, magnesium salts, and bismuth compounds. Aluminum hydroxide is insoluble and has a long duration of activity when compared to other antacids (ACPE, 2003).

- *Anticholinergics*

Anticholinergics may be used as antiulcer agents due to their ability to diminish gastric secretions and to depress gastric motility. However, these muscarinic antagonists require high doses, to inhibit acid secretion. At such doses, they produce a number of side effects such as dry mouth, constipation, blurred vision, and urinary retention. These drugs are not used very often. Pirenzepine is a muscarinic antagonist that produces selective blockade of the

muscarinic receptors that regulate gastric acid secretion. Because of its selectivity, pirenzepine is capable of producing acid inhibition without causing pronounced anticholinergic adverse reactions (ACPE, 2003).

- *Antibiotics*

Several antibiotics may be used to combat *H. pylori* infection. Quadruple therapy consists of a combination of bismuth compounds, antisecretory agents such as proton pump inhibitor or H_2 - receptor antagonists and two antibiotics composed of tetracycline and metronidazole (ACPE, 2003). A triple therapy utilizes a combination of proton pump inhibitors or H_2 - receptor antagonists, clarithromycin,and amoxicillin administered for 14 days. Both therapies have resulted in eradication of *H. pylori* (Sharma and Ahuja, 2003)

2.8 History of *Brassica oleracea*

Brassica oleracea is a cruciferous vegetable and a member of the Brassica family, its common name is Cabbage. It grows in slopes by the sea and cultivated as vegetables, mainly as Herbaceous, annual or perennial plant. Cabbage can be considered one of the most ancient of the more common vegetables. There is evidence that it has been in cultivation for more than 4,000 years. Centuries of cultivation have produced other forms of the cabbage family: kale, kohlrabi, cauliflower, broccoli, and Brussels sprouts. There are many kinds of cabbage: red, white, and green; with smooth or crinkled leaves; and with round, flattened, oblong, or conical heads. The most widely preferred type is the conical shape, or Wakefield cabbage. Other varieties include round or Savoy, flat headed or Dutch, celery or Chinese cabbage (Gomez, 2004).

The *Brassica oleracea* plant is native to the Mediterranean region of Europe. After the domestication of plants began, people in the Mediterranean region began growing this first

ancient "cabbage" plant as a leafy vegetable. Because leaves were the part of the plant which was consumed, it was natural that those plants with the largest leaves would be selectively propagated for next year's crop, this resulted in large and larger-leafed plants slowly being developed as the seed from the largest-leafed plants was favoured. By the 5th century B.C., continued preference for ever-larger leaved had led to the development of the vegetable known as kale. Kale is known botanically by the name *Brassica oleracea* variety acephala which translates to mean "cabbage of the vegetable garden without a head." A continued favoritism of these plants for hundreds of successive generations resulted in the gradual formation of a more and more dense cluster of leaves at the top of the plant. Eventually, the cluster of leaves became so large, it tended to dominate the whole plant, and the cabbage "head" we know today was born. This progression is thought to have been complete in the 1st century A.D. This plant was named *Brassica oleracea* variety *capitata,* which translates to "cabbage of the vegetable garden with a head" (Baldwin, 1995).



Plate 2.1 ***Brassica oleracea l***

2.8.1 Pytochemical Constituents in Cabbage:

4-Me-glucobrassicin, 4-OH-glucobrassicin, Dithiolthiones, Flavonoids, Glucoiberin Gluconapin, Glucoraphanin, Glutamine, Indole-3-carbinol, Isothiocyanates Oxazolidinethione , Phenolic compounds , Progoitrin, R-goitrin, Sinigrin and Thiocyanates (Steinmetz and Potter ,1996; Raloff , 2000).

Cabbage composition per 100g of raw edible portion

Energy	**25.0 kcal=105kj**
Carbohydrate	3.13g
Protein	1.44g
Fat	-
Fiber	2.30g
Vitamin A	13.0µg
Vitamin B_1	0.050mg
Vitamin B_6	0.096mg
Niacin	0.550
Vitamin B_{12}	--
Folate	43.0µg
Vitamin C	32.2mg
Vitamin E	0.105mg
Phosphorus	23.0mg
Iron	0.590mg
Calcium	47.0mg
Magnesium	15.0mg
Zinc	0.180mg
Cholesterol	-
Sodium	18mg
Calorific Value	27

(Pamplona-Roga, 2004)

2.8.2 Medicinal uses and Benefits of cabbage

Cabbage's best-known medicinal use is as a poultice - the leaves of the wild or cultivated plant are blanched, crushed, or chopped, and applied to swellings, tumors, and painful joints. Cabbage leaves eaten raw or cooked aid digestion and the breakdown of toxins in the liver- so the Romans' eating it to ease a hangover was in fact quite sensible. Cabbage is also detoxifying and helpful in the long-term treatment of arthritis. The high vitamin C content of cabbage has made it useful in the prevention of scurvy. Other medical uses include Prostate cancer, Stomach cancer, and Toxic shock syndrome. Raw cabbage is a fair source of vitamin A, a good source of vitamin B1. Cabbage is alkaline in reaction, high in cellulose or roughage, and has very low calorie content. The outside leaves of cabbage (those leaves that

are very green) have as much as 40 percent more calcium than the inside leaves (Pamplona-roga, 2004)

Raw cabbage juice is a hallowed folk remedy for ulcers. It turns out that cabbage and its juice contain considerable amounts of two compounds with anti-ulcer activity, glutamine and S-methyl-methionine (Cheney, 1952). Cabbage juice or broth, or the cooked leaves are used in the treatment of stomach and intestinal ulcers, various kinds of stones and for diseases of the respiratory system (Gomez, 2004). There have been studies which found raw cabbage juice to be useful in treating ulcers. The studies most often mentioned were conducted between 1949 and 1952 at Stanford University by Dr. Garrett Cheney had 100 peptic ulcer patients who drink four glasses of raw cabbage juice daily. The patients reported dramatically less pain, and x-ray examination confirmed faster healing time. There was no other change in there diet, and they did not have drug therapy. 81% of the patients were symptom-free within one week; over two–thirds were better in just four days. The average healing time for patients given standard hospital treatment was over a month .He called his cabbage juice "vitamin U" (Cheney, 1952).

Cabbage contains glucosinolates and their breakdown products that alter the induction of glutathione S-transferase (GST), NADPH, and Quinone oxidoreductase (NQO). The GST family of detoxification enzymes is responsible for conjugating electrophilic compounds with glutathione, creating a more water-soluble and usually non-cytotoxic compound to be excreted. Cabbage juice stimulated tumor necrosis factor a ($TNF_{\propto}$) and interleukin-1 (IL-1) in the normal rats (Komatsu, 1997).$TNF_{\propto}$ and IL-1 are the primary cytokines produced by activated macrophages. Additionally $TNF_{\propto}$ plays an important role in antitumor, antiviral, immunoregulatory, and inflammatory responses. Cabbage contains substances capable of preventing the formation of malignant tumors and can even stop the growth. A variety of

experimental and statistical studies are being conducted that demonstrate the anticarcinogenic effect of cabbage and other cruciferous plant (Chen, 1995). The effect is due to two types of pytochemicals: sulfurated glycosides and Indole derivative compounds (Stoewsad, 1995). When administered orally both substances are capable of inhibiting the formation of malignant tumors in laboratory animals that have been previously treated with carcinogens such as benzopyrene (Metha, 1995).

These protective effects of *brassicas* against cancer are plausible due to their relatively high contents of glucosinolates, which are broken down by hydrolysis to species like indoles and isothiocyanates that are able to influence antioxidant response element, and thereby influence several processes related to chemical carcinogenesis (Van Poppel et al., 1999).Ethanol extract of the roots of *Brassica rapa* (Chinese cabbage) (EBR) treatment significantly inhibited increases in the activities of kidney tissue aldehyde oxidase (AO) and xanthine oxidase (XO) activity. Based on available evidence it was conclude that the (EBR) eliminates oxidative stress and benefits renal function (Yang-Hee et al., 2006).

Clinical results coupled with the results of earlier trials strongly suggest that Glutamine-rich foods and particularly cabbage: Support immune function, especially in trauma and critically ill Patients, and in high-training athletes (Gleeson, and Bishop, 2000), Inhibit oxygen free radical formation that causes inhibition of bactericidal activity of neutrophils; reduced proliferation of T and B lymphocytes; and inhibition of natural killer cell cytotoxic activity, protect and improve the function of the gastrointestinal tract (Hickman, 1998), Help prevention of clinical infection related to bacterial translocation across the gut wall (Buchman, 1996; Buchman, 1999) and also improve healing of the small intestinal mucosa after chemotherapy and protect lymphocytes and attenuate gut permeability during radiochemotherapy (Elia and Lunn, 1999).

CHAPTER THREE

MATERIAL AND METHODS

3.1 Instruments

The instruments used are as follows; water bath (Grand JB Series. Sherpreth Cambridgeshire SGB, 6GB.england), Spectrophotometer (6405uv/vis.Jenway), centrifugation machine, digital top loading balance (GF-2000), Cages with high wide- meshed bottom. All other equipment and materials used were routine laboratory models.

3.2 Chemicals and drugs

- Casein (BDH chemical company Ltd England).
- Trichloroacetic acid crystals (MSerk).
- Phenol red (Rhone-poluenc) powder.
- Hydrochloric acid, 0.01N. BDH Chemicals Ltd Poole England.
- Sodium hydroxide (Na 0H) granule (40gm).

3.3 Plant Material

The plant was obtained from an irrigation farm in Sabon-Gari Zaria. It was identified and authenticated in the herbarium of the Department of Biological Sciences, Ahmadu Bello University, Zaria, and Voucher number 2427 was deposited.

3.4 Experimental animals

Animals used in this study were obtained from the animal house of the Department of Anatomy, Faculty of medicine, Ahmadu Bello University, Zaria. Ninety six (96), male and female albino rats weighing 150-200g were used. All animals were housed in environmentally controlled room (25 ± 2^{o}C, 12 hours' light/dark cycles) in the animal house of Department of Human Physiology, Faculty of Medicine, Ahmadu Bello University, Zaria. They were maintained in dry cages and fed with their usual commercial rat chow. All animals

were deprived of food for 36hrs but allowed water ad-libitum before subjecting them to ulcerogens.

3.5 Methods

3.5.1 Extract Preparation

About 100g of pounded of *Brassica Oleracea* was chopped to small pieces and 100 ml of distilled water added and thoroughly mixed in a blender. The mixture was placed in a mechanical shaker and exhaustively macerated. The extract was concentrated in water bath at 40°C and the filtrate was evaporated in-vacuum to a residue which was subsequently preserved as the extract.

3.5.2 Acute toxicity studies of extracts *Brassica oleracea* in rats

The plant extract was screened for toxicity using the method of Lorke (1983) for oral routes in rats. The method consists of two phases using 13 rats. In the first phase 3 groups of 3 rats each was administered the extract in doses of; 10, 100, and 1000 mg/kg body weight orally and were observed for signs of toxicity and death for 24hours. In the second phase, 4 groups each containing 1 rat was administered new doses of the extract: 1,600, 2,900 and 5000 mg/kg b.w orally, they were also observed for any sign of toxicity and death for 24 hours.

3.5.3 Preliminary phytochemical screening

3.5.3.1 *Test for Flavonoids*

- *Shinoda test:* 2.0 g of EBO was dissolved in 50% methanol by heating. Magnesium metal and 5-6 drops of concentrated hydrochloric acid were added. Appearance of an orange colour would indicate the presence of flavonoids (Ciulie, 1994).
- *Using 10% NaOH and dilute HCl:*

1.0 ml of 10% NaOH was added to 2.0 ml of extract. A yellow precipitate which turns colourless on addition of excess dilute HCl indicates presence of flavonoids (Ciulie, 1994).

3.5.3.2 *Test for Alkaloids*

2.0g EBO was taken into a test tube and dissolved in 2.0 ml of distilled water then it was separated into three separate test tubes, 2-3 drops of Dragendoff's, Wagner's and Mayer's reagents were added seperately. An orange red precipitate/turbidity with Dragendoff's reagent, a brown precipitate with Wagner's reagent or white precipitates with Mayer's reagent would denote the presence of alkaloids (Ciulie, 1994).

3.5.3.3 *Test for Saponins*

0.5 g of EBO was taken in a test tube. 5.0 ml of water was added and vigorously shaken.

A persistent froth that lasted for at least 15 minutes would indicate the presence of Saponins (Brian and Turner, 1975).

3.5.3.4 *Test for Carbohydrates*

2.0 ml of EBO was taken in a test tube and Molisch's reagent was added followed by concentrated H_2SO_4. A brown ring formed at the interface indicates presence of carbohydrates (Brian and Turner, 1975).

3.5.3.5 *Test for reducing Sugars*

2.0 g of EBO was placed into a test tube and dissolved in 2.0ml of distilled water. This was diluted with another 2.0 ml of distilled water followed by addition of Fehling's solution and the mixture warmed. Brick red precipitate at the bottom of the test tube denotes the presence of reducing sugars (Brian and Turner, 1975).

3.5.3.6 *Test for Tannins*

- *Using ferric chloride solution:*

2.0g of the EBO was taken into a test tube and dissolved in 2.0ml of distilled water. 2-3 drops of 5% ferric solution was added. A green or greenish-black colouration indicates the presence of tannins.

- *Using lead sub acetate solution:*

Extract was dissolved in a little amount of ethanol and 2.0ml of H_2O. A few drops of lead sub acetate were then added. A white precipitate indicates presence of tannins (Ciulie, 1994).

3.6 In vivo studies to investigate the effect of aqueous extract of *Brassica oleracea* on ethanol induced gastric mucosal ulcers in albino rats.

Design of the study

A total of 96 albino rats were used in the study. They were classified into two groups, consisting of forty-eight rats each as follows:

Group A (N = 48) considered as gastric ulcer study group.

Group B (N = 48) considered as gastric secretion study group

Each of the group (A and B) was further divided into 6 groups of 8 rats and marked as groups 1, 2, 3, 4, 5 and 6 as follows:

Group 1 (n = 8) was given distilled water orally.

Group 2 (n = 8) was treated with 70% (EtOH) at 1ml/200g of the rat.

Group3 (n = 8) was pre-treated with extract of *B. oleracea* orally at 100mg/kg b.w. daily prior to oral administration of 70% (EtOH) at 1ml/200g of the rat.

Group 4 (n = 8) was pre-treated with extract of *B. oleracea* orally at 200mg/kg b.w. daily prior to oral administration of 70% (EtOH) at 1ml/200g of the rat.

Group 5 (n = 8) was pre-treated with extract of *B. oleracea* orally at 400mg/kg b.w. daily prior to oral administration of 70% (EtOH) at 1ml/200g of the rat.

Group 6 (n = 8) was given cimetidine orally at 50mg/kg b.w. for 1hr Prior to oral administration of 70% (EtOH) at 1ml/200g of the rat.

Groups (1, 3, 4 and 5) were given their respective treatments for seven days prior to oral administration of ethanol to induce ulcer. However, group 6 was given cimetidine at 50mg/kg b.w. orally for 1hr only, before ethanol administration while group 2 was treated with ethanol only to serve as control.

3.6.1 Group a: gastric ulcer studies

3.6.1.1 *Ethanol (EtOH)-induced ulcers in rats*

The rats to be experimented were kept fasting for 36hr in separate cages with raised wide-meshed wire bottom to ensure complete emptying of the stomach and to prevent coprophagia before subjecting them to ulcerogen, but allowed water ad-libithum and the extract of *B. oleracea* was given to pre-treatment groups. Subsequent, 70% ethanol (1m/200g, 1h) was given orally to induce gastric ulcers (Hollander et al., 1985). An hour after the ethanol administration the animals were sacrificed by cervical dislocation followed by an abdominal incision. The stomach was removed and afterwards incised along the greater curvature. It was washed gently in running tap water and gastric mucosa spread on a filter paper for gastric lesions assessment. A 2x hand lens was used to locate the ulcers. Stomach ulceration was expressed in terms of: ulcer score, ulcer index, preventive index and % of ulceration.

3.6.1.2 *Determination of Ulcer parameters*

Ulcer score was assessed using the method of Ohara *et al.*, (1995) severity of mucosal damage was assessed on a score grade as follows;

Grade 0 = No lesions

Grade 1 = Hemorrhagic erosions < 5

Grade 2 = Hemorrhagic erosions >5 or small linear ulcers

Grade 3 = many small linear ulcers (>2mm) or a single linear ulcer (>2mm)

Grade 4 = many linear ulcer of marked size.

Mean Ulcer score: it's calculated by dividing total number of ulcers in animals in a group by number of animals in that group.

Ulcer index: (U.I)

The mean ulcer score of a group of animals similarly treated multiplied by hundred

U.I = mean ulcer score × 100

Preventive index:

The preventive effect on the severity of ulceration was calculated according to the method of Hano et al., (1976).

Preventive index = U.I control –U.I. treated ⁄ U.I. control × 100

Percentage of ulceration:

The ulcer incidence (%) was calculated by dividing the number of animals with ulcer by the total number of animals times one hundred.

3.6.2 Group b: gastric secretion studies

3.6.2.1 *Collection of gastric juice*

Gastric Juice was collected by pyloric ligation using the method described by Raymond et al., (1995). Animals were anaesthetized using diethyl ether an hour after ethanol administration. The abdomen was incised (about 2.5cm long) below xiphistenum and the pylorus ligated taking care not to interfere with blood supply to the stomach and duodenum. The abdomen was closed and the animals were kept back into their cages and received neither food nor water. Four hours after which the animals were killed by decapitation. A ligature was placed around the cardia of the stomach and the stomach was removed, washed with saline and dried

by plotting on a filter paper. Small opening was made at the fundic end along the greater curvature and gastric secretions collected, centrifuged and studied for gastric juice parameters.

3.6.2.2 *Analysis of gastric juice*

i) Determination of the volume of gastric juice after centrifugation

ii) Determination of titratable acidity and total acid output.

To a given volume of gastric juice (0.2ml) was titrated against 0.01N NaoH using an end point of pH7.0 as determined colorimetrically with phenol red as indicator it was calculated as milliequivalent per liter (mEq/L) which is equal to the number of ml of 0.1 NaoH required to neutralize 100ml of gastric juice(Davenport,1972).

Titratable acidity = Volume of 0.01N NaoH (ml) which neutralize 1ml of gastric juice ⁄ 10 ×100

- *Total acid output*

Total acid output was calculated as milliequivalents by multiplying the volume in milliliters by the acid concentration in milliequivalents per liter (Okabe et al., 1975).

Total acid output = Volume of gastric juice × volume of 0.01NaoH which neutralize gastric juice

iii) Determination of proteolytic activity of gastric secretion:

The proteolytic activity of pepsin in the gastric juice was determined by spectrophometric method (Jorgensen, 1954).

Principle:

Pepsin activity was assessed in terms of the amount of proteases produced after incubation of the casein (substrate) with pepsin for thirty minutes at temperature 37^{o}C

Reagents:

- Casein solution 1%(w/v):

1gm of pure casein was dissolved in 100ml of 0.1N HCl and boiled for 15minutes.after boiling the volume was adjusted to 100ml by 0.1N HCl in a volumetric flask.

- Tricloroacetic acid solution (T.C.A.A) 6% (w/v)

Aqueous solution of tricloroacetic acid was prepared by dissolving 6grams of T.C.A.A crystals in 100ml of distilled water.

Procedure

1. 3ml of the casein solution was added to a test and blank test tubes containing 0.2ml sample of gastric juice from the same animal. Enzymatic reaction was stopped in the blank test tube by immediately adding 10ml of T.C.A.A solution.
2. The two test tubes were incubated in a water bath at 37 ^{o}C for 30 minutes
3. At the end of incubation period the tubes were taken out from the bath and enzymatic reaction was stopped in the test, test tube by addition of 10ml T.C.A.A solution and shaken.
4. The solution in the test tubes was allowed to stand for 10minutes and was filtered using No.1 whattman filter paper.
5. The absorbance readings of the filtrate were measured at 280mu wave length of ultraviolet light using the sphectrophotometer.

3.6 Statistical Analysis

Results were expressed as mean $\pm$ S.E.M. Statistical analysis was performed by analysis of variance (ANOVA). $P < 0.05$ was considered as significant. When a significant result was obtained, all pairwise multiple comparison procedures (Tukey test) was performed. Sigma Stat 2.0 (Systat Software, Inc. Point Richmond, CA).

CHAPTER FOUR

RESULT

4.1 Preliminary Phytochemical screening

Phytochemical constituent of aqueous extract *Brassica oleracea* (EBO).

Constituents	Remark
Flavonoids	+
Alkaloids	–
Saponins	–
Carbohydrates	+
Proteins	+
Tanins	–

+ = present - = absent

4.2 Acute toxicity studies

Oral administration of EBO for 24hrs showed no symptoms of toxicity and none of the rats died up to a dose of 5000 mg/kg b.w.

4.3 Effect of aqueous extract of *Brassica oleracea* and Cimetidine on gastric mucosal membrane of ethanol induced gastric ulcers in albino rats

The gastric mucosal membrane of ethanol induced gastric ulcers in albino rats was assessed based on ulcer parameters from the Mean ± S.E.M (P> 0.05) of ulcer scores. The effect of EBO and cimetidine with normal and control groups are shown in tables 4.2 and 4.3 respectively.

4.3.1 *Control*

Normal rats showed a mean ulcer score of 1.00 ± 0.44 SEM while 70% EtOH treatment in the control rats, significantly induced gastric ulcers with mean ulcer score of 5.75± 1.87 SEM (Table 4.1). There was a significant increase of percentage difference in all the ulcer parameters between the normal and control group as shown in Table 4.1.

Table 4.1: Comparison of gastric ulcer parameters in Normal and control (70% EtOH)

	Normal	Control EtOH	% Difference
Ulcer score	1.00	5.75	475 ↑[a]
Ulcer index	100	575	475 ↑[a]
% ulceration	33.3%	100%	66.7 ↑
Preventive index	-	-	-

↑- increase in % difference,
a = significant at $p < 0.05$

Table 4.2: Effect of extract *Brassica Oleracea* (EBO) and Cimetidine on gastric mucosal membrane of ethanol induced ulcers in albino rats with the Normal group.

	Normal	Control EtOH	100mg (EBO)+ EtOH	200mg (EBO)+ EtOH	400mg (EBO)+ EtOH	50mg Cimetidine + EtOH
Ulcer score	1.00 ± 0.44	5.75 ± 1.87[a]	4.88 ± 0.93[a]	3.38 ± 0.94[a]	1.00 ± 0.54[ns]	3.75 ± 0.94[a]
Ulcer index	100	575[a]	488[a]	338[a]	100[ns]	375[a]
%ulceration	33.3%	100%	100%	75%	38%	100%
Preventive index	-	-	15%	41%	83%	35%

Data represent the Mean ± SE of Mean. n = 8
a = significant at $p < 0.05$, ns = not significant

Table 4.3: Effect of EBO and Cimetidine on gastric mucosal membrane of ethanol induced gastric ulcers in albino rats with the control group

	Control EtOH	100mg (EBO) + EtOH	200mg (EBO) + EtOH	400mg (EBO)+EtOH	50mg Cimetidine + EtOH
Ulcer score	5.75 ± 1.87	4.88 ± 0.93^{ns}	3.38 ± 0.94^{a}	1.0 ± 0.54^{a}	3.75 ± 0.94^{ns}
Ulcer index	575	488^{ns}	338^{a}	100^{a}	375^{ns}
% ulceration	100%	100%	75%	38%	100%
Preventive index	-	15%	41%	83%	35%

Data represent the Mean ± SE of Mean. n = 8

a = significant at $p< 0.05$. ns = not significant

Table 4.4: Effect of EBO on gastric mucosal membrane of ethanol induced gastric ulcers in albino rats with Cimetidine group.

	50mg Cimetidine + EtOH	100mg (EBO) + EtOH	200mg (EBO) + EtOH	400mg (EBO) + EtOH
Ulcer score	3.75 ± 0.94	4.88 ± 0.93^{ns}	3.38 ± 0.94^{ns}	1.00 ± 0.54^{a}
Ulcer index	375	488^{ns}	338^{ns}	100^{a}
% ulceration	100%	100%	75%	38%
Preventive index	35%	15%	41%	83%

Data represent the Mean ± SE of Mean. n = 8

a = significant at $p< 0.05$. ns = not significant

4.3.2 Effect of EBO and cimetidine on mean ulcer score

Mean ulcer score expresses the degree of ulceration on mucosal membrane. It is determined by naked eye as described in materials and method. Pre-treatment of the gastric mucosa with oral administration of extract *Brassica oleracea* at various concentrations of 100, 200 and 400mg/kg b.w., for 7 days showed a significant dose-dependent decrease in mean ulcer score, induced by 70% ethanol (1ml/200gm b.w.) for 1hr (Fig 4.1). Pre-treatment with a reference drug cimetidine, a histamine receptor-2 antagonist (50mg/kg b.w) for 1hr, also significantly reduced gastric ulcers with mean ulcer score of 3.75 ± 0.9 SEM (Table 4.3), which is comparable to 200mg/kg b.w of EBO (Fig 4.1). The percentage difference in mean ulcer score, between: (100 and 200 mg/kg b.w. EBO; 100 and 400mg/kg b.w. EBO; 200 and 400mg/kg b.w. EBO) was 30.7%↓, 80%↓; and 70.4%↓ respectively. Cimetidine 50mg/kg b.w with 400mg/kg b.w EBO, the difference in ulcerative score was 275%↑ (Appendices 3a, 3b, 3c and 3d).

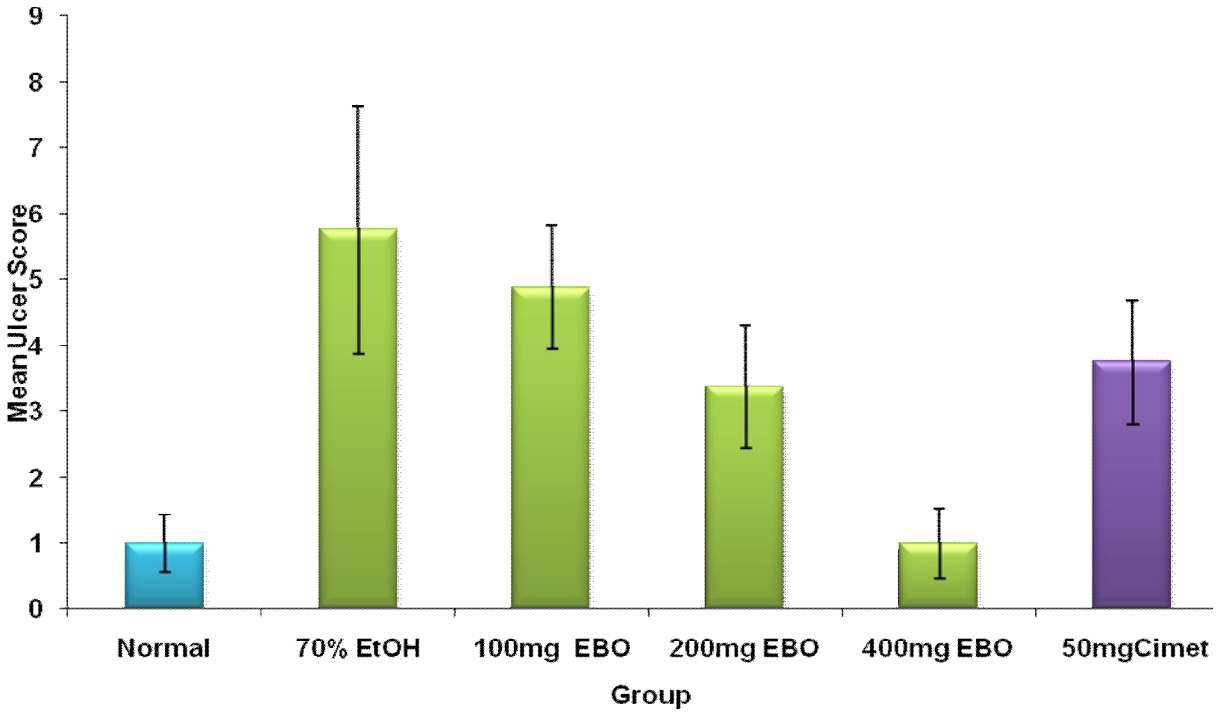


Fig4.1: The Effect of orally administered Brassica oleracea and Cimetidine on mean ulcer score in albino rats

4.3.3 Effect of EBO and Cimetidine on preventive index

The preventive index was calculated from ulcer index of control and treated group. Pretreatment with EBO at various concentrations 100, 200 & 400 mg/kg bw, produced a significant dose-dependent protective effect against gastric ulcers induced by 70% EtOH (1ml/200gm b.w.), (Fig4.2). Pretreatment with cimetidine (50mg/kg b.w) for 1hr, has a preventive index of 35% which falls in between the preventive indices of 100 and 200 mg/kg b.w of the EBO (Fig 4.2). There was an increase in percentage difference of preventive indices between: 100 and 200 mg/kg b.w; 100 and 400mg/kg; 200 and 400mg/kg b.w EBO, but decreases between 400mg/kg b.w EBO and cimetidine 50mg/kg b.w (Appendices 3a, 3b, 3c and 3d).

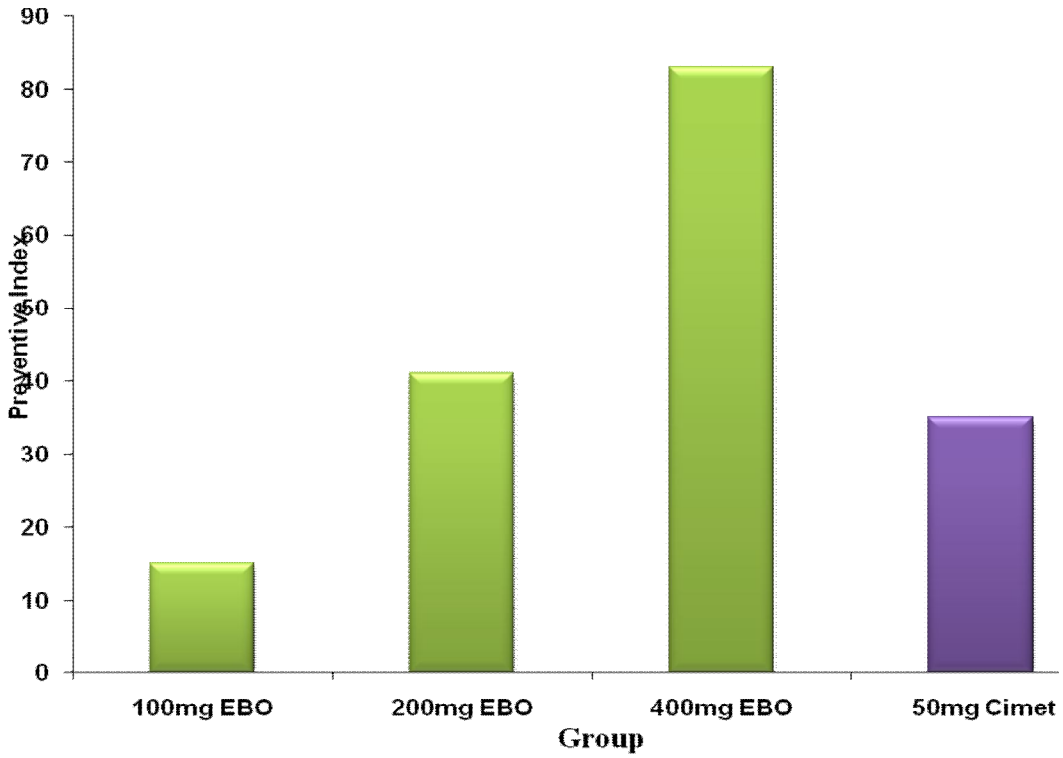


Fig 4.2 The effect of orally administered Brassica oleracea and Cimetidine on Preventive Index in albino rats

4.3.4 Effect of EBO and Cimetidine on incidence of gastric ulcers

Ulcer incidence of the normal group was found to be 33.3%; it was significantly increased to 100% by the control rats with oral administration of 70% EtOH (table 4.2). Pre-treatment with EBO at 100, 200 and 400 mg/kg b.w., produced a significant dose-dependent decrease in occurrence of ulceration and at the highest concentration (400mg/kg b.w) ulcer incidence was comparable with the normal group, while cimetidine a standard drug at 50mg/kg b.w afforded an ulcer incidence of 100%, which was comparable with the control group (70%EtOH) (Fig 4.3). There was a decrease in percentage difference in ulcer incidence between: 100 and 200mg/kg b.w EBO; 100 and 400mg/kg; 200 and 400mg/kg b.w EBO, and an increase between 400mg/kg b.w EBO and 50mg/kg b.w cimetidine (Appendix 3a, 3b, 3c and 3d).

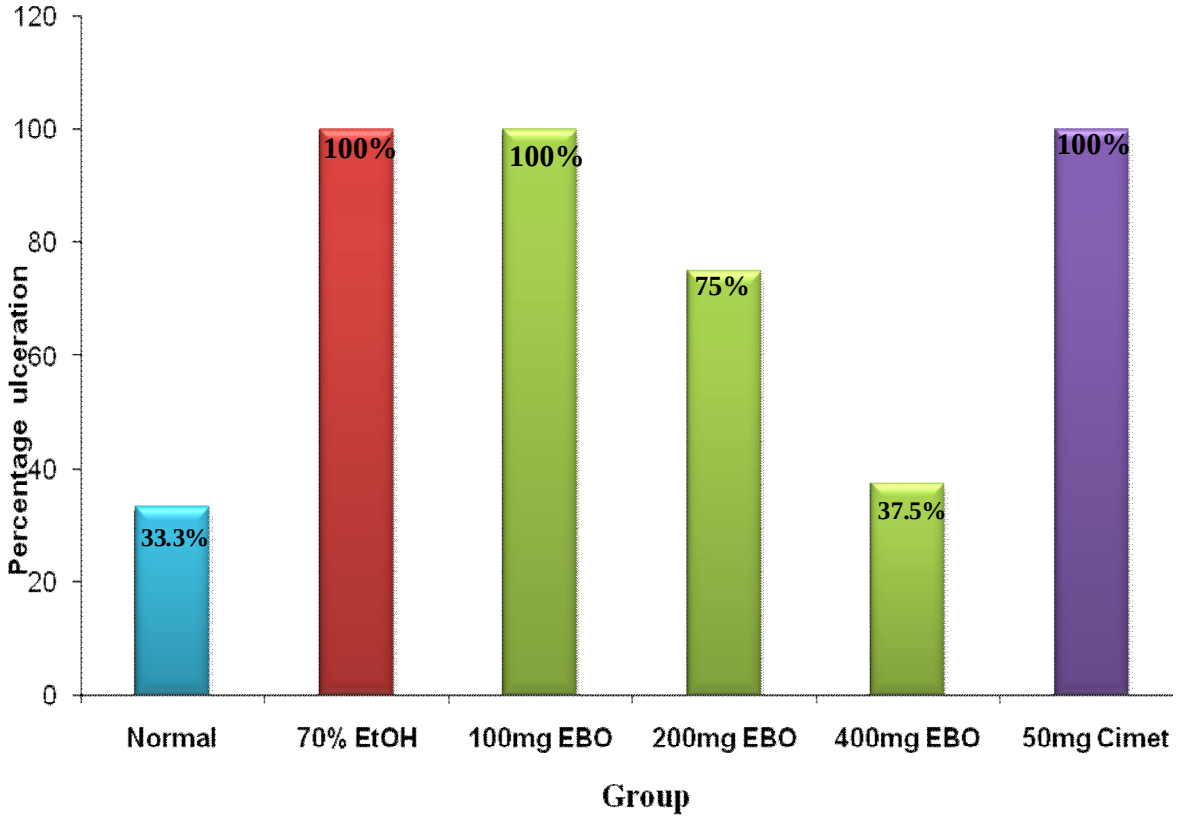


Fig 4.3 : Effect of orally administered Brassica oleracea and Cimetidine on incident of gastric ulcers in albino rats

4.4 Effect of aqueous extract of *Brassica oleracea* (EBO) on gastric secretion of ethanol induced gastric ulcers in albino rats

Normal rats showed; Mean gastric juice volume of 3.76 ± 0.60 SEM, acid concentration of 1.838 ± 0.24 SEM, calculated acid output of 6.78 ± 0.69 and pepsin secretion of 608 ± 226 (Table 4.5). There was a significant ($p<0.05$) decrease in statistical data of acid concentration, acid output and pepsin secretion but no significant difference in the gastric juice volume in the control (70% EtOH) rats when compared with normal rats (Table 4.5). Pretreatment with EBO (100, 200, and 400mg/kg b.w.) given orally for 7 days showed no statistical significant difference in gastric juice volume and pepsin secretion but significantly ($p<0.05$) decreased acid concentration and acid output when compared with the normal rats (Table 4.5). Cimetidine (50mg/kg b.w.) given orally for 1hr significantly ($p<0.05$) decreased gastric juice volume and acid output but no significant difference in acid concentration and pepsin secretion. There was a general increase in Percentage difference of gastric secretion parameters (acid concentration, acid output and pepsin secretion) between: 100 and 200mg/kg b.w. EBO; 100 and 400mg/kg; 400mg/kg b.w. EBO and cimetidine 50mg/kg b.w. however, decreases between: 200 and 400mg/kg b.w. EBO (Appendix 5a, 5b, 5c and 5d).

4.4.1 Control

The control group (70% EtOH) administered orally for 1hr showed; Mean gastric juice volume of 3.88 ± 1.53, acid concentration of 0.83 ± 0.12, acid output of 3.28 ± 1.38 and pepsin secretion of 5388 ± 186. Pretreatment with EBO (100, 200, and 400mg/kg b.w.) orally for 7days showed no significant difference in mean volume, gastric acidity, acid concentration, acid output and pepsin secretion when compared with the control group (Table 4.6). Cimetidine, at 50mg/kg b.w. afforded a significant decrease in mean volume and gastric acidity, with no significant difference in acid output and pepsin secretion (Table 4.6).

Table 4.5: Effect of extract *Brassica Oleracea* (EBO) and cimetidine on gastric secretion of ethanol- induced ulcers in albino rats with the normal group

	Normal	**Control 70% EtOH**	**100mg (EBO) + EtOH**	**200mg (EBO) + EtOH**	**400mg (EBO) + EtOH**	**50mg Cimetidine + EtOH**
Volume (ml/3hrs)	3.76 ± 0.60	3.88 ± 1.53^{ns}	4.85 ± 1.44^{ns}	3.03 ± 0.23^{ns}	3.28 ± 0.30^{ns}	1.38 ± 0.24^{a}
Acid concentration (mEq/l)	1.88 ± 0.24	0.83 ± 0.12^{a}	0.63 ± 0.13^{a}	1.00 ± 0.20^{a}	0.70 ± 0.14^{a}	2.00 ± 0.20^{ns}
Acid output (mEq/3hrs)	6.78 ± 0.69	3.28 ± 1.38^{a}	2.58 ± 0.62^{a}	2.98 ± 0.52^{a}	2.60 ± 0.69^{a}	2.83 ± 0.63^{a}
Pepsin concentration (Mg/ml)	6.1 ± 0.23	5.4 ± 0.19^{a}	6.1 ± 0.12^{ns}	7.4 ± 0.11^{ns}	7.0 ± 0.36^{ns}	12.1± 0.39^{a}

Data are express as mean ± SEM, n = 8.

a = significant at $p< 0.05$. ns = not significant

Table 4.6: Effect of EBO and Cimetidine on gastric secretion of ethanol-induced ulcer in rats with control group

	Control 70% EtOH	100mg (EBO) + EtOH	200mg (EBO) + EtOH	400mg (EBO) + EtOH	50mg Cimetidine + EtOH
Volume (ml/3hrs)	3.88 ± 1.53	4.85 ± 1.44^{ns}	3.03 ± 0.23^{ns}	3.28 ± 0.30^{ns}	1.38 ± 0.24^{a}
Acid concentration (mEq/l)	0.83 ± 0.12	0.63 ± 0.13^{ns}	1.00 ± 0.20^{ns}	0.70 ± 0.14^{ns}	2.00 ± 0.20^{a}
Acid output (mEq/3hrs)	3.28 ± 1.38	2.58 ± 0.62^{ns}	2.98 ± 0.52^{ns}	2.60 ± 0.69^{ns}	2.83 ± 0.63 ^{ns}
Pepsin Concentration (Mg/ml)	5.4 ± 0.12	6.1 ± 0.11^{ns}	7.4 ± 0.11^{ns}	7.0 ± 0.36^{ns}	12.1 ± 0.39^{a}

Data are express as mean ± SEM, n = 8

a = significant at $p < 0.05$. ns = not significant

4.4.2 Effect of EBO and Cimetidine on acid concentration of gastric secretion in ethanol induced ulcer in albino rats

Acid concentration (mEq/l) of gastric juice in the normal rats was found to be 1.88 ± 0.24 SEM, this was significantly decreased in the control rats (70% EtOH) (Table 4.5). Pretreatment with EBO at concentrations of 100, 200, 400mg/kg b.w. showed no significant difference in acid concentration when compared with control group, but there was a significant increase with Cimetidine at 50mg/kg. Percentage difference in acid concentration increases between: 100 and 200mg/kg b.w. EBO; 100 and 400mg/kg; 400mg/kg b.w. EBO and Cimetidine 50mg/kg b.w. by 58.7, 11.1 and 185.7% respectively. Meanwhile between: 200 and 400mg/kg b.w. EBO. It decreases by 42.8% (Appendix 5a, 5b, 5c and 5d).

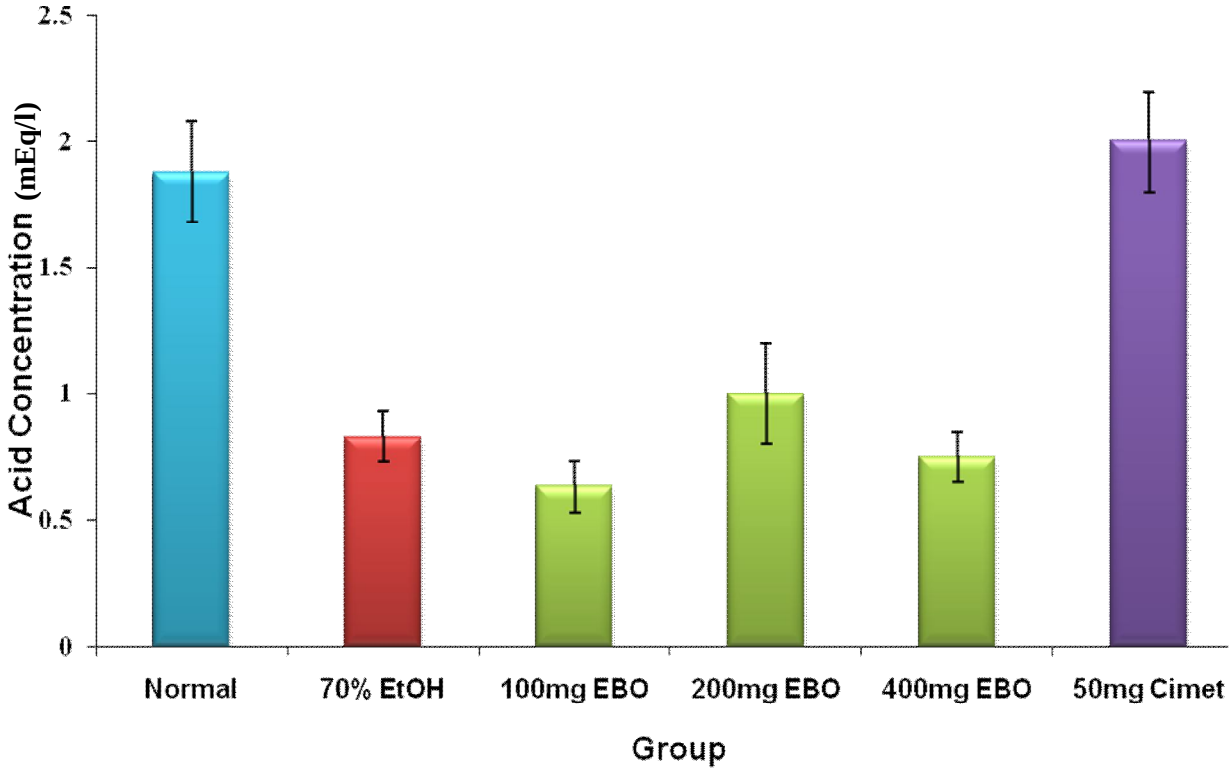


Fig 4.4 Effect of orally administered Brassica oleracea and Cimetidine on Acid Concentration of basal gastric secretion of albino rats

4.4.3 Effect of EBO and Cimetidine on total acid output of gastric secretion in ethanol induced ulcer in albino rats

Total acid output (mEq/3hrs) of normal rats was found to be 6.78 ± 0.69 SEM, which was significantly decreased to 3.28 ± 1.38 SEM in the control rats (Table 4.5). Pretreatment with EBO (100, 200, 400 mg/kg b.w.) and Cimetidine (50 mg/kg b.w.) showed no significant difference when compare with control group (Fig4.5). Similarly, there was no significant difference in percentage difference of acid output between the groups (Appendix 5a, 5b, 5c and 5).

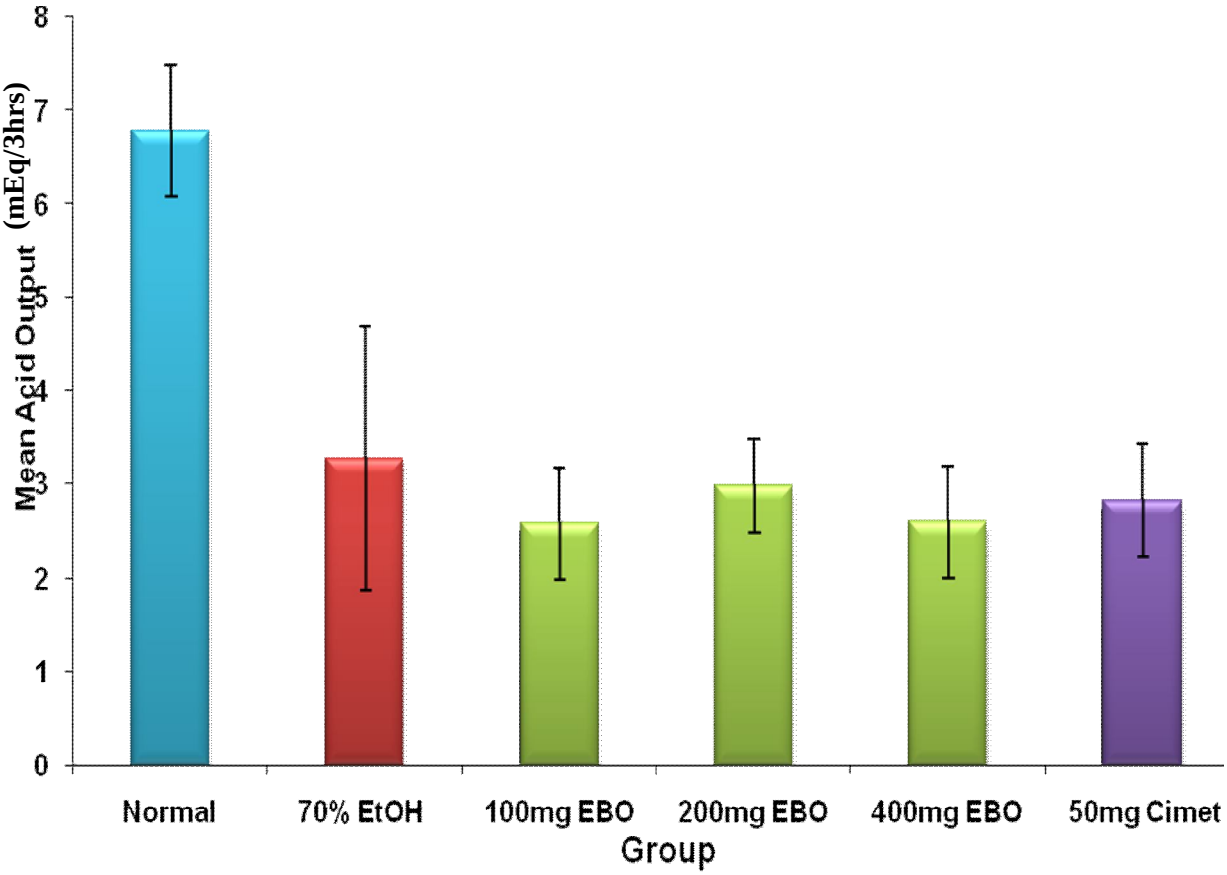


Fig 4.3 Effect of orally administered Brassica oleracea and Cimetidine on Mean Acid Output of basal gastric secretion of albino rats

4.4.4 Effect of EBO and Cimetidine on pepsin concentration in ethanol induced ulcer in albino rats

Pepsin concentration (Mg/ml) was assessed in terms of the amount of proteases produced after intubation of casein (substrate) with pepsin from the gastric juice. There were no significant difference in pepsin concentration with normal, control and EBO treated group at various concentrations; 100, 200, and 400mg/kg b.w. However, cimetidine 50mg/kg b.w. afforded a significant increase in pepsin concentration as compared with all the groups (fig 4.5). Percentage difference in pepsin concentration increases between: 100 and 200 mg/kg b.w. EBO; 100 and 400 mg/kg; 400 mg/kg b.w. EBO and cimetidine 50mg/kg b.w. by 22.0, 14.0 and 74.0% respectively. Meanwhile between: 200 and 400 mg/kg b.w. EBO. It decreases by 6.5% (Appendix 5a, 5b, 5c and 5d).

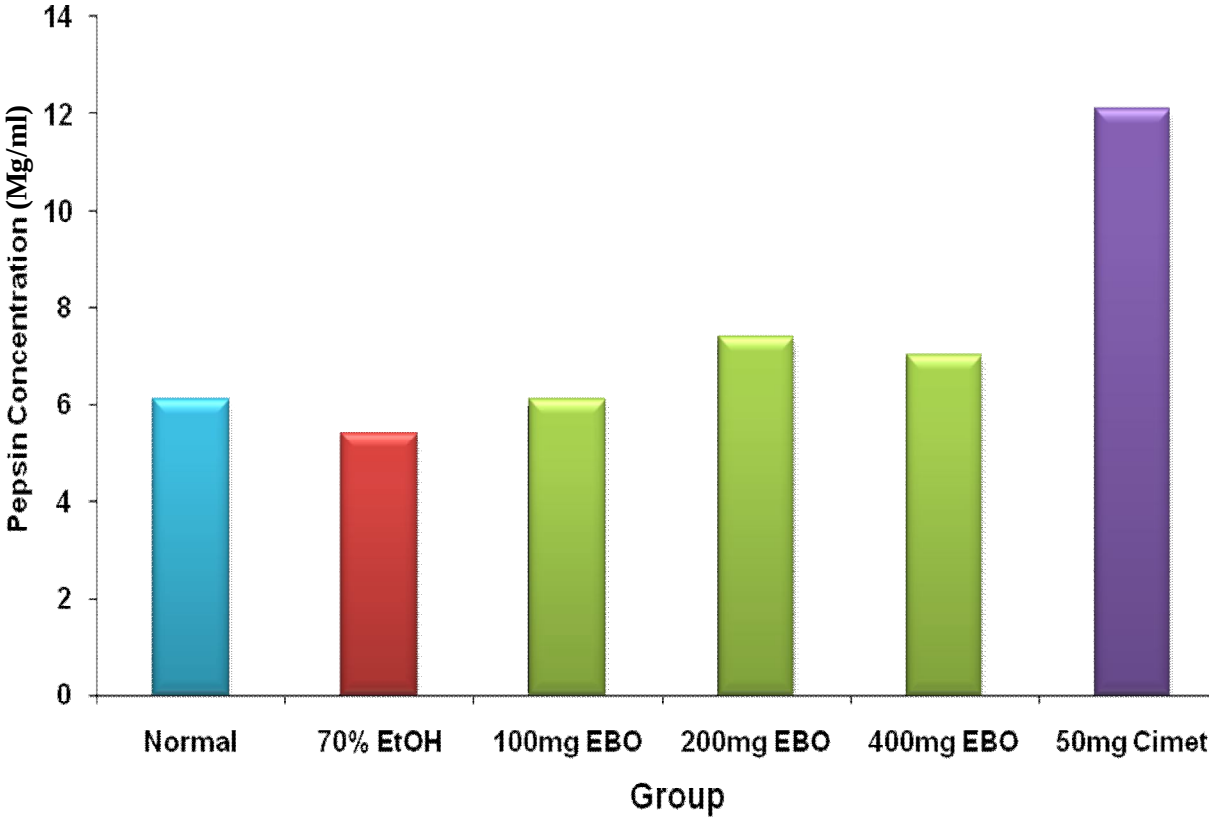


Fig 4.6 Effect of orally administered Brassica oleracea and Cimetidine on Pepsin Concentration of basal gastric secretion of albino rats

4.5 Histological assessment of the gastric mucosa

Histological evaluations on the effect of EBO and cimetidine on ethanol (EtOH) induced gastric lesions in rats (hematoxylin and eosin, 25×). Photomicrograph of gastric mucosa from normal rats demonstrates an intact surface mucosal cells lining lumen of the stomach and an intact gastric pits (Plate 4.6a). The glandular region of the stomach from control group (70% EtOH) had hemorrhagic ulcerated gastric mucosal epithelial cells that extended and involved the gastric glands and submucosa. Muscularis region has been replaced by necrotic tissue and heavy infiltration (Plate 4.6b). The section of gastric mucosa from rat pre-treated with EBO at 100 and 200mg/kg b.w. had eroded mucosal epithelial cells of the glandular region of the stomach, that exfoliate into the gastric lumen but less infilteration and haemorrhage, as shown in Plates 4.6c and 4.6d respectively. In the 400mg/kg b.w. EBO pre-treated rat there is no observable haemorrhagic necrosis of gastric mucosa and showed protection against the histopathological changes observed in ethanol treated group with an intact gastric pits, maintenance of mucosa and epithelization are apparent even after exposure of EtOH (Plate 4.6e). Cimetidine (50mg/kg b.w.) pre-treated group demonstrates less hemorrhagic necrosis, infiltration and edema in the gastric mucosa of rat. There is disruption of gastric pits (Plate 4.6f).

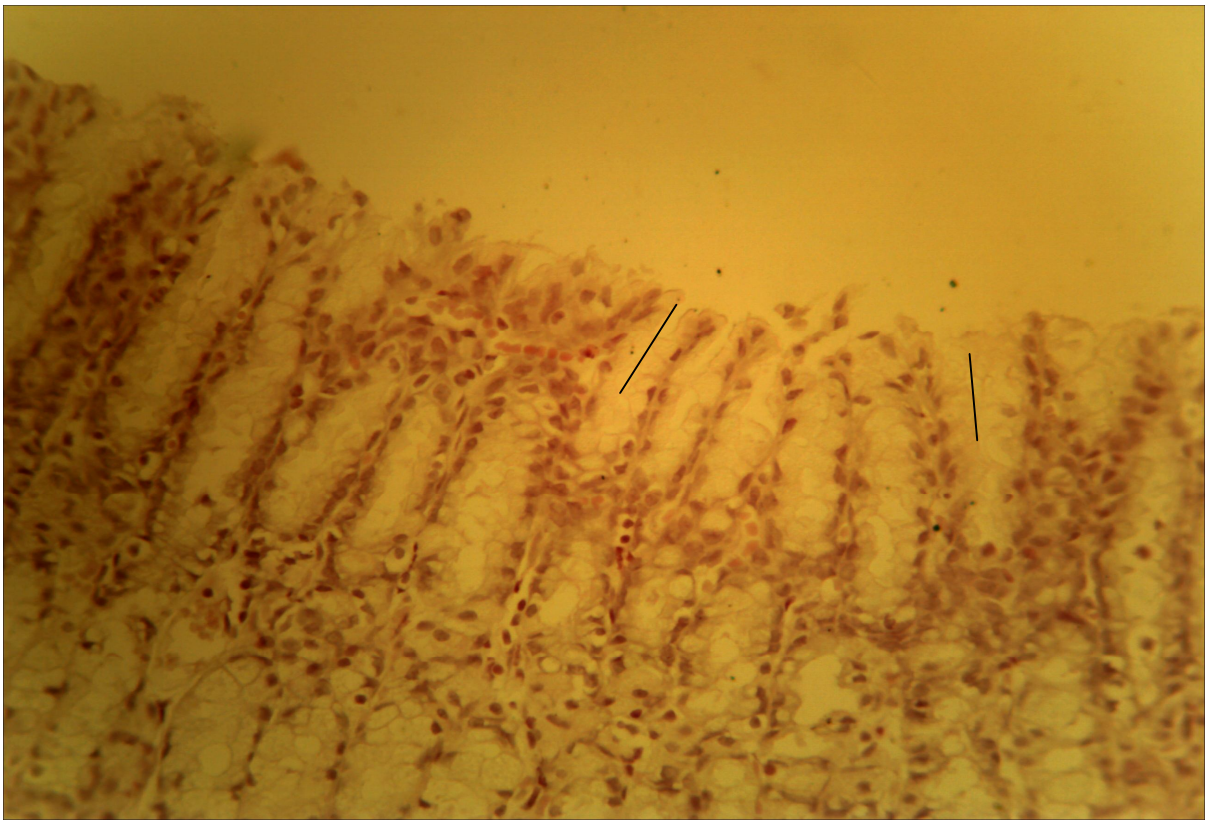


Plate 4.6a: Photomicrograph of normal gastric mucosa of rat. H & E stain x 250

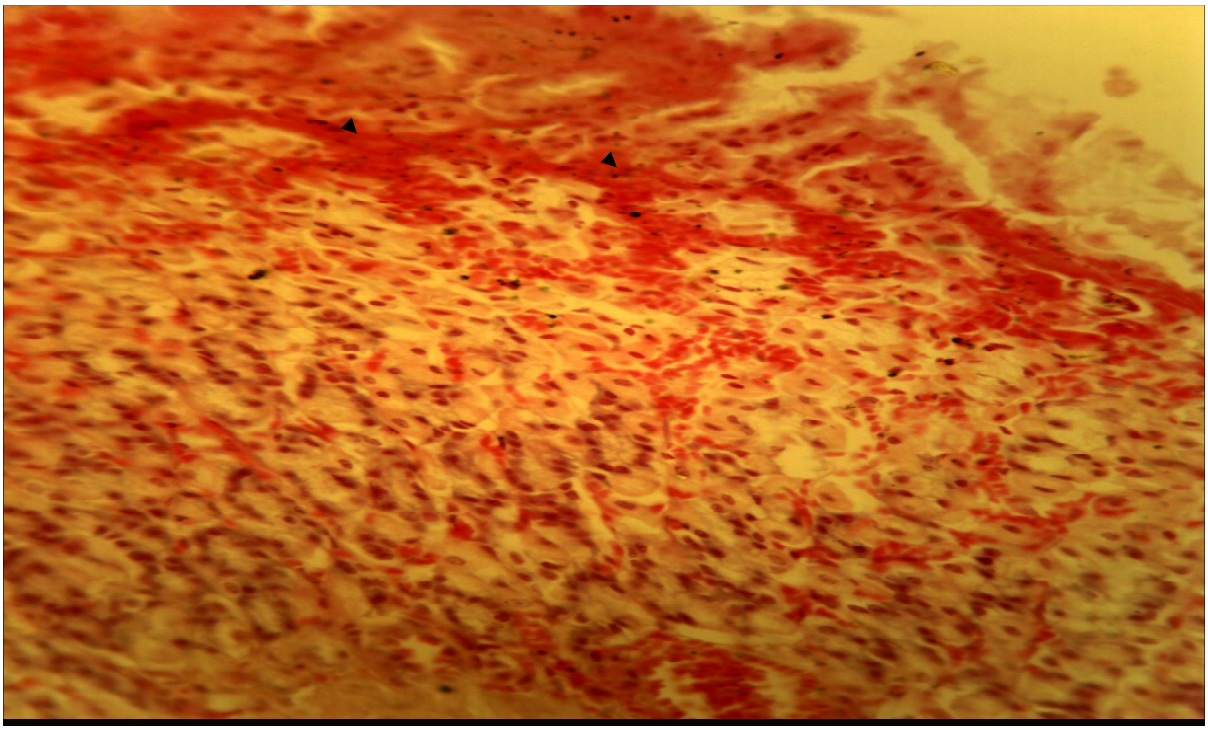


Plate 4.6b: Photomicrograph of gastric mucosa of 70% EtOH treated rat. H & E stain x 250. Note the necrotize haemorrhagic ulcerated mucosa that involve submucosa and gastric glands

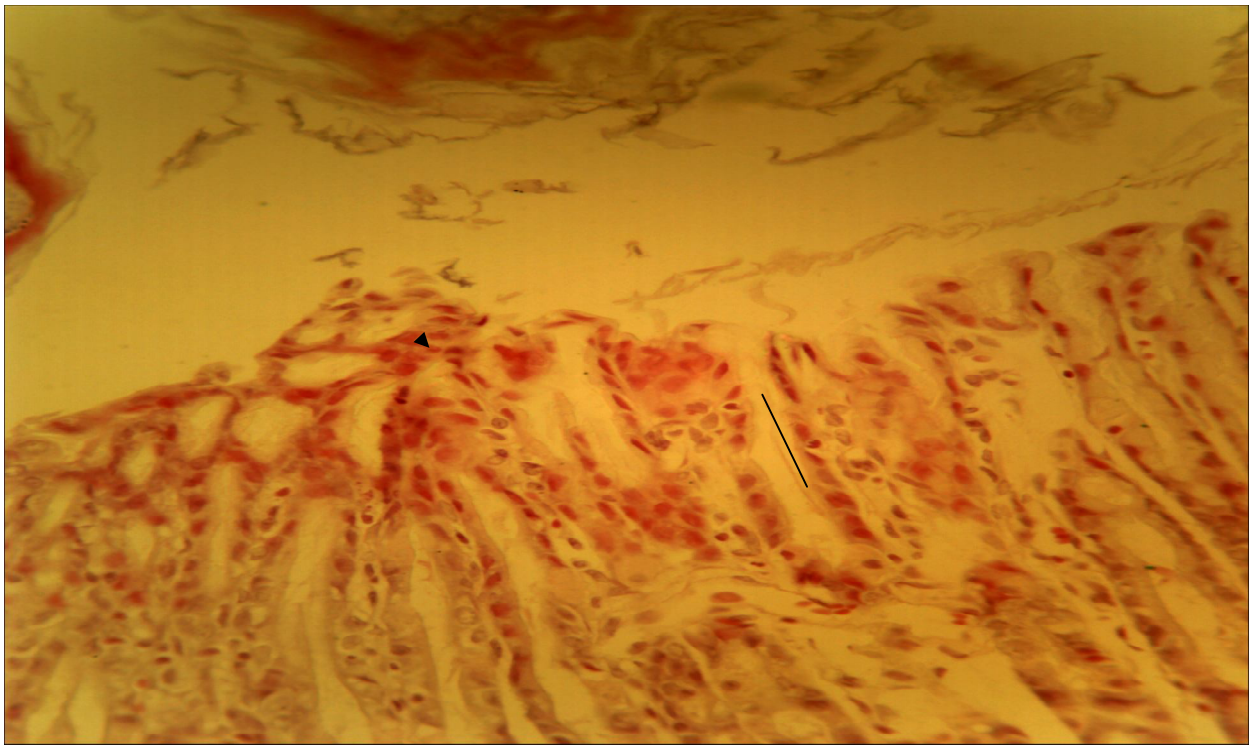


Plate 4.6c: Photomicrograph of gastric mucosa of rat pretreated with 100mg EBO prior to EtOH administration. H & E stain x 250. Ulceration (▲), gastric pits (\ )

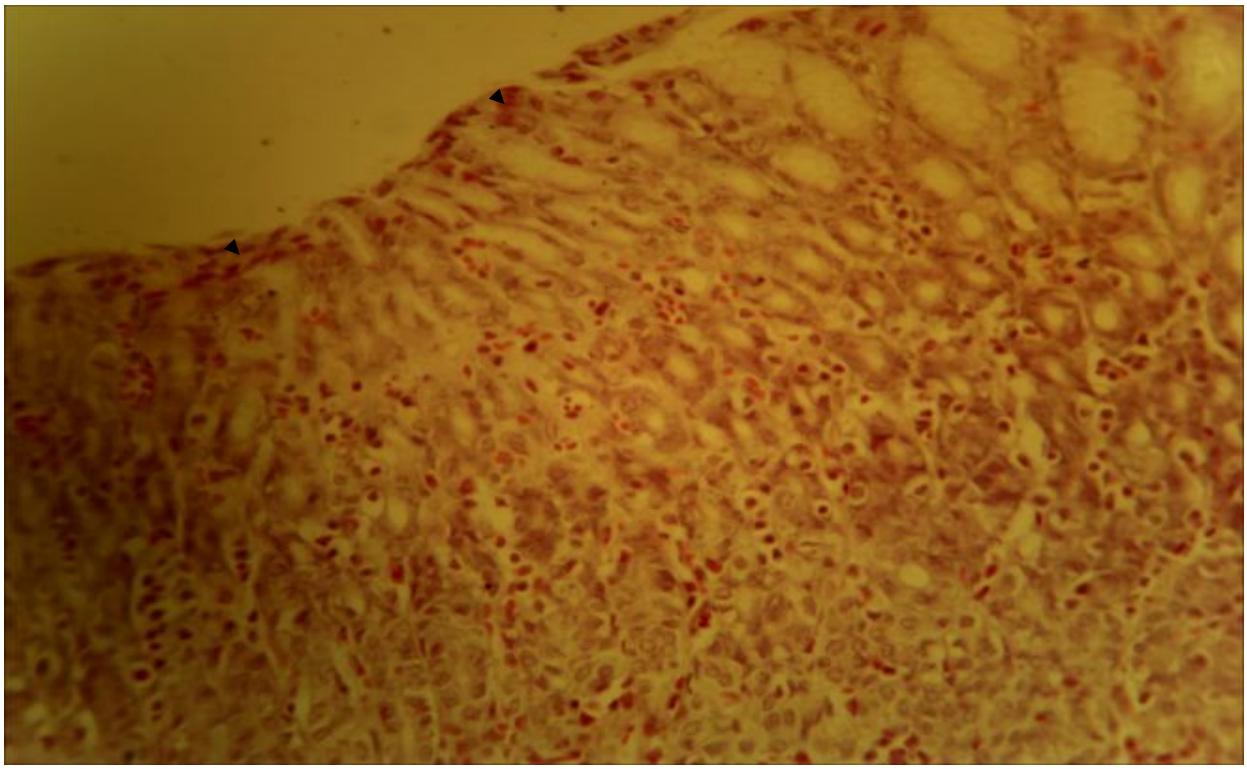


Plate 4.6d: Photomicrograph of gastric mucosa of rat pretreated with 200mg EBO prior to EtOH administration. H & E stain x 250. Ulceration (►)

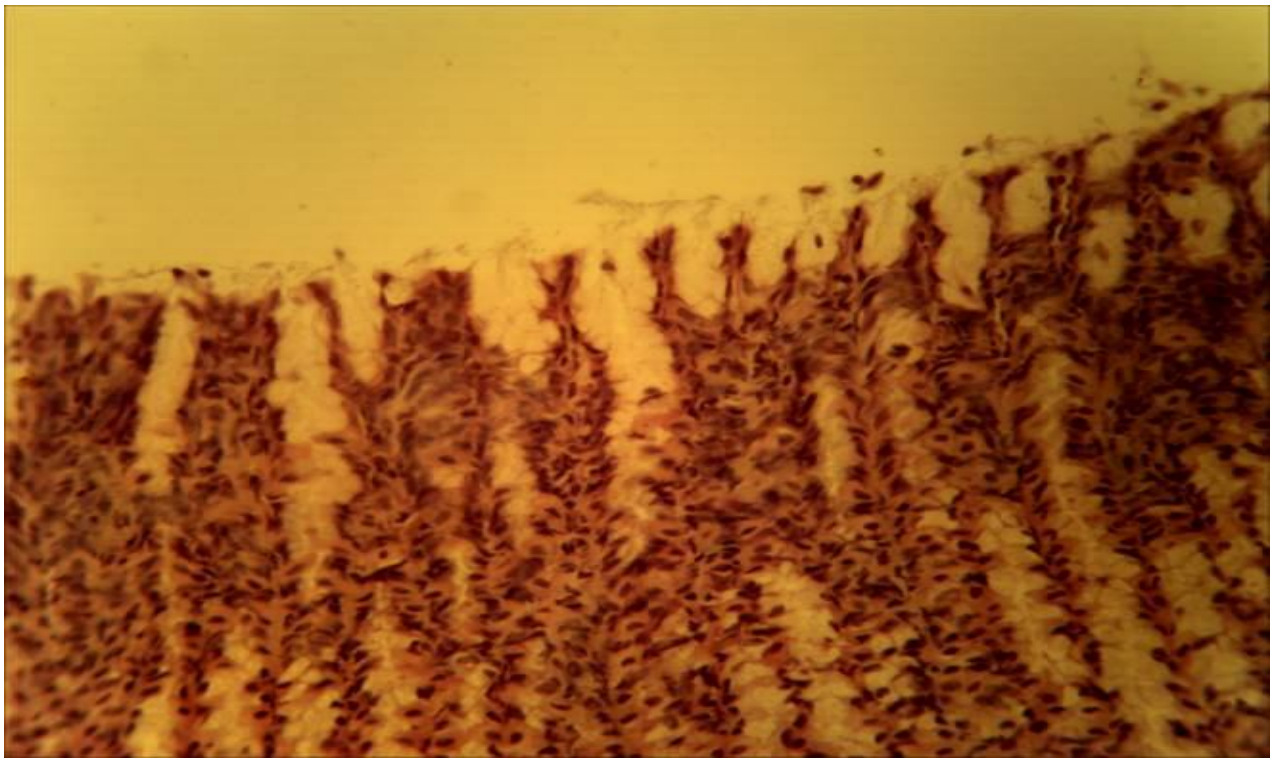


Plate 4.6e: **Photomicrograph of gastric mucosa of rat pretreated with 400mg EBO prior to EtOH administration. H & E stain x 250**

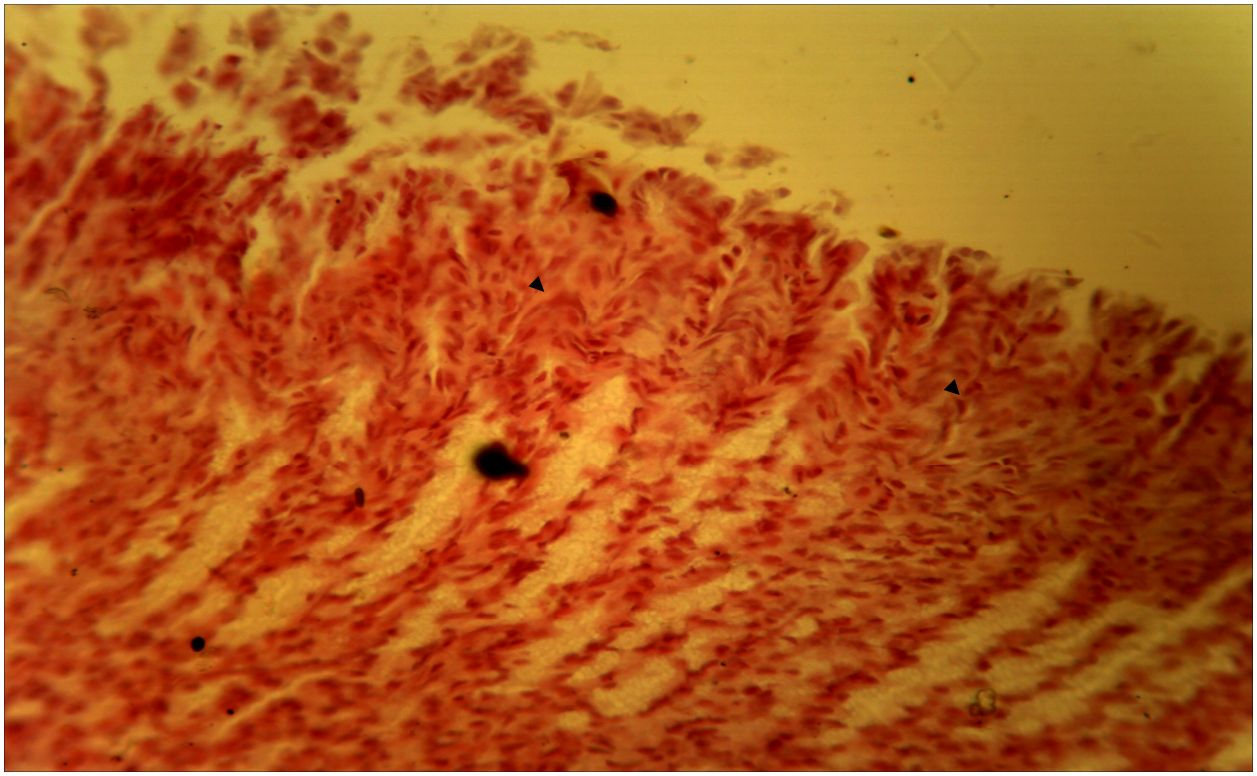


Plate 4.6f: **Photomicrograph of gastric mucosa of rat pretreated with 50mg cimetidine prior to EtOH administration. H & E stain x 250. Ulceration (◂)**

CHAPTER FIVE

DISCUSSION

The present study demonstrated a significant protective effect of Extract *Brassica oleracea* (EBO) on gastric ulceration in ethanol-induced ulcer model. *Brassica oleracea* (Cabbage) is a cruciferous vegetable and a member of the *Brassica* family. It's among the rich unexploited world flora with anti-ulcerogenic properties (Alanso, 1998). The plant juice has been used for the treatment of many kinds of cancers and ulcers (Chen, 1995).

Gastric ulcers are defined as lesion in the mucosa of the stomach. Gastric ulcers have multiple etiopathogenesis, however it is generally agreed that gastric lesions develop when the delicate balance between some gastroprotective and aggressive factors are lost, with either an increase in aggressive insults, or a relative decrease in protective factors (Hoogerwerf and Pasricha 2001). The gastric mucosa is challenged by a variety of both endogenous and exogenous irritants (aggressive factors), including ethanol, gastric acid, pepsin, reactive oxygen species, non-steroidal anti-inflammatory drugs (NSAIDs), stress and Helicobacter pylori (Holzer, 1998).

Toxicity studies of EBO carried out in rats indicated no lethal effect at least up to an oral dose of 5000mg/kg b.w. for 24 hrs, indicating that LD_{50} of EBO will be higher than that dose. This suggests that EBO though the plant itself is widely and highly consumed as food has a wide margin of safety.

In the present study, oral administration of 70% EtOH to rats produced gastric mucosal lesions, which correspond to score grade 4 (many linear ulcer of marked size) according to the method of Ohara *et al.*, (1995) of severity of mucosal damage assessment. Ulcers caused by ethanol (EtOH) may possibly arise as a result of direct damage of gastric mucosal cells,

resulting in the development of free radicals and hyperoxidation of lipid (Terano et al., 1989). Similarly Oates and Hakkinen, (1988) reported that the incidence of ethanol-induced ulcers predominate the glandular part of the stomach and stimulate leukotriene C_4 (LTC_4), mast cell secretory products. This are involved in inflammatory responses and histamine been a secretory product of mast cell could stimulate an increase in gastric acid secretion ensuing mucosal damage.

Pre-treatment with extract of *Brassica oleracea* (EBO) at concentrations of 100, 200 and 400 mg/kg, b.w showed a significant dose-dependent protective effect against ethanol-induced gastric ulcer, while pre-treatment with a reference drug Cimetidine (50mg/kg) also showed a significant protection which was comparable with the effect conferred by 200mg/kg concentration of EBO. The histopathology of the stomach tissue from a rat from each treated group indicate that, the treatment with 100 and 200mg/kg b.w. of extract *Brassica oleracea* (EBO) were less protective to the gastric mucosa cells but at 400mg/kg b.w. of the EBO it conferred a highly protective beneficial effect on the gastric mucosa cells. Pre-treatment with cimetidine was protective; however there were some necrosis, infiltration and edema in the gastric mucosa of the stomach.

Inhibition of gastric acidity is an important aspect of peptic ulcer disease, as such gastric secretory studies was carried out on gastric juice of ethanol-induced ulcer. There was no significant difference in volume of the gastric juice in normal rats when compared with the control rats (70% EtOH) but there was a significant decrease in gastric acidity and pepsin secretion. Pretreatment with EBO at various concentration; 100, 200, and 400mg/kg showed no significant difference in mean volume, acid concentration, acid output and pepsin concentration when compared with the control group. Cimetidine, at 50mg/kg b.w. afforded a

significant decrease in mean volume, but, a significant increase in pepsin concentration and acid concentration with no significant difference in acid output.

Gastric ulcers induced by ethanol have been apparent by the results of histopathology examination. The 70% ethanol administered orally as was done in the control rat, was indeed ulcerogenic, marked with necrotized ulcerated gastric mucosal epithelial cells that extended and involved the gastric glands and submucosa. These could be attributed to the significant decrease in gastric juice parameter with the control rat when compared to the normal rats. EtOH is also reported to produce a significant increase in gastric mucosal H^+/K^+ ATPase activity (Jainu and Devi 2004). H^+/K^+ ATPase is an enzyme responsible for regulating the formation of acid secretion by the gastric parietal cells. It was anticipated that an increase in gastric acidity be observed with ethanol administration but, the damage of cells and glands due to concentration of ethanol could be the possible explanation of a significant decrease in gastric acidity. However, the reference drug cimetidine showed an increase in acid concentration with a decrease volume of gastric juice and acid output. As cimetidine is an H_2 receptor blocker inhibiting histamine release, a potent acid secretagogoue. The stimulatory action of histamine is mediated by the H_2 receptor as previously demonstrated in other pharmacological studies (Berglindh, 1977; Chew et al., 1980; Bottcher et al., 1989). Different ligands and their corresponding receptors on the parietal cell leads to activation of different signalling pathway which in turn regulates a series reaction that control the acid secreting pumps, H^+, K^+ - ATPase, and the magnitude of stimulus appears to result from a complex additive interaction of signals (Del Valle, 2001). cimetidine at 50mg/kg did not decreased acid concentration and the insignificant effect on acid output could be attributed to the damage of mucosal glands by ethanol. However, there was a significant increase in pepsin concentration with cimetidine. Although cimetidine markedly tends to inhibit gastric acid output, but it has a lesser effect on gastric pepsin output and depending on the dose, it may

increase gastric pepsin concentration (Taylor et al., 1982). The reality is that because of marked inhibition of gastric acidity with cimetidine peptic activity was favoured.

Damage of gastric mucosal cells induced by ethanol has been associated with generation of reactive oxygen species which produces an unbalanced oxidant/ antioxidant cellular processes (Repetto and Liesuy, 2002). Free radicals affect lipids by initiating peroxidation. Superoxide O_2^-, hydrogen peroxide (H_2O_2) and hydroxyl radical (OH^-) are important reactive oxygen species causing tissue damage (Fridovich, 1986). Hydroxyl radical combines with the hydrogen atom to form water, most of the toxicity of O_2^- and H_2O in vivo is due to their reaction with metal ion to form OH^- subsequently the toxic nature of OH- and H_2O_2 is affected by iron catalyse within the cell. Ethanol releases H_2O_2 which can react with Cl^- to generate hypochlorus acid (Stvtinova et al., 1995). Hypoclorus acid is 100-1000 times more toxic than superoxide. This destroys glandular cells thus decreasing gastric secretion in volume and content.

Studies by Terano et al. (1989), indicated that intragastric administration of superoxide dismutase was able to protect the gastric mucosal against the damaging effect of ethanol. In addition Kato et al. (2000), in a study examining alterations of ulcerogenic and healing responses in adjuvant-induced arthritic rats, reported that the ulcerogenic response to ethanol was prevented by increased production of nitric oxide and inducible nitric oxide synthase. These would suggest the involvement of free radicals in the pathogenesis of ethanol-induced gastric ulcers. Moreover various studies have also implicated the generation of oxygen derived free radicals and lipid peroxidation as one of the most important mechanisms involved in the pathogenesis of gastric ulcer (Di Carlo et al., 1999).

Phytochemical studies by Steinmetz (1996) and Raloff (2000), showed that *Brassica oleracea* is such plant with high level of glutamine, S-methionine, flavonoids and many antioxidant

micronutrients; Cu, Mn, Zn, vitamin C, E . Preliminary phytochemical screening of EBO in the present study verified the presence of flavonoids. Among the principal properties that may account for the potential health benefit of flavonoid is their significant antioxidant activity (Repetto and Llesuy, 2002). Several in vitro studies have demonstrated that flavonoids can scavenge superoxide, dihydroxyl, peroxide radicals. They have membrane stabilizing properties and also inhibit lipid peroxidation in different systems (Alanko, 1999). Galati et al., (2003) also describe that there is a correlation between the antioxidant and the antiulcer activity of the flavonoids. In addition *Brassica oleracea* contains glucosinolates and their breakdown products that alter the induction of glutathione S-transferase (GST), NADPH, and quinine oxidoreductase (NQO). The GST family of detoxification enzymes is responsible for conjugating electrophilic compounds with glutathione, creating a more water-soluble and usually non-cytotoxic compound to be excreted. NQO is a flavoprotein its protective action is result of its successful competition with single-electron reduction reactions and the inhibition of oxidative metabolic intermediates. The presence of these antioxidant compounds may probably explain the protective effect of EBO in this study. Given that antioxidants are known to inhibit lipid peroxidation and scavenge free radicals (Repetto and Llesuy, 2002).

Intracellular antioxidant such as gluthathione and -tocopherol protect the gastric mucosa from ethanol-induced mucosal damage (Repetto and Liesuy, 2002). It was however reported by Victor et al. (1991), in a study on the effect of 40% ethanol on gastric mucosa of mongrel dogs that there was a significant decrease in the level of mucosal GSH and marked injury to the mucosal surface. However, Study by Araki et al. (2000), confirmed that the ethanol induced lesions depend on the dose of ethanol and can be prevented.

Therefore, the anti-ulcer effect of EBO against ethanol-induced ulcer, without a significant effect on gastric secretion, as shown in the statistical data may perhaps be attributed to antioxidant activity of its compound among many other properties, considering the fact that the crude extract was been used in this study.

CHAPTER SIX

CONCLUSION AND RECOMMENDATION

6.1 CONCLUSION

The oral administration of extract of *Brassica oleracea* exhibited a significant gastric mucosal protection against ethanol-induced gastric ulcers in rat, without any apparent toxicological effects. This action called gastro- or cyto-protection was not related to the inhibition of gastric acid secretion. Based on the result obtained and evidences from the literature, the gastric mucosal protection is associated in part with antioxidant effect in combination with other properties of the crude plant extract. Various studies reviewed in this study concerning gastric mucosal damage induced by acute / chronic ethanol toxicity have been associated with generation of toxic reactive species. Whereas phytochemical evaluation carried out on EBO has proved the presence of flavonoid, a medicinal property of most plant and a source of natural antioxidant. Furthermore, other studies have indicated the presence of several antioxidants in *Brassica oleracea*.

Therefore in view of the observation that there was no significant effect on gastric secretion with oral administration of EBO in ethanol-induced gastric ulcers, yet there was a significant cyto-protection by the plant. The probable mechanism of action utilized by *Brassica oleracea,* may perhaps be related to factors of gastric mucosal membrane protection.

In summary, the plant *Brassica oleracea* showed a potent antiulcer activity, which justifies the ethnomedical claims.

6.2 Recommendation

Inhibition of gastric acidity is an important aspect in the treatment of peptic ulcer disease. Yet EBO conferred cyto-protection without a significant effect in gastric acidity; as such further work is required using different ulcer models and standard anti-ulcer drugs with respect to the ulcer models, to ascertain the effect on gastric secretion. However, this will also suggest the mechanism of action of the plant. Also the active components of the plant with anti-ulcer property need to be analyzed.

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APPENDICES

Appendix 1: Comparison of gastric ulcer parameters between Normal and control (70% EtOH)

	Normal	Control EtOH	% Difference
Ulcer score	1.00	5.75	475 ↑[a]
Ulcer index	100	575	475 ↑[a]
% ulceration	33.3%	100%	66.7 ↑
Preventive index	-	-	-

a = significant (P>0.05)

Appendix 2a: Comparison of gastric ulcer parameters between control (70% EtOH) and 100mg (EBO)

	Control EtOH	100mg (EBO) + EtOH	% Difference
Ulcer score	5.75	4.88	15 ↓[ns]
Ulcer index	575	488	15 ↓[ns]
% ulceration	100%	100%	0
Preventive index	-	15%	15↑

↑- Increase in % difference, ↓- Decrease in % difference
ns = not significant

Appendix 2b: Comparison of gastric ulcer parameters between control (70% EtOH) and 200mg (EBO)

	Control EtOH	200mg (EBO) + EtOH	% Difference
Ulcer score	5.75	3.38	34 ↓[a]
Ulcer index	575	338	34 ↓[a]
% ulceration	100%	75%	25 ↓
Preventive index	-	41%	41 ↑

a = significant (P>0.05)

Appendix 2c: Comparison of gastric ulcer parameters between control (70% EtOH) and 400mg (EBO)

	Control EtOH	400mg (EBO) + EtOH	% Difference
Ulcer score	5.75	1.0	83 ↓[a]
Ulcer index	575	100	83 ↓[a]
% ulceration	100%	38%	62 ↓
Preventive index	-	83%	83 ↑

↑- Increase in % difference, ↓- Decrease in % difference

a = significant (P>0.05)

Appendix 3a: Comparison of gastric ulcer parameters between treatment groups; 100 and 200 mg/kg EBO

	100mg (EBO) + EtOH	200mg (EBO) + EtOH	% Difference
Ulcer score	4.88	3.38	30.7 $\downarrow^{ns}$
Ulcer index	488	338	30.7 $\downarrow^{ns}$
% ulceration	100%	75%	25.0 ↓
Preventive index	15%	41%	26 ↑

ns = not significant

Appendix 3b: Comparison of gastric ulcer parameters between treatment groups; 100 and 400 mg/kg EBO

	100mg (EBO) + EtOH	400mg (EBO) + EtOH	% Difference
Ulcer score	4.88	1.0	80 $\downarrow^{a}$
Ulcer index	488	100	80 $\downarrow^{a}$
% ulceration	100%	38%	62 ↓
Preventive index	15%	83%	68 ↑

↑- Increase in % difference, ↓- Decrease in % difference

a = significant (P>0.05)

Appendix 3c: Comparison of gastric ulcer parameters between treatment groups; 200 and 400mg/kg EBO

	200mg/kg (EBO)	400mg/kg (EBO)	% Difference
Ulcer score	3.38	1.0	70.4 ↓[a]
Ulcer index	338	100	70.4 ↓[a]
% ulceration	75%	38%	37.0 ↓
Preventive index	41%	83%	42.0 ↑

a = significant (P>0.05)

Appendix 3d: Comparison of gastric ulcer parameters between treatment groups; 400 mg/kg EBO and Cimetidine 50mg/kg

	400mg/kg (EBO)	50mg/kg Cimetidine	% Difference
Ulcer score	1.0	3.75	275 ↑[a]
Ulcer index	100	375	275 ↑[a]
% ulceration	38%	100%	62 ↑
Preventive index	83%	35%	48 ↓

↑- Increase in % difference, ↓- Decrease in % difference

a = significant (P>0.05)

Appendix 4: Effect of orally administered extract of *Brassica oleracea* (EBO) with Cimetidine on gastric juice parameters

	50mg Cimetidine + EtOH	100mg (EBO) + EtOH	200mg (EBO) + EtOH	400mg (EBO) + EtOH
Volume (ml/3hrs)	1.38 ± 0.24	4.85 ± 1.44[a]	3.03 ± 0.23[a]	3.28 ± 0.30[a]
Acid concentration (mEq/l)	2.0 ± 0.20	0.63 ± 0.13[a]	1.0 ± 0.20[a]	0.7 ± 0.14[a]
Acid output (mEq/ 3hrs)	2.83 ± 0.63	2.58 ± 0.62[ns]	2.98 ± 0.52[ns]	2.60 ± 0.69[ns]
Pepsin Concentration (mg/ml)	12.1 ± 39	6.1 ± 11[a]	7.4 ± 11[a]	7.0 ± 36[a]

Data represent the Mean ± SE of Mean
n = 8 a = significant at $p < 0.05$ ns = not significant

Appendix 5: Comparison of gastric secretion parameters between normal and control group

	Normal Distilled Water	Control 70% EtOH	% difference
Volume (ml/3hrs)	3.76	3.88	3.19 ↑[ns]
Acid concentration (mEq/l)	1.88	0.83	53.03 ↓[a]
Acid output (mEq/3hrs)	6.78	3.28	51.62 ↓[a]
Pepsin concentration (mg/ml)	6.1	5.4	11.47 ↓[ns]

↑- Increase in % difference, ↓- Decrease in % difference
a = significant at $p < 0.05$ ns = not significant

Appendix 5a: Comparison of gastric secretion parameters between treatment groups; 100 and 200mg/kg EBO

	100mg (EBO) + EtOH	200mg (EBO) + EtOH	% Difference
Volume (ml/3hrs)	4.85	3.03	37.5 $\downarrow^{ns}$
Acid concentration (mEq/l)	0.63	1.0	58.7 $\uparrow^{a}$
Acid output (mEq/3hrs)	2.58	2.98	15.5 $\uparrow^{ns}$
Pepsin Concentration (mg/ml)	6.1	7.4	21.31 $\uparrow^{a}$

a = significant at $p < 0.05$ ns = not significant

Appendix 5b: Comparison of gastric secretion parameters between treatment groups; 100 and 400 mg /kg EBO

	100mg (EBO) + EtOH	400mg (EBO) + EtOH	% Difference
Volume(ml/3hrs)	4.85	3.28	32.4 $\downarrow^{ns}$
Acid concentration (mEq/l)	0.63	0.7	11.1 $\uparrow^{ns}$
Acid output (mEq/l)	2.58	2.60	0.8 $\uparrow^{ns}$
Pepsin concentration (mg/ml)	6.1	7.0	14.8 $\uparrow^{ns}$

ns = not significan

Appendix 5c: Comparison of gastric secretion parameters between treatment groups; 200 and 400 mg/kg EBO

	200mg (EBO) + EtOH	400mg (EBO) + EtOH	% Difference
Volume (ml/3hrs)	3.03	3.28	9.2 ↑
Acid concentration (mEq/l)	1.0	0.7	42.8 ↓[a]
Acid output (mEq/3hrs)	2.98	2.60	12.8 ↓[ns]
Pepsin concentration (mg/ml)	7.4	7.0	5.4 ↓[ns]

↑- Increase in % difference, ↓- Decrease in % difference

a = significant at $p < 0.05$ ns = not significan

Appendix 5d: Comparison of gastric secretion parameters between treatment groups; 400 mg/kg EBO and cimetidine

	400mg (EBO) + EtOH	50mg Cimetidine + EtOH	% Difference
Volume (ml/3hrs)	3.28	1.38	57.9 ↓[a]
Acid concentration (mEq/l)	0.7	2.0	185.7 ↑[a]
Acid output (mEq/3hrs)	2.60	2.83	8.8 ↑[ns]
Pepsin concentration (mg/ml)	7.0	12.1	73.0 ↑[a]

↑- Increase in % difference, ↓- Decrease in % difference

a = significant at $p < 0.05$ ns = not significant