2 REVIEW OF LITERATURE

This chapter gives a brief review of the anatomy of the stomach and physiology of gastric acid secretion, its involvement in the causation of ulcer. Also we have reviewed the existing literature related to the occurrence, symptoms, risk factors and pathogenesis of peptic ulcer disease, followed by the detailed mechanism of ulcer healing with separate briefings on each of the factors involved.

2.1 Peptic Ulcer disease:

Peptic ulcer disease (PUD) is a very common global problem today that encompasses both gastric and duodenal ulcer. An ulcer is defined as the disruption of the mucosal integrity leading to a local defect or excavation due to active inflammation. The pathophysiology of these ulcers involves an imbalance between offensive (acid, pepsin and *Helicobacter pylori*) and defensive factors (mucin, bicarbonate and prostaglandin and growth factors) (Valle, 2005).

Peptic ulcer disease is caused via two ways:

1) Excess secretion of acid and pepsin by the gastric mucosa or
2) Diminished ability of the gastroduodenal mucosal barrier to protect against the digestive properties of the acid-pepsin complex.

![Figure 2.1: Peptic ulcer disease (PUD): PUD encompasses both gastric and duodenal ulcers.](image)
Gastric and duodenal ulcers share many common features in terms of pathogenesis, diagnosis and treatment, but several factors and pathophysiology distinguish them from one another (Table 2.1).

<table>
<thead>
<tr>
<th>Gastric ulcer</th>
<th>Duodenal ulcer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Decrease in defensive factors</td>
<td>Increase in acid production</td>
</tr>
<tr>
<td>Lesser curvature of stomach</td>
<td>First portion of duodenum</td>
</tr>
<tr>
<td>45-55 years</td>
<td>35-45 years</td>
</tr>
<tr>
<td>Epigastric pain increases with food intake</td>
<td>Epigastric pain decreased with food intake</td>
</tr>
<tr>
<td>Weight loss (anorexia)</td>
<td>Weight gain</td>
</tr>
<tr>
<td>Haemorrhage is common</td>
<td>Bloating and excessive gas and nausea</td>
</tr>
</tbody>
</table>

Table 2.1: Difference between Gastric ulcer and Duodenal ulcer

![Image](imageurl)

**Figure 2.2:** Imbalance between the aggressive and defensive factors results in the pathogenesis of Peptic ulcer disease.
2.2 Epidemiology of peptic ulcer disease:

PUD is the most common chronic infection of humans because of its high morbidity, mortality and economic loss. In the United States about four million people have active peptic ulcers and about 350,000 new cases are diagnosed each year. Four times as many duodenal ulcers as gastric ulcers are diagnosed. Approximately 3000 deaths per year in the United States are due to duodenal ulcer and 3000 to gastric ulcer. There has been a marked decrease in reported hospitalization and mortality rates for peptic ulcer in the United States. Changes in criteria for selecting the underlying cause of death might account for some of the apparent decrease in ulcer mortality rates. Hospitalization rates for duodenal ulcers decreased nearly 50 per cent from 1970 to 1978, but hospitalization rates for gastric ulcers did not decrease. Although this decrease in hospitalization rates may reflect a decrease in duodenal ulcer disease incidence, it appears that changes in coding practices, hospitalization criteria, and diagnostic procedures have contributed to the reported declines in peptic ulcer hospitalization and mortality rates. There is no good evidence to support the popular belief that peptic ulcer is most common in the spring and autumn. The most consistent pattern appears to be low ulcer rates in the summer. There is strong evidence that cigarette smoking, regular use of aspirin, and prolonged use of steroids are associated with the development of peptic ulcer. There is some evidence that coffee and aspirin substitutes may affect ulcers, but most studies do not implicate alcohol, food, or psychological stress as causes of ulcer disease. Genetic factors play a role in both duodenal and gastric ulcer. The first-degree relatives of patients with duodenal ulcer have a two- to threefold increase in risk of getting duodenal ulcer and relatives of gastric ulcer patients have a similarly increased risk of getting a gastric ulcer. About half of the patients with duodenal ulcer have elevated plasma pepsinogen I. A small increase in risk of duodenal ulcer is found in persons with blood group O and in subjects who fail to secrete blood group antigens into the saliva. In most Western countries, morbidity from duodenal ulcer is more common than from gastric ulcer, even though deaths from gastric ulcer exceed or equal those from duodenal ulcer. In Japan, both morbidity and mortality are higher for gastric ulcer than for duodenal ulcer.

The prevalence of PUD has shifted from predominance in males to similar occurrences in males and females. Lifetime prevalence is approximately 11-14% in men and 8-11% in women. Age trends for ulcer occurrence reveal declining rates in
younger men, particularly for duodenal ulcer, and increasing rates in older women. Trends reflect complex changes in risk factors for PUD, including age-cohort phenomena with the prevalence of H pylori infection and the use of NSAIDs in older populations.

2.3 Pathophysiology of PUD:

Historically, pathophysiology of peptic ulcer disease focused on abnormalities in the secretion of gastric acid and pepsin, and on the suppression of acid as a treatment strategy. Today, gastric hyper secretion associated with gastrinoma in Zollinger–Ellison syndrome, antral G-cell hyperplasia, an increase in parietal-cell mass, and a physiological imbalance between the antagonistic gastric hormones gastrin and somatostatin is still an important issue in peptic ulcer disease. Moreover, it is known that cholinergic hypersensitivity and parasympathetic dominance are related to the stimulation not only of hydrochloric acid but also pepsin, which is often neglected as a cofactor in the development of erosive injury to the gastric mucosa.

![Image of Pathogenesis of Peptic Ulcer Disease]

**Figure 2.3:** Pathogenesis of Peptic Ulcer Disease
2.4 *Gastric Anatomy:*

The stomach is an expanded section of the digestive tube located between the esophagus and small intestine in the upper quadrant of the abdomen (Guyton *et al.*, 1998).

Anatomically, the stomach is divided into four parts –

- cardiac (narrow conical portion distal gastro esophageal junction)
- fundus (dome shaped proximal stomach)
- body or corpus (remainder of stomach) and
- a pyloric part

The medial curvature of stomach is known as lesser curvature while the lateral curvature is called as greater curvature. The stomach secretes 2 liters of gastric juice per day, a fluid mainly consisting of $\text{H}^+$, $\text{Cl}^-$, $\text{K}^+$, and water (Forte, 1986; Lipkin, 1985).

The wall of the stomach is composed of four layers:

- mucosa
- submucosa
- muscularis propria and
- serosa

The mucosa membrane of the stomach is thick and vascular carrying smooth, soft and velvety surface. The submucosa comprises of a dense tissue layer, supporting large plexus of blood and lymph vessels. In the empty state, the stomach is contracted and the mucosa and submucosa are thrown up into distinct folds called *rugae*. The rugae present on the gastric epithelial lining contain microscopic gastric pits; each branching into four or five gastric glands made up of highly specialized epithelial cells. The makeup of gastric glands varies with their anatomic locations. The gastric mucosa consists of three important types of tubular glands:
2.4.1 Cardiac glands:

They are heavily branched tubular glands, which mainly contain the mucus secreting cells.

2.4.2 Oxyntic glands:

The oxyntic glands are located on the inside surfaces of the body and fundus of the stomach, constituting the proximal 80 percent of the stomach.

A typical oxyntic gland is composed of three types of cells:

a) The mucous neck cells:

These cells extend into the rugae and secrete mucin, which protects the epithelium against shear stress and acid. The continuous shedding and replacement is found in this layer.

b) The peptic or chief cells

It secretes large quantities of pepsinogen, which is an inactive precursor of protein digesting enzyme, pepsin. These cells are present in the deepest part of the gastric mucosa.

c) The parietal or oxyntic cells which secrete hydrochloric acid and intrinsic factor. These are located on the upper end of the glands. Hydrochloric acid activates pepsinogen, stimulates hunger and also effectively sterilizes the contents of stomach.
d) A group of endocrine cells are also present in the epithelial lining of the glands, which are called enterochromaffin-like (ECL) cells. These cells are the source of gastric histamine.

2.4.3 Pyloric glands:

The pyloric glands mainly consist of mucous neck cells along with endocrine cells like gastrin secreting G cells and somatostatin secreting D cells. G cells possess gastrin-containing granules while D cells are present in the antral region of the stomach secrete somastostatin that regulates the acid secretion.

![Gastric Gland](image)

**Figure 2.4:** Different types of cells present in the gastric gland

2.5 Components of Gastric secretion:

Gastric acid secretion is found to be the major prerequisite for peptic ulcer disease and other acid-related diseases (Mossner et al., 2005). Gastric mucosal integrity is maintained through a balance between aggressive and defensive factors. The aggressive factors include acid, pepsin, Helicobacter pylori, nonsteroidal anti-inflammatory drugs (NSAIDS), smoking, alcohol, ischemia and corticosteroids where
Review of Literature

as the defensive factors are mucus, bicarbonate, blood flow, prostaglandins and growth factors. Among these aggressive factors, acid and pepsin confers the contributory role in ulcerogenesis (Forte, 1986). In our study, we have focused in determining the role of acid secretion in the pathophysiology of ulcer disease.

Gastric fluid contains enzymatic (Pepsinogen, Gastric lipase, Amylase and Renin) and non-enzymatic (Mucin, Hydrochloric acid) secretions, intrinsic factor, lactic acid and inorganic ions such as Na+, K+, Mg2+, HPO4−, SO42− and HCO3−. Out of these constituents Hydrochloric acid, Pepsinogen, Mucus, Intrinsic factor and bicarbonate ions play major roles in various physiological conditions. Oxyntic (or gastric) glands and pyloric glands are the two important tubular glands of the stomach mucosa (Forte, 1986). The oxyntic glands secrete hydrochloric acid, pepsinogen, intrinsic factor and mucus. The pyloric glands secrete mainly the mucus for protection of the pyloric mucosa, some pepsinogen, and mainly gastrin.

2.5.1 Aggressive factors:

2.5.1.1 Hydrochloric acid:

Hydrochloric acid is one of the most important offensive factors in ulcerogenesis, which not only regulates normal functioning of stomach but also plays in etiologic role in producing various forms of discomfort and inciting gastro duodenal mucosal injury. Hydrochloric acid is secreted by the stimulated parietal cells. Capacity of the stomach to secrete HCl is almost linearly related to parietal cell numbers. The primary function of HCl is to kill and minimize ingested bacteria with a notable exception of H. pylori. Gastric acid plays a significant role in protein hydrolysis and other aspects of digestive process. It aggravates the mucosal lesion mainly by back diffusion and by activating pepsinogen into pepsin, which increase the size of lesion by its proteolytic action. Acid back diffusion release histamine, which causes inflammation and further increase in acid output to cause more gastric damage.

2.5.1.2 Pepsinogen:

Pepsinogen, an inactive zymogen is a precursor of proteolytic enzyme pepsin and is secreted into gastric juice from both mucus cells and chief cells. Once secreted, pepsinogen is activated by stomach acid into active protease pepsin, which is largely responsible for the stomach ability to initiate digestion of the protein. Pepsinogen
(42,500 Dalton) gets cleaved in presence of HCl to yield active pepsin molecule (32,500 Dalton) (Van et al., 1956). Pepsin acts on proteins in highly acidic medium (pH 1.8-3.5). Pepsin also acts as one of the aggressive factors involved in mucosal injury (Allen et al., 1993).

2.5.1.3 Gastrin:

Gastrin stimulates acid secretion from parietal cells either directly or indirectly by enhancing histamine release from the ECL cells through gastrin/cholecystokinin receptors. Enhancement of inositol phospholipid turnover and the resulting increase in Ca\(^{2+}\) concentration play an important role in histamine releases from parietal cells induced by gastrin.

2.5.2 Defensive factors:

2.5.2.1 Mucus:

The most abundant epithelial cells are mucous cells, which cover the entire luminal surface and extend down into glands as mucous neck cells. These cells secrete bicarbonate rich mucus that coats and lubricates and serves an important role in protecting the epithelium from acid, pepsin and other chemical insults. Mucus gel is capable of maintenance of pH gradient so that the pH of the cell membrane is slightly alkaline (~7.3).

2.5.2.2 Bicarbonate:

Bicarbonate ions, secreted by the surface epithelial cells of mucosal lining of gastric mucosa, forms a pH gradient ranging from 1 to 2 at the gastric luminal surface and reaching to 6 to 7 along the epithelial cell surface. Bicarbonate affords far more protection than mucus alone and constitutes the first line of stomach defense against the gastric fluid. Bicarbonate secretion is stimulated by calcium, prostaglandins, cholinergic input and luminal acidification.

2.6 Phases of gastric secretion:

The secretion of gastric juice occurs in three phases:

2.6.1 Cephalic Phase:

The cephalic phase of gastric secretion occurs even before the food enters the stomach when it is being eaten. It results from the sight, smell, thought or taste of food.
The greater the appetite, the more intense is the stimulation. This phase of secretion accounts for 20 percent of gastric secretion.

2.6.2 Gastric Phase:

Once the food enters the stomach, it excites the long vagovagal reflexes, the local enteric reflexes and the gastrin mechanism, which in turn cause secretion of gastric juice throughout the several hours for which the food remains in the stomach. The gastric phase of secretion accounts for about 70 percent of total gastric secretion which is about 1.5 liters.

2.6.3 Intestinal Phase:

A small amount of gastric juice is secreted due to the presence of food in the upper portion of small intestine and in duodenum. This is due to the release of gastrin from the gastric mucosa in response to distention or chemical stimuli. In addition, amino acids absorbed in the blood and other hormones or reflexes play minor roles in causing secretion of gastric juice.

2.7 Regulation of Gastric secretion:

Gastric secretion is a complex, continuous process controlled by multiple central (neural) and peripheral (endocrine and paracrine) factors. Each factor attributes to a common final physiological event - secretion of $H^+$ by parietal cells, which are located on the fundus of the stomach. The most important structures in the central nervous system (CNS) involved in central stimulation of gastric acid secretion are the dorsal motor nucleus of the vagal nerve (DMNV), the hypothalamus, and the nucleus tractus solitarius (NTS). Efferent fibers originating in the DMNV descend to the stomach via the vagus nerve and synapse with ganglion cells of the enteric nervous system (ENS) (Hoogerwerf et al., 2001; Wallace et al., 2011).

2.7.1 The neuronal control:

The gastrointestinal system has a nervous system of its own called the enteric nervous system. It lies entirely in the wall of the gut, beginning in the esophagus and extending all the way to anus. The enteric system especially controls the gastrointestinal movements and secretions and consists of about 100 million neurons. The enteric system consists of two major plexus: the Myenteric- plexus and the Meissner’s plexus. The plexuses are interconnected and their ganglion cells receive
preganglionic parasympathetic fibres from the vagus which are mostly cholinergic and are mostly excitatory, though some are inhibitory in effect. The neurotransmitters secreted by the enteric neurons include Acetyl Choline, Noradrenaline, 5-Hydroxytryptamine, Purines, Nitric oxide and a variety of pharmacologically active peptides. Although the enteric system can function on its own, stimulation by the parasympathetic and sympathetic systems can further activate or inhibit the gastrointestinal functions.

2.7.2 Hormonal control:

The hormonal control includes hormones of gastrointestinal tract that include endocrine and paracrine secretions. The important endocrine secretion includes gastrin. The paracrine secretion includes hormones released from special cells found throughout the gastrointestinal tract. The most important of these hormones is Histamine.

2.7.3 The Parietal Cell and acid secretion:

The parietal/oxyntic cells are highly specialized epithelial cells located on the upper end of the glands and secrete hydrochloric acid. The gastric parietal cell secretes HCI into the lumen of the stomach at concentrations in excess of 150 mm. The structure of parietal cell contains many large branching intracellular canaliculi. When this cells secrete the acid juice, the membranes of canaliculi open widely to empty their secretion directly in to the lumen of stomach (Hoogerwerf et al., 2001). Parietal cells possess an extensive secretory membrane system that comprises 50% of the membrane mass of the cell. The major protein constituent of these membranes is the gastric proton pump, the heterodimeric gastric H+ K+ ATPase (Dunbar et al., 2001; Sachs, 1994; Van Driel et al., 1995; Yao et al., 2003). A central role has been established for the gastric (H+ + K+)-ATPase (EC 3.6.1.36) in acid secretion (Forte et al., 1977). When isolated, the (H+ + K+)-ATPase is found in vesicles orientated with their ATP- hydrolyzing site exposed on the exterior face, such that, in the presence of Mg2+ and internal K+, addition of ATP results in the accumulation of protons (Sachs et al., 1976). Stimulation of acid secretion is associated with extensive morphological changes within the parietal cell. There is a 5-10-fold increase in the apical surface of the parietal cell with the concomitant depletion of the extensive intracellular tubulovesicular membranes. Withdrawal of the stimulus results in a cessation of acid secretion, accompanied by a reversion of the parietal cell ultrastructure back to the resting state. A membrane-
recycling hypothesis has been offered to account for these changes (Forte et al., 1981; Forte et al., 1977). Subcellular fractionation of gastric mucosa in the resting and stimulated state reveals that the ultrastructural changes are accompanied by biochemical and functional alternations. Fractionation of resting gastric mucosa yields the majority of the \((H^+ K^+)\)-ATPase in low density, microsomal membrane vesicles. In contrast, the \((H^+ K^+)\)-ATPase from stimulated gastric mucosa is redistributed to larger, denser membrane vesicles (Wolosin et al., 1981a). The membrane vesicles from stimulated tissue show a greater permeability to KCl than those from resting tissue (Wolosin et al., 1981b).

2.8 Stimulation of gastric acid secretion:

The basic neurotransmitters or factors that directly stimulate the secretion by gastric glands are Acetylcholine, Gastrin and Histamine. All these function by binding first with specific receptors for each on the secretory cells.

2.8.1 Stimulation of acid secretion by Acetylcholine:

Acetylcholine excites secretion by all the secretory cell types in the gastric glands, including secretion of pepsinogen by the peptic cells, hydrochloric acid by the parietal cells and mucus by the mucus cells. Gastrin and Histamine stimulate strongly the secretion of acid by the parietal cell.

2.8.2 Stimulation of acid secretion by Gastrin:

Gastrin stimulates acid secretion predominantly in an indirect fashion by causing the release of histamine from ECL cells through cholecystokinin (CCK\(_2\)) receptors. Enhancement of inositol phospholipids turnover and the resulting increase in \(Ca^{2+}\) concentration play an important role in histamine release from parietal cells induced by gastrin. Gastrin is the major endocrine regulator of gastric acid secretion because changes in circulating gastrin can account for most of the gastric secretory response to feeding (Hoogerwerf et al., 2001). The nerve signals from the vagus nerve and the local enteric reflexes stimulate the mucosa of the stomach antrum to secrete the hormone Gastrin. This hormone is secreted from the gastrin cells or the G cells present in the pyloric glands.
2.8.3 **Role of Histamine in acid secretion:**

Histamine, an amino acid derivative stimulates acid secretion by the parietal cells. Histamine is released from the enterochromaffin-like (ECL) cells through multifactorial pathways and is a critical regulator of acid production through the H₂ subtype of receptor. Histamine activates the parietal cell in paracrine fashion (Hoogerwerf et al., 2001).

2.8.4 **Histamine H₂-Receptor Antagonists:**

Gastric acid secretion by parietal cells of the gastric mucosa has a complex control mechanism. The H₂ receptor antagonists inhibit acid production by reversible competing with histamine for binding to H₂ receptors on the basolateral membrane of parietal cells. All four of the H₂RAs: cimetidine, ranitidine, famotidine and nizatidine, which differ mainly in their pharmacokinetics and propensity to cause drug interactions, are available as prescription and OTC formulation for oral administration. These drugs are less potent than PPIs but still suppress 24-h gastric acid secretion by ≈70%. The H₂ receptor antagonists predominantly inhibit basal acid secretion, which accounts for their efficacy in suppressing nocturnal acid secretion. These are approved for acute treatment of episodic heartburn, or for prophylaxis before an activity that may potentially result in reflux symptoms (Heavy meal or exercise in some patients). H₂RA therapy is generally safe; the most commonly reported adverse events were diarrhea and headache. Cimetidine inhibits cytochrome P450 and can slow metabolism of several drugs (for example, warfarin, phenytoin, diazepam), thus sometimes resulting in serious adverse clinical effects. Most H₂RAs cross the placenta thus they are advised to use with caution during pregnancy. The rapid development of pharmacological tolerance (within 7–14 days) is a further disadvantage of H₂RAs, and the loss of gastric acid secretion suppression obtained with these agents may partially explain their unsatisfactory use in patients with GERD (Wallace et al., 2011).
A few other factors that stimulate the gastric acid secretion are:

- Caffeine
- Alcoholic beverages
- Circulatory amino acids

The stimulatory effects of these factors are slight in comparison with acetylcholine, gastrin and histamine.

2.9 **Basic Mechanism of Gastric acid secretion:**

When stimulated, the parietal cells secrete an acid solution that contains about 160 millimoles of hydrochloric acid per liter. The pH of this acid is about 0.8, demonstrating its extreme acidity. At this pH, the hydrogen ion concentration is about 3 million times that of the arterial blood. It requires a tremendous amount of more than 1500 calories of energy per liter of gastric juice (Guyton *et al.*, 1998).

The following are the steps involved in the mechanism of Hydrochloric acid formation.

- Chloride ion is actively transported from the cytoplasm of the parietal cell in to the lumen of the canaliculus, and sodium ions are actively transported out
of the lumen. These two effects together create a negative potential of -40 to -70 millivolts in the canaliculus, which in turn causes passive diffusion of positively charged potassium ions and a small number of sodium ions from the cell cytoplasm also in to the canaliculus. Thus, in effect, potassium chloride and smaller amounts of sodium chloride enter the canaliculus (step 1 in the diagram).

- Water becomes dissociated into hydrogen ions and hydroxyl ions in the cell cytoplasm. The hydrogen ions are then actively secreted in to the canaliculus in exchange for potassium ions; this exchange is catalyzed by H⁺, K⁺-ATPase (step 2). In addition, sodium ions are actively reabsorbed by a separate ATPase, the Na⁺, H⁺-ATPase. Thus, most of the potassium and sodium ions that had diffused in to the canaliculus are reabsorbed, and the hydrogen ions take their place, giving a strong solution of hydrochloric acid in the canaliculus, which is then secreted in to the lumen of the gland.

- Water passes into the canaliculus by osmosis because of the secretion of the ions into the canaliculus. Thus, the final secretion entering the canaliculus contains hydrochloric acid in a concentration of 150 to 160 mEq/liter, potassium chloride in a concentration of 15 mEq/liter and a small amount of sodium chloride.

- Finally, carbon dioxide, either formed during metabolism in the cell or entering the cell from the blood, combines under the influence of carbonic anhydrase with the hydroxyl ions (formed in step 2) to form bicarbonate ions, which then diffuse out of the cell in to the extracellular fluid in exchange for chloride ions that enter the cell and later will be secreted in to the canaliculus (steps 3, 4, 5).
2.10 Physiological and pharmacological regulation of gastric secretions:

Acid is secreted by the parietal cell in response to a variety of influences both central and peripheral. Figure 7 depicts the role of various neuronal (acetylcholine), paracrine (histamine), and endocrine (gastrin) factors in the regulation of acid secretion. Their respective specific receptors (M₃, H₂, and CCK2) have been localized on the basolateral membrane of the parietal cell. The binding of ligands to receptors on the surface of the parietal cell generates changes in second messengers, which determine the location and the activity of the gastric proton pump, gastric H⁺-K⁺-ATPase (Ito et al., 1974).

Two major signaling pathways are present within the parietal cell: the cyclic Adenosine Mono Phosphate (AMP)-dependent pathway and the Ca²⁺-dependent pathway. Histamine uses the first pathway, while gastrin and ACh exert their effect via the latter. The cyclic –AMP dependent pathway results in the phosphorylation of the parietal cell effector proteins. Ca²⁺-dependent pathway leads to an increase in the cytosolic Ca²⁺. Both the pathways activate the gastric H⁺, K⁺-ATPase (Hoogerwerf et al., 2001).

ACh release from postganglionic vagal fibers can stimulate directly the gastric acid secretion through a specific muscarinic cholinergic receptor subtype, M₃, located...
on the basolateral membrane of the parietal cell. Ach also indirectly affects the parietal cell through the stimulation of histamine release from the enterochromaffin-like (ECL) cells in the fundus and the stimulation of gastrin release from the G cells in the gastric antrum.

Histamine is released from ECL cells through multifactorial pathways and is a critical regulator of acid production through the H₂ subtype of receptor. ECL cells are usually found in close proximity to the parietal cells. Histamine activates the parietal cell in a paracrine fashion; it diffuses from its site to the parietal cell. Its involvement in the gastric acid secretion has been convincingly demonstrated by the inhibition of acid secretion with the use of H₂ receptor antagonists. The ECL cells are the sole source of gastric histamine involved in acid secretion.

Gastrin primarily is present in the antral G cells. As with histamine, the release of gastrin is regulated through multifactorial pathways involving, among other factors, central neural activation, local distention, and chemical components of the gastric content. Gastrin stimulates acid secretion predominantly in an indirect manner by causing the release of histamine from ECL cells; a less important, direct effect of gastrin on parietal cells is also seen.

Somatostatin, localized in the antral D cells, may inhibit gastrin secretion in a paracrine matter, but its exact role in the inhibition of acid secretion remains to be defined. There appears to be a decrease in D cells in patients with *Helicobacter pylori* infection, and this may lead to excess gastrin production due to a reduced inhibition by somatostatin.
Figure 2.7: Physiological and pharmacological regulation of gastric secretions: the basis for therapy of peptic ulcer drugs

2.10.1 Proton Pump:

The gastric proton pump generates a pH of 0.8 in the gastric lumen, creating one of the largest ion gradients known to biology. Gastric acid secretion is conducted by proton pump the H⁺-K⁺-ATPase, which exchanges H⁺ on the cytoplasmic side with K⁺ on the opposite side using energy supplied by ATP hydrolysis. A central role has been established for the gastric H⁺ K⁺-ATPase in acid secretion (Forte et al., 1977). When isolated, the H⁺ K⁺-ATPase is found in vesicles orientated with their ATP-hydrolysing site exposed on the exterior face, such that, in the presence of Mg²⁺ and internal K⁺, addition of ATP results in the accumulation of protons (Sachs et al., 1976).

2.10.2 Subunits of H⁺-K⁺-ATPase:

The gastric H⁺-K⁺-ATPase is a member of the P₂-type ATPase family and undergoes a cycle of phosphorylation and dephosphorylation coupled to the outward and inward transport of hydrogen and potassium ions, respectively, in the secretory
The canalicular of the parietal cell (Tyagarajan et al., 1996a). The gastric H+–K+-ATPase consists of α- and β-subunits. The heterodimeric H-K-ATPase has a remarkable ability to resist the harsh conditions on the external surface of the functioning parietal cell. The α subunit (HKα), is responsible for ATP-catalyzed exchange of H+ for K+. It is a multispansing membrane protein with most of its 114-kDa mass located in the cytoplasm (Okamoto et al., 1990; Shull, 1990). The closely associated β-subunit (HKβ) is a glycoprotein; on the basis of the predicted topology from a single transmembrane segment, 70% of its glycosylated mass is oriented in the extracellular space (Canfield et al., 1990; Okamoto et al., 1990; Shull, 1990). HKβ has six or seven N-linked sites of glycosylation (Canfield et al., 1990; Shull, 1990), although there are reported differences for the nature of the oligosaccharides for different species (Arnold et al., 1998). Previous studies have suggested that glycosylation has some influence on enzymatic activity (Klaassen et al., 1997); however, this activity may be secondary to some more basic requirement for proper subunit folding and interaction and/or trafficking through the cell (Chow et al., 1995). It has also been proposed that the carbohydrate moieties of HKβ may play a role in protecting the holoenzyme from the harsh extracellular environment (Chow et al., 1995; Forte et al., 1970).

Striking morphological transformations characterize the stimulation of the gastric parietal cell; the canalicular apical membrane greatly expands at the expense of an extensive intracellular membrane network. This membrane network, rich in the gastric proton pump (H+–K+-ATPase), has conventionally been called tubulovesicles.

2.11 Present therapeutic strategies of Peptic ulcer:

Current therapeutic strategies for treating peptic ulcer disease are focused on decreasing the acid secretion and/or strengthening the mucosal defense system. Understanding the physiology of gastric acid secretion and the pathophysiology of acid related diseases (e.g. gastroesophageal reflux and peptic ulcer) has led to the development of numerous ways to decrease acid exposure. Pharmacologically one can try to neutralize secreted acid by antacids, prevent stimulation of the parietal cell, improve mucosal defenses and block the functioning of the proton pump. New approaches that block acid secretion are now being developed. Gastrin (CCK2) receptor antagonists and potassium-competitive acid blockers (P-CABs) are in clinical development. The various categories of drugs for treating gastric ulcer include:

1) Acid neutralizing/inhibitory drugs: This category of drugs includes
(a) H₂ Receptor antagonists: eg: Cimetidine, Ranitidine and Femotidine.
(b) Proton Pump Inhibitors: eg: Omeprazole, Lansoprazole, Rabeprazole.
(c) Antacid.

2. **Cytoprotective agents:** eg: Sucralfate, Colloidal bismuth subcitrate, Ranitidine bismuth citrate.

3. **Prostaglandin analogue:** eg: Misoprostol, Enprostil, Rioprostil.

4. **Anti-cholinergic drugs:** Propantheline, Oxyphenonium, Pirenzepine, Telenzepine.

5. **Miscellaneous drugs:** eg: rebapimide.

2.12 *Importance of proton pump inhibitors:*

Proton pump inhibitors (PPIs) are the most effective pharmacologic agents for the treatment of gastric ulcer. There are currently 5 PPIs available: omeprazole, esomeprazole, lansoprazole, pantoprazole, and rabeprazole, of which only omeprazole is available as an OTC. PPIs are prodrug which is converted into sulphinamide by an actively secreting gastric parietal cell. The trapped sulphonamide then binds irreversibly to the H⁺-K⁺-ATPase and blocks the secretion of protons. Because they block the final step in acid production, the PPI are effective in acid suppression regardless of other stimulating factors. In typical doses, these drugs diminish the daily production of acid (basal & stimulated by 80-90%) (Wallace *et al.*, 2011). All currently available PPIs are substituted benzimidazoles. All PPIs are prodrugs, and thus have to be protected against acid degradation with encapsulation, i.e. enteric-coated microgranules, or tablets. When PPIs reach the acidic environment of the parietal cells they are entrapped and form an irreversible cationic sulphenamide bond with the hydrogen potassium ATPase, thus inhibiting its action. This inhibition is independent of the mode of stimulation of the parietal cell (Valle, 2005).

Proton pump inhibitors are more effective than H₂ Receptor Antagonists for the treatment of peptic ulcers (Chow *et al.*, 1995) and are the most effective drugs to treat all grades of GERD and peptic ulcer disease. Proton pump inhibitors promote faster symptom relief not only in reflux disease (Wolosin *et al.*, 1981a) but also in nonerosive reflux disease (Tyagarajan *et al.*, 1996b). Continuous therapy with PPIs is efficient in preventing relapse of oesophagitis. There are currently five PPIs available on the market; omeprazole, esomeprazole, lansoprazole, pantoprazole and rabeprazole. A further PPI, tenatoprazole is currently in phase III development.
Long-term use of PPIs has potential areas of concern which includes hypergastrinemia and rebound, hypersecretion following drug withdrawal, high ulcer relapses, tolerance and various drug interactions (Wallace et al., 2011). However recent review of the potential gastrointestinal effects of long-term acid suppression with PPI showed that these agents rarely, if ever, produce adverse events (Klinkenberg-Knol et al., 2000).

Now a days racemic mixture of S and R isomers of proton pump are gaining recognition. These isomers of proton pump inhibitors show superior therapeutic efficacy and have better metabolic and pharmacokinetic profile compared to their parent racemates. The new PPIs are enlisted below (Table 2.2)

<table>
<thead>
<tr>
<th>Isomeric PPIs</th>
<th>Parent Racemates</th>
<th>Symptom Relief Compared to parent PPIs</th>
<th>Healing Efficacy Compared to parent PPIs</th>
</tr>
</thead>
<tbody>
<tr>
<td>Esomeprazole</td>
<td>Omeprazole</td>
<td>Better</td>
<td>Better</td>
</tr>
<tr>
<td>S-pantoprazole</td>
<td>Pantoprazole</td>
<td>Better</td>
<td>Equally effective</td>
</tr>
<tr>
<td>Dxrabeprazole</td>
<td>Rebeprazole</td>
<td>Better</td>
<td>Better</td>
</tr>
<tr>
<td>Dxlsansoprazole</td>
<td>Lansoprazole</td>
<td>Better</td>
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Table 2.2: New isomeric PPIs and comparison of their therapeutic and healing efficacy with parent PPIs

2.13 The gastric mucosal defense system:

Gastric mucosal layers form a barrier that limits exposure of the gastric mucosal cells to numerous injurious luminal agents and irritants of exogenous and endogenous origins (Zayachkivska et al., 2005). However, if the barrier is weakened and/or corroding challenge is increased, the epithelial layers will be overwhelmed and the underlying tissue is digested leading to formation of lesion or ulcer (Playford et al., 2005). Anything that breaches the mucosal lining results in the inflammation of underlying tissue and erosion of the stomach wall which ends in gastric ulceration (Kwiecien et al., 2002b). The endogenous gastroprotective components of the gastrointestinal mucosa against aggressive factors mainly consist of functional, humoral
and neuronal factors. Alkaline mucus secretion, mucosal microcirculation and motility act as functional factors, while prostaglandin, bicarbonate and nitric oxide act as humoral factors, all of which are known to contribute to mucosal protection against injurious luminal agents (Repetto et al., 2002a). The physiological basis of mucosal barrier function involves several factors and mechanisms. These are: 1) mucus coating of epithelial cells, 2) HCO$_3$- component that neutralizes the acid, 3) epithelial cells joined by tight junction, and 4) high epithelial cell turnover rate. They could be envisioned as pre-epithelial, epithelial and sub-epithelial components of mucosal protective barrier (Zayachkivska et al., 2005).

![Figure 2.8: Components involved in providing gastroduodenal mucosal defense and repair (Source: Valle, 2005).](image)

The first line of defense is a mucus-bicarbonate layer which serves as a physicochemical barrier to multiple molecules including H$^+$. The mucous-gel functions as a non-stirred water layer impeding diffusion of ions and molecules such as pepsin and H$^+$. Gastric mucus consists of a viscous, elastic, adherent and transparent gel formed by 95% water and 5% glycoproteins that covers the entire gastrointestinal mucosa. Mucus is capable of acting as an antioxidant, and thus can reduce mucosal damage mediated by oxygen free radicals (Guha et al., 2002; Valle, 2005). The protective properties of the mucus barrier depend not only on the gel structure but also on the amount or thickness of the layers covering the mucosal surface. When cells containing mucus are damaged by extra-cellular oxygen radicals, the intracellular mucus may be released into the gastric tissue and prevent additional damage by scavenging them (Repetto et al., 2002b). Thus a decrease in gastric mucus makes
epithelial cells susceptible to injuries induced by acid or chemicals like aspirin. The surface epithelial cells, the second layer, provide the next line of defense through several factors including mucus and bicarbonate production, and formation of intercellular tight junctions. Several growth factors such as epidermal growth factor (EGF), transforming growth factor alpha (TGFα), and basic fibroblast growth factor (FGF) modulate the process of restoring the damaged regions (restitution) of the mucosa (Guha et al., 2002).

The sub-epithelial defense/repair system is an elaborate microvascular system within the gastric sub-mucosal layer. Mucosal blood flow is also an important component of the gastroduodenal barrier function. In the stomach, the presence of luminal acid increases the delivery of vascular bicarbonate into the overlying mucous layer by the mucosal microcirculation, thereby neutralizing H+ invading from the lumen. The circulatory bed in the sub-mucosa provides HCO₃⁻, micronutrients and O₂ while removing toxic metabolic byproducts. The endogenous PGs play an important role in the maintenance of mucosal integrity, which includes continuous secretion of HCO₃⁻ and a mucus production in the stomach and duodenum (Kwiecien et al., 2002a; Valle, 2005).

2.13.1 Sucralfate:

Sucralfate, a mucosal protective agent prevent mucosal injury, inflammation and heals existing ulcers by improving the mucosal protection mechanism. Sucralfate consist of the octasulfate of sucrose to which Al(OH)₃ has been added. In an acid environment (pH<4), it undergoes extensive crosslinking to produce a viscous, sticky polymer that adheres to epithelial cells and ulcer crater for upto 6 hrs after a single dose. In addition to inhibiting hydrolysis of mucosal protein by pepsin, sucralfate may have additional cytoprotective effects, including stimulation of local production of PGE₂ and epidermal growth factor. Sucralfate also binds bile salts, thus some clinicians use sucralfate to treat individuals with the syndrome of biliary esophagitis. Sucralfate provides similar level of symptomatic relief to that of H2RAs; however, studies evaluating sucralfate in the healing of GERD have produced inconsistent results, with reported healing rates varying from 17%–67% (Chiba et al., 1997; Tytgat, 1987). A systemic review evaluated the effectiveness in healing erosive esophagitis of mucosal protective agents compared with H₂RA therapy alone or H₂RAs combined with mucosal protective agents did not show statistically significant benefit of the
combination therapy compared to sucralfate monotherapy in healing of peptic reflux esophagitis. Mucosal protective agents are inferior to antacid/alginates, H₂RAs and PPIs in the treatment of erosive esophagitis and in relieving symptoms of GERD. The efficacy of mucosal protective agents in healing esophagitis has not been documented in systemic database review.

2.14 Miscellaneous Agents:

2.14.1 Potassium-competitive acid blockers:

Potassium-competitive acid blockers (P-CABs) represent a new class of drugs acting through a reversible binding mechanism different from the PPIs. There are several compounds (soraprazan, AZD0865, revaprazan), known initially as ‘reversible’ PPIs and categorized now as potassium-competitive acid blockers (P-CABs), which bind to the proton pump at or near the site of the potassium channel (Mossner et al., 2005; Vakil, 2004). P-CAB binding to the proton pump is competitive and reversible, and these compounds inhibit acid secretion rapidly, within 30 min of administration; whereas classical PPIs need several days to reach their steady-state effect. Moreover, P-CABs are active in the absence of stimulated acid secretion and their effect is rapidly reversible. Therefore, P-CABs generated considerable research interest as potential new therapies for GERD. However, recent study with AZD0865 showed that despite of pronounced effects on acid secretions, marginal effects were observed on healing rates and symptoms control compared to PPI (Kahrilas et al., 2007; Klinkenberg-Knol et al., 2000). Therefore, with the exception of TAK-438 (Takeda) and revaprazan (Yuhan), most pharmaceutical companies have discontinued clinical development of their P-CAB compounds. Clinical data for both agents in the setting of GERD are currently lacking, further research will show whether these two compounds are able to overcome the disappointing results seen with AZD0865.

2.15 Risk factors for Peptic Ulcer Disease:

Peptic ulcer disease is a chronic disease with a high rate of relapsing. As it is a multifactorial disease, lots of factors contribute in its progress. The two predominant causes of gastric injury are H pylori and NSAID ingestion. Apart from these two factors several factors like- Psychologic stress, cigarette smoking, alcohol consumption, use of nonsteroidal antiinflammatory drugs (NSAIDs) including aspirin, oral bisphosphonates,
potassium chloride, immuno suppressive medications, and an age related decline in prostaglandin levels have all been shown to contribute to peptic ulcer disease.

2.15.1 Genetic factors:

Johnsen et al. reported that inheritance is a risk factor for PUD. In a study performed also before the “H. pylori era”, the first-degree relatives of patients with DU had a two- to threefold increase in risk of getting DU and first-degree relatives of GU patients had a similarly increased risk of getting a GU (Kurata et al., 1984). However, in a more recent twin cohort study by Räähä et al. (Raiha et al., 1998) controlling also for H. pylori infection, it was found that familial aggregation of PUD is modest, and attributable almost solely to genetic factors (Valle, 2005).

2.15.2 Psychological factors:

There is some evidence for an association between PUD and psychological factors. In one study depression was the variable that best discriminated PUD patients from non-ulcer controls; a negative perception of life events also had discriminating value for PUD risk. Emotional stress might also predispose for PUD development (Valle, 2005). In a study by Levenstein et al (Levenstein et al., 1998), they found that depression, maladjustment and hostility are prospectively associated with PUD. These associations are partially accounted for by confounding or mediation by standard risk factors and are to some extent related to socioeconomic status (Desai et al., 1997). Goodwin et al. 1986 (Goodwin et al., 1986) in an US population based survey reported a clear dose-response relationship between generalized anxiety disorder and self-reported PUD among adults. Some studies have shown that psychological factors may contribute to delayed ulcer healing and on the other hand psychotherapy may have a positive role in healing of PUD (Levenstein et al., 1996).
Figure 2.9: Factors involved in Psychological stress

2.15.3 Socioeconomic factors:

According to some earlier reports, DU is a more common condition in persons with a low level of education and heavy work (Friedman et al., 1974; Langman, 1979), often explained by the fact that the latter are more often smokers, but also by difficulties of being able to respond to demands, inability to exert any influence on or ability to find satisfaction in work (Mason et al., 1986). Shift work and a trying private life also seemed to predispose to PUD (Nasiry et al., 1987; Walker et al., 1988). A lower frequency of PUD, in people with a higher education, has been described from the US. These weak associations mainly apply to DU disease and all these studies have been done before the “H. pylori era”, and subsequently not controlled for this prosperity dependent infection. More relevant is therefore for example the study by Jones at al. that showed that the lowest rate for ulcer diagnosis (4.7%) was found in the highest social class and the highest (17.1%) in the lowest social class, and the study by Levenstein et al. showed that psychological stress, health risk behaviors, analgesic use and hard physical labor may contribute to the increased risk of ulcer in low socioeconomic populations (Levenstein et al., 1998) and also that low socioeconomic status and concrete life difficulties are associated with peptic ulcer in the general population (Levenstein et al., 1995). In a recent Danish study, they found that poor
socioeconomic status is an important risk factor for PUD exerting its effect independently of *H. pylori* infection and that strenuous work may increase the risk of PUD in people with *H. pylori* infection (Rosenstock et al., 2004). Researchers in China concluded recently that the incidence of PUD in the Wuhan area of China is highly associated with age, gender, occupation and geographic environmental factors (Dong et al., 2004).

2.16 *Helicobacter pylori*-associated ulcer:

During the 1980s, *H. pylori* infection was found in more than 90% of patients with duodenal ulcers, and some 70% of patients with gastric ulcers (Ernst, 2000). The declining incidence and prevalence of peptic ulcer in developed countries has paralleled the falling prevalence of *H. pylori* infection, especially in populations with high infection rates (Goodwin et al., 1986). Only *H. pylori* eradication is an effective treatment for both duodenal and gastric ulcers. Antisecretory drugs work well for controlling symptoms and allowing ulcers to heal, and the absolute benefit of eradicating *H. pylori* infection is small with respect to healing alone. In a Cochrane meta-analysis the eradication of *H. pylori* infection combined with the use of an ulcer-healing drug significantly increased duodenal healing to 83% (intent-to-treat analysis), with the relative risk of the ulcer persisting being 0.66 (95% CI 0.58–0.76) compared with the ulcer-healing drugs alone; but eradication was not significantly superior to ulcer-healing drugs for gastric-ulcer healing (relative risk 1.32; 95% CI 0.92–1.90).

It was, however, the isolation of *H. pylori* and its identification as the most important cause of peptic ulcer disease that led to exploration of the role of inflammation and its associated cytokine cascade in gastric acid secretion. *H. pylori* evade attack by the host immune system and causes chronic, indolent inflammation by several mechanisms. *H. pylori* can damage the mucosal defense system by reducing the thickness of the mucus gel layer, diminishing mucosal blood flow and interacting with the gastric epithelium throughout all stages of the infection. *H. pylori* infection can also increase gastric acid secretion; by producing various antigens, virulence factors, and soluble mediators, *H. pylori* induces inflammation, which increases parietal-cell mass and, therefore, the capacity to secrete acid. The *H. pylori* cytotoxin associated gene CagA also has an important role: it interferes with gastric epithelial cell-signaling pathways, thereby regulating cellular responses and possibly contributing to apical
junction barrier disruption, interleukin-8 secretion and phenotypic changes to gastric epithelial cells.

Understanding the pathophysiology of peptic ulcer disease is at something of a crossroads: mechanisms of injury differ distinctly between duodenal and gastric ulcers. Duodenal ulcer is essentially an H. pylori-related disease and is caused mainly by an increase in acid and pepsin load, and gastric metaplasia in the duodenal cap (Dore et al., 2000). Gastric ulcer, at least in Western countries, is most commonly associated with NSAID ingestion, although H. pylori infection might also be present (Laine, 1996). Chronic, superficial and atrophic gastritis predominate in patients with gastric ulcers, when even normal acid levels can be associated with mucosal ulceration (Wolfe et al., 1988). In both conditions, ulcer is associated with an imbalance between protective and aggressive factors, with inflammation being a leading cause of this imbalance.

The isolation of H. pylori in the early 1980s was one of the most exciting advances in the history of peptic ulcer disease (Marshall et al., 1984), and it has dramatically changed the management of peptic ulcer. Eradication of H. pylori infection is now the
mainstay of treatment for peptic ulcer disease, and has resulted in very high ulcer healing rates and recurrence rates that have dropped dramatically, especially for individuals with a duodenal ulcer. The greater recognition of the role of NSAIDs and aspirin in gastrointestinal tract injury has led to the development of therapeutic and preventive strategies that rely on the use of antisecretory drugs, the prostaglandin analog misoprostol, or selective cyclo-oxygenase (COX)-2 inhibitors (coxibs).

2.17 Drugs:

2.17.1 Non steroidal anti-inflammatory drugs induced injury:

Despite their well-accepted anti-inflammatory and analgesic benefits, NSAID use is probably the most common cause of gastrointestinal mucosal injury in Western countries. NSAIDs, including aspirin, significantly increase the risk of adverse gastrointestinal events, particularly those related to gastric and/or duodenal mucosal injury: erosions, ulcers and ulcer complications, especially bleeding (Laine, 2001). About 15–30% of regular NSAID users have one or more ulcers when examined endoscopically, and 3–4.5% of NSAID users have clinically significant upper gastrointestinal events, including ulcers and ulcer complications. Patients taking low-dose aspirin for the prevention of a cardiovascular event, such as myocardial infarction or thrombotic stroke, are also at increased risk of gastrointestinal injury and complications (Weisman et al., 2002). In asymptomatic patients taking low-dose aspirin (75–325 mg/day) for ≥3 months, endoscopically observed ulcers or erosions are reported in 47.83% of cases.19 The risk of upper gastrointestinal bleeding events is dose-dependent, with an odds ratio (OR) of 3.3 for 300 mg of aspirin (95% CI 1.2–9.0) and an OR of 6.4 for 1.2 g of aspirin (95% CI 2.5–16.5) (Slattery, 1995). In multivariate models adjusted for age, sex, and clinical risk, low-dose aspirin alone was independently associated with an increased risk of ulcer bleeding, with an OR of 2.4 (95% CI 1.8–3.
Figure 2.10: Mechanism by which NSAIDs induce mucosal injury

NSAID cause gastric injury by various different means such as topical application increases permeability of the mucosa, allowing aggressive factors access to the mucosa. Most of the NSAIDs are weak organic acid so in the acidic milieu they are converted to more lipid soluble unionized acids that penetrate into the gastric epithelial cells. There at neutral pH, they are reionized and trapped within the cell causing local injury. Other action of NSAIDs on different cell types is exhibited in Figure 2.10.

Figure 2.11: Mechanism of NSAID induced gastric injury
Other mechanism through which NSAIDs cause gastromucosal injury is independent of COX inhibition, which mainly includes: neutrophils, gastric hypermotility, microcirculatory disturbances, oxygen derived free radicals and luminal acid. Different mechanism through which NSAIDs contributes to the genesis of mucosal damage are shown in Figure 2.11.

**Figure 2.12:** Diagrammatic presentation of mechanism of action of non-steroidal anti-inflammatory drugs

The injurious gastrointestinal effects of NSAIDs are largely caused by the inhibition of COX1 and its role in normal mucosal defense mechanisms (discussed above), and also through the inhibition of thromboxane A2, which compromises platelet function and results in gastrointestinal bleeding. Clinical trials have repeatedly demonstrated that coxibs are associated with fewer ulcers, less gastrointestinal bleeding and fewer ulcer complications than nonselective NSAIDs, but concurrent use of low-dose aspirin blunts this benefit (Hunt, 2003; Schnitzer, 2004). It is expected that the withdrawal of several coxibs will lead to many patients switching back to nonselective NSAIDs, with an anticipated increase in cases of gastrointestinal bleeding, especially in elderly patients.
2.18 Endogenous mediators:

Several factors that are formed endogenously under normal physiological conditions and by various inflammatory processes have been implicated in gastric ulceration. These can be lipid metabolites, amines, reactive oxygen species, proinflammatory cytokines or chemoattractants.

2.18.1 Lipid mediators:

2.18.1.1 Role of leukotrienes:

Leukotrienes are another group of mediators that might contribute to the increase in neutrophil adherence and the mucosal injury that occurs after NSAID administration. Leukotrienes, like prostaglandins, are derived from arachidonic acid. They have been shown to increase the susceptibility of the gastric mucosa to injury (Asako et al., 1992; Pihan et al., 1988; Rainsford, 1987; Vaananen et al., 1992), at least in part through stimulatory effects on neutrophil adherence to the vascular endothelium (Asako et al., 1992). Inhibitors of leukotriene synthesis and leukotriene receptor antagonists have been shown to exert protective effects in experimental NSAID-gastropathy (Pihan et al., 1988; Rainsford, 1987). There is also evidence of elevated
leukotriene B4 production following NSAID administration to laboratory animals and humans (Hudson et al., 1993). Inhibitors of leukotriene synthesis and an LTB4 receptor antagonist have been shown to prevent NSAID induced neutrophil adherence to the vascular endothelium in vivo and in vitro (Yoshida et al., 1993).

2.18.1.2 Platelet activating factor (PAF):

PAF is released by most of cell types and can exert effects on a wide range of target cells and organs (Snyder, 1990). Among its most potent actions, is ability to modulate smooth muscle tone and to activate neutrophils. PAF is also reported to be extremely potent ulcerogenic agent (Rosam et al., 1986). PAF stimulates leukocyte adherence to the vascular endothelium and activates granulocytes to release reactive oxygen metabolites (Wallace et al., 1990). PAF can themselves act as adhesion molecule.

2.18.1.3 Thromboxane A2:

Thromboxane A2 is generated by the sequential degradation of arachidonic acid by the enzyme cyclooxygenase and thromboxane synthetase. It is a very potent vasoconstrictor and renders gastric mucosa susceptible to the injury by gastric irritants. It also produces ischemia, which in turn causes injury due to decreased supply of nutrients and energy. Takahashi et al. (1999) (Takahashi et al., 1999) has also demonstrated that thromboxane A2 synthesis in the stomach was significantly elevated in ulcerated tissue, and a thromboxane synthase inhibitor markedly accelerated ulcer healing by promoting regeneration of the mucosa.

2.19 Oxidative stress and free radicals gastric ulceration:

Oxygen free radicals are detrimental to the integrity of biological tissues. The mechanism of damage involves lipid peroxidation, which destroys cell membranes with the release of intracellular components, such as lysosomal enzymes, leading to further tissue damage. The radicals also promote mucosal damage by causing degradation of the epithelial basement membrane components, complete alteration of the cell metabolism and DNA damage. Moreover, lipid peroxidation leads to loss of membrane fluidity and impairment of ion transport and membrane integrity on the surface of epithelial cells and helps to generate gastric lesion (Demir et al., 2003; Dokmeci et al., 2005). The body has developed several endogenous antioxidant systems to deal with the production of ROS. Antioxidants act as radical scavengers, inhibit lipid
peroxidation and other free radical-mediated processes, and thereby protect the human body from several diseases attributed to the reactions of radicals (Dokmeci et al., 2005; Repetto et al., 2002b). They can be divided into enzymatic and nonenzymatic groups. The enzymatic antioxidants include superoxide dismutase (SOD) which is the major antioxidative enzyme, catalase, and glutathione peroxidase that work as a system to protect the body against the deleterious effects of free radicals. These enzymes require trace metal co-factors for maximum efficiency, including selenium for glutathione peroxidase, copper, zinc or manganese for SOD and iron for catalase (Demir et al., 2003; Nasuti et al., 2006). The non-enzymatic antioxidants include the lipid soluble vitamins, vitamin E and A, and the water-soluble vitamin C and glutathione (GSH). Glutathione, which is synthesized intracellularly from cysteine, glycine, and glutamate, is capable of scavenging ROS either directly or enzymatically via glutathione peroxidise (Demir et al., 2003). Oxygen handling cells have different systems, e.g. SOD, peroxidase, catalases and tissue thiol group which are able to protect them against the toxic effects of free radicals, one of the most devastating being O2 (Repetto et al., 2002b). Several mucosal defense mechanisms protect the stomach and duodenum from noxious agents. The ROS generated by the metabolism of arachidonic acid, platelets, macrophages, and smooth muscle cells may contribute to gastric mucosal damage. Neutrophils produce O2•- which reacts with cellular lipids, leading to the formation of lipid peroxides that are metabolized to malondialdehyde (MDA) and 4-hydroxynonenal (4-HNE) (Kwiecien et al., 2002a).

2.19.1 Reactive oxygen intermediates may participate in inflammatory events, such as:

(a) Polymorphonuclear leukocyte (PMN) and monocyte/macrophage chemotaxis;
(b) Specific stimulus related to respiratory burst, especially in inflammatory cells with greater free radical production; (c) low concentration of scavenger enzymes in interstitial spaces; and
(d) Formation of metal immune complexes which can also produce OH.

2.20 Ulcer healing:

It is a complex process, which involves cell migration, proliferation, reepithelialization, angiogenesis, and matrix deposition, all ultimately leading to scar formation. All these processes are controlled by growth factors, transcription factors and cytokines (Wong et al., 2000).
2.20.1 Cellular and molecular events in the ulcer margin:

Mucosa of the ulcer margin forms a characteristic “healing zone”. The epithelial cells lining glands of the ulcer margin undergo de-differentiation, express epidermal growth factor receptor (EGF-R) and actively proliferate (Tarnawski et al., 1992). Proliferation is essential for ulcer healing, because it supplies epithelial cells crucial for re-epithelialization of the mucosal surface and reconstruction of gastric glands (Tarnawski et al., 1990). These cells migrate from the ulcer margin onto the granulation tissue to re-epithelialize the ulcer base. In addition, the epithelial cells from the base of the ulcer margin form tubes composed of ulcer-associated cell lineage, which invade granulation tissue migrate toward the surface, branch and undergo transformation into gastric glands within the ulcer scar (Wright et al., 1990). Growth factors are the major stimuli for cell proliferation, division, migration and re-epithelialization. In addition to the initial pool of growth factors derived from the platelets, macrophages and injured tissue, ulceration triggers in cells lining mucosa of the ulcer margin, genes encoding for the growth factors (e.g. EGF, bFGF, HGF, VEGF and PDGF) and COX-2, in a well synchronized spatial and temporal manner (Tarnawski et al., 1998).

These growth factors produced locally, activate epithelial cell migration and proliferation via autocrine and/or paracrine actions.

2.20.1.1 Re-epithelialization:

The migration of epithelial cells from the ulcer margin to restore epithelial continuity is essential for cutaneous and gastrointestinal wound/ulcer healing, because a continuous epithelial "barrier" protects granulation tissue against mechanical and chemical injury or infection.

The cell migration is dependent on the transcription factors and cytoskeletal rearrangements (Chai et al., 2004). The cytoskeleton (actin filaments, microtubules, intermediate filaments, focal adhesions and their associated proteins) plays an important role in cell structure, shape and mobility (Chai et al., 2004). Cell migration requires polymerization of G-actin into F-actin and formation of stress fibers. Recent study demonstrated that, role of serum response factor (SRF), it is crucial for ulcer re-epithelialization and muscle restoration.

2.20.1.2 Signaling events in the mucosa of the ulcer margin during ulcer healing:

In vivo studies on experimental gastric ulcers in rats, demonstrated that ulceration triggers overexpression of EGF and its receptor, EGF-R, in epithelial cells of
the ulcer margin (Pai et al., 1998) and that healing of the epithelial component of ulcers involves activation of the EGF-R–MAPK (Erk) signal transduction pathway (Pai et al., 1998; Tarnawski et al., 1998).

2.20.1.3 Cellular and molecular events in granulation tissue: angiogenesis:

Granulation tissue develops at the ulcer base within 48-72 hours after ulceration. Granulation tissue consists of proliferating connective tissue cells, i.e. macrophages, fibroblasts and proliferating endothelial cells, which form microvessels through the process of angiogenesis. Migration of fibroblasts into the granulation tissue and their proliferation are triggered by growth factors: TGFβ, PDGF, EGF, FGF and cytokines: TNF-α and IL-1β derived from inflammatory cells, activated endothelial cell and macrophages.

Granulation tissue supplies connective tissue cells (synthesizing extracellular matrix) for restoring the lamina propria and microvessels for the restoration of the microvasculature within ulcer scar (Tarnawski et al., 1990).

2.20.1.4 Angiogenesis:

Formation of new microvessels from pre-existing vessels – is essential for healing of chronic gastroduodenal ulcers (Folkman et al., 1996). Stimulation of angiogenesis in granulation tissue has been shown to accelerate ulcer healing. Blood vessels are especially important during tissue injury. Following acute gastric mucosal necrosis such as erosion or ulcer, it is very important to reconstruct the microvascular network. When there is ulceration, blood delivers nutrients, growth factors and immunocytes to the site of injury. The growth of new microvessel through angiogenesis is promoted by angiogenic growth factors such as bFGF, VEGF, PDGF and angiopoietin (Tarnawski et al., 2000).

Among all the growth factors, bFGF and VEGF are very important in regulating angiogenesis. bFGF is a direct mitogen for vascular endothelial cells, fibroblast and smooth muscle cells (Shing et al., 1984). VEGF expression is elevated during granulation tissue formation (Frank et al., 1995). This growth factor acts specially on vascular endothelial cell proliferation, migration and tube formation (Szabo et al., 2000). Epidermal growth factor (EGF) and transforming growth factor alpha (TGF-α) are two polypeptides of 53 and 50 amino acids respectively, that are synthesized as
large transmembrane precursors and proteolytically activated into smaller mature forms (Barnard et al., 1995). Both EGF and TGF-a stimulate cell proliferation and migration

2.20.1.4.1 Vascular endothelial growth factor:

(VEGF) is a highly specific mitogen for vascular endothelial cells. Five VEGF isoforms are generated as a result of alternative splicing from a single VEGF gene. These isoforms differ in their molecular mass and in biological properties such as their ability to bind to cell-surface heparan-sulfate proteoglycans. The expression of VEGF is potentiated in response to hypoxia, by activated oncogenes, and by a variety of cytokines. VEGF induces endothelial cell proliferation, promotes cell migration, and inhibits apoptosis. In vivo VEGF induces angiogenesis as well as permeabilization of blood vessels, and plays a central role in the regulation of vasculogenesis. Deregulated VEGF expression contributes to the development of solid tumors by promoting tumor angiogenesis and to the etiology of several additional diseases that are characterized by abnormal angiogenesis. Consequently, inhibition of VEGF signaling abrogates the development of a wide variety of tumors. The various VEGF forms bind to two tyrosine-kinase receptors, VEGFR-1 (flt-1) and VEGFR-2 (KDR/flk-1), which are expressed almost exclusively in endothelial cells. Endothelial cells express in addition the neuropilin-1 and neuropilin-2 coreceptors, which bind selectively to the 165 amino acid form of VEGF (VEGF165).

2.21 Appeal for Herbal Therapy:

Treatment of symptomatologies related to gastric ulcers or gastritis with medicinal plants are quite common in traditional medicine worldwide. Number of drugs including proton pump inhibitors, H2 receptor antagonists etc are available for the treatment of peptic ulcer but clinical evaluation of these drugs showed incidence of relapse, side effects and danger of drug interaction during ulcer therapy. In order to overcome these adverse effects, investigation has been extended for the search of new and novel molecules from plant sources which can show better protection with lower rate of incidences of relapse. It has long been recognized that natural product structures have the characteristics of high chemical diversity, biochemical specificity and other molecular properties that make them favorable as lead structures for the remedies of a number of disorders including anti-ulcer activity.
These therapeutic strategies extend from the use of simple conventional antacids to the use of more complex and effective proton pump inhibitors (PPIs). In addition, inclusion of antibiotic in the regimen for the treatment of Helicobacter pylori (H. pylori) associated peptic ulcer is indispensable. However, associated side-effects with these agents are becoming a cause of concern. For instance, the prolonged use of irreversible proton pump inhibitors brings about acid suppression thus upsetting the normal physiology of the gastric mucosa. Extreme acid suppression at recommended doses sometimes leads to achlorohydria and predispose to enteric infections like typhoid, cholera, and dysentery. Nowadays, the search for natural products with medicinal properties, particularly those from plants and honeybees with less toxic anti-ulcerogenic principles, which either supplements modern drugs or is used as an alternative is a topic of interest in different parts of the world.

In this aspect we have identified some of the medicinal plants having potential antiulcer activity.

2.22 Tectona grandis:

*Tectona grandis* (TG), belonging to the family Verbenaceae is locally called as “Thekku maram” and commonly known as Indian teak. In traditional medicine, its leaf is extensively used for wound healing activity when administered orally (Majumdar et al., 2007), it is also beneficial in dyspepsia with burning of stomach. Further reports have shown the presence of a naphthaquinone lapachol, in TG roots to possess anti-ulcer (Goel et al., 1987) and nitric oxide scavenging activity (Jagetia et al., 2004). But the mechanism through which it offers gastroprotective effects remains unexplored.

2.23 Xylocarpus granatum:

*X. granatum* Koen. belongs to the Natural Order Meliaceae. It is a mangrove plant and is commonly known as pussur in Hindi language. *X. granatum*. Koen (Bengali-Dhundul) is a moderate sized evergreen tree with their grey barks, usually grows in coastal forests of Bengal, Andaman’s, Burma, the Malay peninsula and island of Australia and Africa (Kirtikar et al., 1980). Some mangroves have shown insecticidal activity (Miki et al., 1994). The barks are astringent and are used for dysentery, diarrhea, and other abdominal troubles and as febrifuge (Ghani, 1998)). The seed ash mixed with sulphur and coconut oil is applied as ointment for itch (Ghani, 1998). Fruit is used as a cure for swelling of the breast and elephantiasis.
2.24 Annona squamosa:

*Annona squamosa*, belongs to the family Annonaceae and is commonly known as sugar apple. It is a fruit tree native to Central America and is now cultivated throughout tropics mainly for its edible fruit. The taste of the fruit pulp is sweet because of its high sugar content (58% of drymass), and it is clear that the fruit pulp contains a high calorie value (Andrade et al., 2001). This plant is reputed to possess several medicinal properties (Asolkar et al., 1992). Folkloric record reports its use as an insecticide and an anti-tumor agent (Cheema et al., 1985), anti-diabetic (Shirwaikar et al., 2004), anti-oxidant, anti-lipidimic (Gupta et al., 2008), and anti-inflammatory activities due to the presence of cyclic peptides (Yang et al., 2008). In addition, the crushed leaves are sniffed to overcome hysteria and fainting spells, and they are also applied on ulcers and wounds. A leaf decoction is taken in case of dysentery (Yang et al., 2008). The previous phytochemical investigation of this plant has proved that it has a variety of compounds like acetogenins which are responsible for antifeedant, antimalarial, cytotoxic and immunosuppressive activities (Fujimoto et al., 1988; Morita et al., 2000). Diterpenes isolated from the title plant have anti-HIV principle and anti-platelet aggregation activity (Wu et al., 1996; Yang et al., 2002). The partially purified flavonoids reported from the same source are responsible for antimicrobial and pesticidal activities (Kotkar et al., 2002). Some lignans and hydroxyl ketones are also found in this plant (Shanker et al., 2007; Yang et al., 2005). The number of alkaloids reported from this plant belongs to different groups such as aporphine (Bhakuni et al., 1972; Bhaumik et al., 1979) and benzoquinazoline (Morita et al., 2000).

2.25 Need of Scientific approach for the development of anti-ulcer agents from Natural product:

Drugs from indigenous sources of natural products are now a target for development, refinement and pharmacological modification for anti-ulcer treatment. Natural products structurally have characteristic high chemical diversity, biochemical specificity and other medicinal properties that make them favourable as lead structures for the remedies of a number of disorders including anti-ulcer activity. Ayurveda, which literally means the science of life, is one of the oldest systems of medicines in India. This system of using natural resources for betterment of health was developed through the experimentation and experiences of day-to-day life style of Indian people. But scientific evidence to prove the rationale of using these formulations in health care
is essential to develop. Various Indian medicinal plants like Allophylus serratus (Dharmani et al., 2005b), Desmodium gangeticum (Dharmani et al., 2005a), Ocimum sanctum (Dharmani et al., 2004), Hemidesmus indicus (Anoop et al., 2003), Asparagus racemosus (Sairam et al., 2003), Azadirachta indica (Bandhopadyay et al., 2002) etc. have been reported to possess anti-ulcer activity. We have directed our endeavors towards establishing the scientific rational in governing the efficacy of natural products in peptic ulcer, using various animal models to explore their anti-ulcer and ulcer healing properties along with their molecular mechanism of action. To establish the scientific basis for the role of natural products in peptic ulcer disease a number of parameters are assessed involving elucidation of the both anti-secretory and cytoprotective effects imparted by the compound. Furthermore, the efficacy of the compounds is tested against various animal models both acute and chronic to study their anti-ulcer and ulcer healing properties.