1

General introduction

Human digestive system is an enormously complex system made up of a series of hollow organs. Among, stomach plays a pivotal role in the digestion of food. The stomach can resist to a large variety of noxious factors, including hydrochloric acid, refluxed bile salt and alcohol. This high resistance of stomach to injurious factors depends on a number of physiological protective factors. However, when these protective factors are overwhelmed by injurious factors, causes gastric inflammation/peptic ulcers.

1.1. Peptic ulcers

The word ulcer is derived from French word “ulcere” and Latin word “ulcus” which means “sore” or “wound”. Thus ulcers can be defined as “deep necrotic open craters or lesions that develop in the inner lining (mucosa) of the stomach” and makes the person irritating, less tolerant, violent, aggressive, etc. It is a common global problem with increasing incidence and most prevalent disease of the modern society and one of the most rampant gastrointestinal disorder continues to occupy the key position in concern of both, clinical practitioners and researchers.

Based on anatomical location, ulcers are classified into three types. Peptic ulcer in stomach is called as gastric ulcer. Once it occurs in duodenum and esophagus is called as duodenal ulcer and esophageal ulcer respectively (Fig. 1-1). Similar principle, pathophysiology of ulcer and mechanism of ulcer healing can be applied to all types of gastrointestinal ulcers. The present thesis emphasizes more on gastric ulcer.
1.2. History

For thousands of years before discovering ulcers, healthy people had suffered from acute abdominal pain, nausea, vomiting and diarrhoea followed by death. The first gastric ulcer in the human history may be dated back to 1670. Princess Henriette-Anne, daughter of King Charles, had died suddenly at the age of 26 after a day of abdominal pain and tenderness. The autopsy of Henriette-Anne showed the presence of perforated gastric lesions. Since the cause of death was not known (Baron, 1998). Before late 19th century, about ulcer was not clear and it was very rare (Brzozowski, 2003).

Jean Cruveilheir (1835) was the one who described the pathology of ulcer in France for the first time. Therefore, it was referred as 'Cruveilheir disease' (Cruveilheir, 1835). The elucidation of pathophysiology of ulcer and mechanism of ulcer heal had accelerated dramatically within 25 years due to the rapid development of several techniques and extensive research on development of drugs for ulcer treatment.

1.3. Epidemiology and prevalence of ulcer

Epidemiology refers to the branch of medical science dealing with the transmission and control of disease. The increasing incidence and prevalence of gastric ulcers have been attributed to several factors encountered during day-to-day life, such as stress (Miller 1987), exposure to Helicobacter pylori infection (Ernst et al., 2000), use of non-steroidal anti-inflammatory drugs (NSAIDS) (Langman et al., 1991), cigarette smoking, alcoholism. The epidemiology revealed that, prevalence of peptic ulcer is
strongly associated with *H. pylori* infection. 60% of gastric ulcer and 90% of duodenal ulcer is associated with chronic inflammation due to *H. pylori* infection.

Worldwide 14.5 million peoples have gastric ulcers with a mortality rate of 4.08 million (http://digestive.niddk.nih.gov/statistics/statistics.htm/peptic ulcer prevalence, 2008). In western countries, its incidence increased in the early twentieth century but started to decline during the latter part of that century. In developed countries like United States, United Kingdom, Norway, Australia, Germany, Japan, Canada, Singapore, Spain, etc. 0.5-2.5 % of (per 100,000 of deaths) death rate is because of peptic ulcer. Whereas, in developing countries like India, Brazil, China, Indonesia, Mexico, Nepal, Pakistan, Saudi Arabia, Malaysia, Kenya etc. death rate because of peptic ulcer is about 3.3 – 16.2 % per 100,000 deaths. India stands fifth rank in worldwide prevalence of peptic ulcer with 12.4 % of death rate (www.worldlifeexpectancy.com/cause-of-death/peptic-ulcer-disease) (Fig. 1-2). A report by Tovey (1979), showed a marked regional differences in India. Ulcer is being more prevalent in southern India than northern India.

The decline in death rate due to peptic ulcer in developed countries might be because of decrease in prevalence of *H. pylori* infection due to improved hygiene, use of effective therapy to eradicate the infection and potent anti-secretory drugs such as histamine-2 receptor antagonists (H₂ RA) and proton pump inhibitors (PPI) (Lam, 1993; Dutta et al., 2012).

1.4. Physiology of gastric mucosal defense

Many pathological conditions cause peptic ulcer disease (PUD) by disrupting and/or penetrating mucosal surfaces. Except in rare cases, the stomach can withstand exposure to highly concentrated hydrochloric acid, refluxed bile salts, alcohol, and foodstuffs with a wide range of temperatures and osmolarity. This is attributed to a number of physiological responses by the mucosal lining to potentially harmful luminal agents, and to an ability to rapidly repair damage when it occur (Wallace, 2008). The gastric mucosal defense consists of several local and neurohormonal physiological mechanisms to protect the mucosa from exposures to damaging noxious factors (Laine et al., 2008).
**Figure 1-2**: Worldwide prevalence of peptic ulcer.

(URL: www.worldlifeexpectancy.com/asia/peptic-ulcer-disease-cause-of-death)
1.4.1. Local gastric mucosal defense mechanism

1.4.1.1. Mucus-bicarbonate-phospholipid barrier

Mucus-bicarbonate-phospholipid is the first line of defense system where unstirred gastric mucosal layer consist of mucus gel, bicarbonate anion and surfactant phospholipids (Lichtenberger, 1999). The secretion of bicarbonates by surface epithelial cells counteracts protons diffusing from the lumen into the gel, essential to maintain a microenvironment with a pH near to 7 at the mucus-mucosa interface (Allen et al., 1993; Fornai et al., 2011). On the other hand, surfactant phospholipids covers a mucus gel which deliberates hydrophobic property to the mucus layer (Lichtenberger, 1999).

Thus mucous-bicarbonate-phospholipid barrier is the one which segregate epithelium from the gastric lumen, which is able to prevent the penetration of proteolytic enzymes and avoids the proteolytic digestion of epithelium (Allen and Flemstrom, 2005). When this protective barrier breaks down during pathological events upon detrimental action of aggressive factors, a second line of protective mechanisms comes into play.

1.4.1.2. Epithelial cells.

The continuous layers of surface epithelial cells represents the next line of mucosal defense. The epithelial tissue is responsible for the production of mucus, bicarbonate through the secretion of various mucin glycoproteins like MUC2, MUC5AC, MUC5B and MUC6 (Allen and Flemstrom, 2005). Cells are hydrophobic in nature owing to the presence of phospholipids on their surface which helps to repel acid and water soluble injuring agents (Lichtenberger, 1999). Since, epithelial layer formed by close interconnection of epithelial cells by tight junction without any gap, and prevents back diffusion of acid and pepsin (Allen and Flemstrom, 2005). Another important factor, Heat Shock Proteins (HSPs), a molecular chaperons released from epithelial cells in response to temperature increment, oxidative stress and cytotoxic agents plays a crucial role in preventing protein denaturation and protect cells from injury (Tanaka et al., 2007). Another relevant protective factors, cathelicidin and β-defensin are cationic peptides which prevent bacterial colonization at the mucosal surface (Yang et al., 2006). In addition, trefoil factors (TFFs) secreted from epithelial cells controls re-epithelization and exert mucosal protective actions (Taupin and Podolsky, 2003).
1.4.1.3. Mucosal blood flow

Mucosal blood flow (MBF) maintains the structure and function of the stomach by delivering oxygen, nutrients; removing H\(^+\) and toxic metabolites from gastric mucosa (Sorbye and Svanes, 1994; Kawano and Tsuji, 2000). Low MBF results in tissue necrosis, whereas high MBF protects against injurious agents (Sorbye and Svanes, 1994). The experimental evidence demonstrating that, Nitric Oxide (NO) and Prostacyclin (PGI\(_2\)) maintains the viability of endothelial cells and inhibit platelet and leukocyte adhesion to the microvasculature, thus prevents the occurrence of mucosal ischemia (Laine et al., 2008). In addition, PGI\(_2\) and NO produced by endothelial cells which lines microvessels, protects the gastric mucosa against damage and counteract the detrimental effects of various vasoconstrictors like leukotriene C\(_4\), thromboxane A\(_2\) and endothelin 1. The increase in MBF is mediated by NO release and there is experimental evidence that, NO protects the gastric mucosa against injury induced by ethanol or endothelin 1 (Kawano and Tsuji, 2000; Holzer, 2006). It has been also observed that another endogenous compound, H\(_2\)S, can exert protective actions against gastric mucosal injury. This compound in particular has been shown to reduce the expression of tumor necrosis factor \(\alpha\) (TNF- \(\alpha\)), decreases leukocyte adhesion to vascular endothelium and also prevent NSAID induced gastric mucosal damage (Fiorucci et al., 2006).

1.4.1.4. Prostaglandins

Prostaglandins are oxygenated fatty acids derived from arachidonic acid, which has a strong protective effect and plays a crucial role in maintaining gastric mucosal integrity (Wallace, 2008; Whittle, 1980; Hoshino et al., 2003; Wilson, 1996; Halter et al., 2001). The gastrointestinal mucosa contains relatively large amounts of prostaglandin. PGI\(_2\) is the most common prostaglandin formed by the gastric mucosa, and with prostaglandin E\(_2\) (PGE\(_2\)), mainly affects MBF. It have been shown to protect against gastric and duodenal mucosal damage in animals and humans. Inhibition of PGI\(_2\) formation by aspirin-like agents (NSAID) would be expected to reduce MBF and produce areas of local ischemia and tissue damage which would subsequently be the sites of erosions and ulcers (Whittle, 1980). Both PGI\(_2\) and PGE\(_2\) mediates increase in gastric MBF and bicarbonate secretion through the activation of EP\(_1\) (prostaglandin E\(_1\)) receptor
in the damaged mucosa and a decreased gastric motility (Russell, 1986; Takeuch et al., 2002). This protection can occur independently of acid inhibition and may be direct or adaptive. Other EP receptor subtypes are also involved in the protective actions of PGE₂. Like, EP₃ receptors inhibit the gastric acid secretion, while EP₄ receptors stimulate the secretion of mucus (Kato et al., 2005). Prostaglandins are also known for inhibition of mast cell activation as well as leukocyte and platelet adhesion to the vascular endothelium (Halter et al., 2001). PGE₂ is also responsible for gastric mucosal protection under ethanol induced apoptosis in gastric mucosal cells via induction of an increase in cAMP (Cyclic adenosine monophosphate) and activation of PKA (Protein Kinase A) (Hoshino et al., 2003).

1.4.1.5. Sensory innervation

Sensory innervation plays a prominent role in the protection of gastric mucosa from injury. The vasculature of gastric mucosa and submucosa is innervated by extrinsic primary afferent sensory neurons, which are arranged in a plexus at the base of the mucosal layer (Holzer, 2007). The nerve fibers stemming from this plexus run along with capillary vessels and reach the basal membrane of surface epithelial cells. These nerves can detect luminal acidity or back-diffusing acid through acid-sensing channels. The activation of such sensory nerves modulates the contraction tone of submucosal arteriols, thus regulating the MBF. Also, stimulation of sensory nerves leads to release of calcitonin gene-related peptide (CGRP) which in turn contributes to the maintenance of mucosal integrity through the vasodilation of submucosal vessel mediated by release of NO as demonstrated by studies where the ablation of sensory transmission impaired the vasodilatory response and increased the sensitivity of gastric mucosa to injuring agents (Holzer, 2007).

1.4.1.6. Mucosal cell renewal

The integrity of gastric epithelium is maintained by a continuous process of cell renewal ensured by mucosal progenitor cells. These cells are subjected to a continuous, well co-ordinated and controlled proliferation, which ensures the replacement of damaged or aged cells on the epithelial surface. The process of complete epithelial renewal takes about 3-7 days, while the overall glandular cell replacement requires
months. However, the restoration of surface epithelium after damage occurs very quickly and results by migration of preserved cells located in the neck area of gastric glands (Laine et al., 2008). The process of cell turnover is regulated by growth factors. In particular, a marked expression of epidermal growth factor receptor (EGF-R) has been detected in gastric progenitor cells. Such a receptor can be activated by mitogenic growth factors, such as transforming growth factor-α (TGF-α) and insulin-like growth factor-1 (IGF-1) (Nguyen et al., 2007). In addition, PGE2 and gastrin are able to transactivate the EGF-R and promote the activation of mitogen-activated protein kinase (MAPK) pathway, with consequent stimulation of cell proliferation (Pai et al., 2002). Remarkably, the presence of EGF has not been detected in the normal mucosa, although it is contained in the gastric juice, as a product of salivary and esophageal glands and can stimulate mucosal cell proliferation in case of injury (Milani and Calabrò, 2001). In addition, mucosal progenitor cells express survivin, an antiapoptotic factor, which inhibits apoptotic cell death (Chiou et al., 2005).

1.4.2. Neurohormonal defense mechanism

Central nervous system (CNS) and hormonal factors plays a crucial role in gastric mucosal defence to maintain mucosal integrity (laine et al., 2008). Corticotropin-releasing factor (CRF) acts within the CNS to inhibit gastric acid secretion by a specific receptor-mediated (CRF2) event (Druege et al., 1989; Chatzaki et al., 2006). In addition, other mediators, including gastrin-17, cholecystokinin, thyrotropin-releasing hormone, bombesin, EGF, peptide YY and neurokinin A, play significant roles in the regulation of gastric protective mechanisms, which can be blunted by afferent nerve ablation, CGRP receptor blockade, and inhibition of NO synthase (Peskar, 2001; Moszik et al., 2001).

1.5. Causes/Risk factors

A coating of mucus and other chemicals normally shield the stomach and duodenum from digesting themselves. When these protective mechanisms are disrupted, powerful digestive acids can erode into the lining of these organs and cause gastric ulcers. Gastric lesions develop due to loss of delicate balance between gastro-protective and aggressive factors.
Reduction in gastro protective factors, such as mucus, bicarbonate secretion, gastric MBF and enhancement of aggressive factors, such as increase of acid/pepsin secretion and *H. pylori* infection results in gastric ulceration (Fig. 1-3) (Miller 1987; Ernst et al., 2000).

![Figure 1-3: Pictorial representation of ulcer development due to the imbalance between aggressive and protective factors (Ref: Rajesh et al., 2013).](image)

It is a heterogeneous group of disorder, the increasing incidence and prevalence of gastric ulcers attributed to several factors encountered during day-to-day life. Stress plays a major role in causing gastric ulcer (Miller 1987). In general, exposure to bacterial infection (Ernst et al., 2000), and use of non-steroidal anti-inflammatory drugs (NSAIDS) (Langman et al., 1991) are responsible for majority of gastric ulcer. However, there are many other risk factors causing gastric ulcers such as, smoking cigarettes, drinking too much alcohol, cocaine, caffeine, stress and radiations.

1.5.1. *Helicobacter pylori*

*Helicobacter pylori*, is a gram negative, microaerophilic, rod shaped bacterium (Fig. 1-4) which infects about 50% of the world population (Suzuki et al., 2007; Brooks et al., 1995; Martinon et al., 2000). Previously it was referred as *Campylobacter pylori*, known to cause a various gastro duodenal diseases such as chronic active gastritis. In 1984, Barry Marshall and Robin Warren found and identified *H. pylori* for the first time
in patients with gastritis and gastric ulcer. The discovery resulted in the award of Nobel Prize (2005) in physiology and medicine.

It is a major cause of both acute and chronic gastritis, gastric ulcer and gastric carcinoma (Parsonnet et al., 1991; Talley et al., 1991). The World Health Organization (WHO) and International agency for Research on Cancer consensus group (IARC) have classified this bacterium as biological carcinogen (Schistosomes, 1994).

Propensities to develop gastro duodenal diseases depend upon a complex set of factors viz., (i) bacterial associated virulence strains, (ii) host genotype, (iii) environmental parameters and (iv) inter specific associations among the above mentioned factors.

1.5.1.1. Pathophysiology of H. pylori infection

Although H. pylori infection is highly prevalent in the global human population, the majority of infected individuals remain asymptomatic. A complex combination of host, environmental, bacterial factors are considered to determine susceptibility and severity of outcome in the subset of individuals that develop clinical disease (Fig. 1-5). These factors collectively determine the ability of H. pylori to colonize the gastric mucosa and profoundly influence the nature of the interaction that ensues. Many studies over the previous years provide a new insight of H. pylori virulence strategies and the activities of critical bacterial determinants that modulate the host environment (Delahay and Rugge, 2012).

The three virulence factors in H. pylori, vacuolating cytotoxin (VacA), blood group antigen-binding adhesion molecule (BabA) and gene products of the pathogenicity island (cagA PAI) are associated with severe diseases caused by the bacterium (Delahay and Rugge, 2012, Kusters et al., 2006).
1.5.1.1. CagA PAI. CagA protein is a highly immunogenic protein encoded by CagA gene (Covacci et al., 1993). This gene is present in approximately 50 to 70% of H. pylori strains (Ching et al., 1996; Cover et al., 1996, Ummuru et al., 1993) and is a marker for the presence of a genomic PAI of about 40 KB, depending on the strain analyzed that encodes between 27 to 31 proteins (Akopyants et al., 1998; Censini et a., 1996). Strains carrying the cag PAI are referred to as CagA⁺ strains, as they are commonly identified in patients by their potential to induce significant antibody titers against the CagA marker protein. Patients infected with CagA⁺ strains usually have a higher inflammatory response and are significantly more at risk for developing a symptomatic outcome (peptic ulcer or gastric cancer).

CagA PAI plays, a important role which facilitate the transfer of CagA, peptidoglycan (PGN) and other bacterial factors into host cell cytoplasm through type IV secretory system (Fig. 1-6) (Asahi et al., 2000; Christie et al., 2000; Covacci et al., 1993; Fischer et al., 2001). Once delivered inside the cell, the CagA is functionally activated by being phosphorylated at tyrosine residues in EPIYA motifs (Asahi et al., 2000; Higashi et al., 2002; Odenbreit et al., 2000; Segal et al., 1999; Stein et al., 2000) by Src family kinases (Selbach et al., 2003; Stein et al., 2002).
Phosphorylated CagA, which then interacts with a range of host signaling molecules such as the tyrosine phosphatase SHP-2 (Higashi et al., 2002; Yamazaki, 2003), resulting in morphological changes in the epithelial cells (Moese et al., 2004; Naumann et al., 1999; Selbach et al., 2004) (Fig. 1-6).

The Cag PAI also affects the immune response due to its ability to induce apoptosis of T cells (Paziak-Domanska et al., 2000; Wang et al., 2001). The interaction between the type IV structure and the host cell results in the induction of pro-inflammatory cytokines in epithelial cells (Odenbreit et al., 2000; Segal et al., 1999). It is believed that CagA plays only a minor role, if any in their activation (Al-Ghoul et al., 2004; Fischer et al., 2001). Whereas, PGN fragments play a major role, which induce NOD-1-dependent activation of NF-kB and thereby transcription of inflammatory program by recruiting inflammatory cells particularly, neutrophils, which cross the epithelial barrier to eradicate the bacteria thereby inducing chronic gastritis (Sansonetti, 2004).
1.5.1.1.2. **VacA** The VacA (vacuolating cytotoxin) is a major virulence factor of *H. pylori* and contributes significantly to murine gastric colonization of *H. pylori* (Salama et al., 2001). Approximately, 50% of *H. pylori* strains secrete VacA, a highly immunogenic 95-kD protein that induces massive vacuolization in epithelial cells (Cover and Blaser, 1991) and plays a very important role in the pathogenesis of both peptic ulcer and gastric cancer (Atherton et al., 1995; Marais et al., 1999; Ogura et al., 2000).

The activities of VacA include membrane channel formation, disruption of endosomal and lysosomal activity, effects on integrin receptor-induced cell signaling, interference with cytoskeleton-dependent cell functions, induction of mitochondrial apoptosis and immune modulation (Fig. 1-7).

![Diagram](image)

**Figure 1-7:** The VacA protein influences cellular processes via different routes, thus assisting in chronic colonization of the gastric mucosa by *H. pylori*. (1) Surface-bound VacA may be directly delivered to the cell membrane. Secreted VacA may either (2) bind to a cell membrane receptor and initiate a pro-inflammatory response, (3) taken up directly by the cell and be trafficked to the mitochondria and induce apoptosis, (4) taken up by pinocytosis and induce vacuolization, (5) form a membrane channel resulting in leakage of nutrients to the extracellular space, or (6) pass through the tight junctions and inhibit T-cell activation and proliferation. (Ref: Kusters et al., 2006).

VacA forms pores in epithelial cell membranes, thus inducing the release of urea and anions from the host cells. It also increases transcellular permeability, leading to the release of nutrients and cations (Montecucco and Bernard, 2003). Upon bacterial contact with host cells, toxin clusters are transferred to the host cell surface and exert their toxic action (Ilver et al., 2004). VacA also enters the cytosol, subsequently accumulates in the
mitochondrial inner membrane, and activates endogenous mitochondrial channels, thereby inducing apoptosis (Cover et al., 2003; Galmiche et al., 2000). The pro-apoptotic effect of VacA is a cell type dependent and may be limited to gastric epithelial cells such as parietal cells (Boncristian et al., 2003; Gebert et al., 2003; Sundrud et al., 2004). This may result in reduced acid secretion (Kobayashi et al., 1996; Neu et al., 2002), thereby pre-disposing for development of gastric cancer.

The proteolytic cleavage of secreted VacA forms 33-kD N-terminal and 55-kD C-terminal fragments. The N-terminal protein performs an essential function in the formation of anionic channels, while the C-terminal protein mediates cell binding (Ji et al. 2000; Kim et al., 2004; Torres et al., 2005).

1.5.1.1.3. BabA. 78-kD BabA encoded by BabA gene represents the best characterized H. pylori adhesion protein. The animal studies suggest that BabA mediated adhesion is relevant for the colonization, pathogenesis of H. pylori (Guruge et al., 1998; Rad et al., 2002) and also have a role in the virulence of H. pylori as the BabA2 allele is strongly associated with peptic ulcer and adenocarcinoma (Gerhard et al., 1999).

1.5.1.1.4. LPS (Lipopolysacharides) The overall composition of the cell envelope of H. pylori is similar to that of other gram-negative bacteria. It consists of an inner (cytoplasmic) membrane, periplasm with peptidoglycan and an outer membrane. The outer membrane consists of phospholipids and LPS. The majority of H. pylori strains contain fucosylated oligosaccharide antigens that are structurally and immunologically related a very close to human blood group antigens and contribute immune evasion (Mahdavi et al., 2003). H. pylori LPS stimulates NF-κB and IL-8 production in both epithelial cells and immune cells in a CagA-independent manner (Lepper et al., 2004; Maeda et al., 2001). Consequently, H. pylori LPS is unlikely to represent a major factor in the immune activation.

1.5.2. Non-steroidal anti-inflammatory drugs (NSAIDs)

NSAIDs represent the second-most important cause of PUD. The risk of developing an NSAID induced peptic ulcer increases with dose, frequency, use of multiple NSAIDs, age (more likely in those age 60 or older), gender (more common in
women than men), smoking, alcohol, use of corticosteroids, such as prednisone (Huang et al., 2002).

NSAIDs can cause damage to the gastro-duodenal mucosa via several mechanisms, including the topical irritant effect of these drugs on the epithelium, impairment of the barrier properties of the mucosa, suppression of gastric prostaglandin synthesis, reduction of gastric mucosal blood flow and interference with the repair of superficial injury. The presence of acid in the lumen of the stomach also contributes to the pathogenesis of NSAID induced ulcers and bleeding by impairing the restitution process, interfering with homeostasis and inactivating several growth factors that are important in mucosal defense and repair (Wallace, 2000). The detailed mechanism of NSAIDs induced ulceration is described below.

1.5.2.1. Pathophysiology of gastric mucosal damage by NSAIDs

The pathophysiology of gastric injury associated with NSAID administration depends partly on cyclooxygenase dependent and cyclooxygenase independent mechanisms (Scarpignato and Hunt, 2010) (Fig. 1-8). In cyclooxygenase dependent mechanism, inhibition of cyclooxygenase increases the risk of gastric mucosal injury through the suppression of numerous prostaglandin mediated protective functions.

NSAIDs can shift the mucosal balance towards the recruitment (activation) and endothelial adhesion of circulating neutrophils through the inhibition of beneficial effects of prostaglandin as described above (section: 1.4.1.4). Adhered neutrophils clog the microvasculature causing a local decrease in MBF and marked release of tissue damaging factors including proteolytic enzymes and leukotriene. These factors enhance the vascular tone, exacerbate tissue ischaemia, stimulate the production of ROS and promote the destruction of intestinal matrix, leading to a severe degree of local tissue necrosis particularly in the presence of a low luminal pH (Whittle, 2002; Jimenez et al., 2004).

A number of studies have demonstrated the expression of bicarbonate in gastric epithelial cells through the activation of EP1 receptors by cyclooxygenase-derived prostaglandins (Takeuchi et al., 1997; Rossmann et al., 1999). As described above (section 1.4.1.1), mucosal bicarbonate represents the first line of defense mechanism for
mucosal damage and cyclooxygenase dependent inhibition of bicarbonate secretion through the prostaglandin blockade which contributes to gastric mucosal injury elicited by NSAIDs.

On the other hand, NSAIDs induce gastric ulcer through cyclooxygenase independent mechanism. Generally, NSAIDs are weakly acidic in nature. In gastric lumen acidity, undissociated lipophilic form of acidic NSAIDs can impair the hydrophobic surface barrier of the stomach. This transformation of the gastric mucosal surface from a non-wettable to a wettable state appears to be linked with the ability of acidic NSAIDs to destabilize the extracellular lining of zwitterionic phospholipids, particularly phosphatidylycholine which are present within and on surface of the mucus layer (Lichtenberger et al., 2007). Such an effect contributes significantly to NSAID-induced gastric injury which can even persist for prolonged periods after discontinuation of NSAID administration (Lichtenberger, 2001).

There is also evidence that, the protonophore action of aspirin and other acidic NSAIDs take a significant part in the topical damage to gastric mucosa. In particular, upon exposure to the acidic environment of gastric lumen, an undissociated lipid-soluble form of aspirin is able to penetrate cell membranes and accumulate into epithelial cells, where the inner pH is at a physiological level of 7.4. At this pH, aspirin dissociates and remains segregated within cells. This accumulation enhances the inhibition of prostaglandin biosynthesis, and it brings into play other properties of aspirin such as an uncoupling of mitochondrial oxidative phosphorylation. The consequences of such mitochondrial dysfunction leads to decrease in ATP production and an increase in AMP and ADP levels, which are then responsible for increments of intracellular calcium concentration. These changes are followed by mitochondrial injury, increased generation of ROS and alterations in the Na+/K+ balance. Which lead to weakening of the mucosal barrier and cellular necrosis (Wallace, 2001; Bjarnson et al., 2007). An additional mechanism involved in the injurious effects of NSAIDs on gastrointestinal mucosa is related to the detrimental actions of these drugs on the integrity of epithelial tight junctions which are known to segregate the apical from basolateral cell surface domains in order to establish cell polarity, provide a barrier function against the back diffusion of
acid and other solutes through the paracellular space (Schneeberger and Lynch, 2004). It has been suggested that, cyclooxygenase inhibition may be implicated in NSAID-induced alterations of intercellular epithelial permeability (Joh et al., 2003). However, recent evidence indicates that aspirin can elicit gastric epithelial barrier dysfunction through down-regulation of claudin-7, a member of the claudin protein family, which plays an important role in the formation of tight junctions (Oshima et al., 2008).

![Pathophysiology of gastric mucosal damage by NSAIDs](image)

**Figure 1-8:** Pathophysiology of gastric mucosal damage by NSAIDs.

### 1.5.3. Other risk factors

#### 1.5.3.1. Smoking

Worldwide, cigarette smoking is found to be a common habit and is considered as the largest cause of premature death (Peto, 1994). Many investigations revealed a strong correlation between cigarette smoking and PUD. Compared with nonsmokers, smokers are roughly twice as likely to develop PUD. Cigarette smoking is coupled with aninitiation, prolongation and reoccurrence of gastric ulcers (Eastwood, 1997; Endoh and Leung, 1994). Epidemiologic studies show that cigarette smoking increases both the incidence and decline rate of PUD and delays ulcer healing in humans (Ko and cho, 2000). Many components in cigarette smoke acts as an ulcerogen, among these nicotine has received a special emphasis (Brenna et al., 1993). Several studies reveal that ROI
generated due to smoking and nicotine is linked intimately with the pathogenesis of peptic ulcer (Salim, 1992).

1.5.3.1.1. Pathophysiology of cigarette smoking

Smoking and nicotine have significant adverse effects, potentiate gastric aggressive factors and attenuate defensive factors, thus playing a potential role in the pathogenesis of PUD (Endoh and Leung, 1994) (Fig. 1-9). In smokers having duodenal ulcer, acid and pepsin, (Walker and Taylor, 1979) output is more pronounced. This increased gastric acid secretion is mediated through the stimulation of H₂-receptor by histamine released by degranulation of mast cells and in the increase of the functional parietal cell volume or secretory capacity in smokers (Chow et al., 1998; Ogle et al., 1993; Ligny et al., 1989). In smokers, the increase in bile salt reflux rate and gastric bile salt concentration has been observed; thereby duodenogastric reflux raises the risk of gastric ulcer (Muller-Lissner, 1986; Endoh and Leung, 1994).

Smoking and nicotine not only induce ulceration, but they also potentiate ulceration caused by H. pylori, alcohol, NSAIDs or cold restrain stress (Chow et al., 1997, Wong et al., 2002, Tariq et al., 1996). ROS, potentiated by cigarette smoking and nicotine play an important role in ulcerogenesis through oxidative damage of the mucosa by gastric mucosal cell apoptosis (Salim, 1992; Etienne et al.; 1988; Kim et al., 2000). Smoking increases the production of platelet activating factor (PAF) (Eastwood, 1997; Endoh and Leung, 1994), vasopressin secretion (Laszlo et al., 1998) and endothelin (Wallace et al., 1989) which are potent gastric ulcerogens, reduce the level of circulating epidermal growth factor (EGF) which are necessary for gastric mucosal cell renewal (Endoh and Leung, 1994, Jarvis and Whitehead, 1980). Nicotine also decreases prostaglandin generation in the gastric mucosa of smokers, thereby making the mucosa susceptible to ulceration (Ma et al., 1998; Quimby et al., 1986; Lam, 1987; Lindell et al., 1997). Both smoking and nicotine reduce angiogenesis in gastric mucosa through inhibition of NO synthesis thereby arresting cell renewal process (Cho, 2001; Jones et al., 1999). Smoking, not only induce but also impairs both spontaneous and drug-induced healing of ulcer (Holstege, 1987; Ma et al., 1999).
1.5.3.2. Alcohol

Throughout the world, alcohol has been used for centuries in social, medical, cultural and religious settings. Currently, it is responsible for 3.8% of deaths and 4.6% of disability. The World Health Organization (WHO) has estimated that, there are about 2 billion people worldwide who consume alcoholic beverages and 76.3 million with diagnosable multiple pathological consequences (Stermer, 2002; WHO, 2004, 2008; Rehm et al., 2009). Alcoholism is associated with increased risk of cancer of mouth, tongue, throat, stomach, pancreas and colon, liver cirrhosis etc. (Stermer, 2002). Among the various organ systems that mediate alcohol effects, gastrointestinal tract plays a particularly important role. Ethanol induced gastric ulcers have been widely used for the experimental evaluation of anti-ulcer activities because of its rapid and convenient way of screening plant extracts as anti-ulcer potency.

1.5.3.2.1. Pathophysiology of alcohol consumption

Influence of alcohol in the stomach includes; acid secretion, gastric emptying and certain acid-related diseases including peptic ulcer. The alcohol absorption into the bloodstream occurs throughout the gastrointestinal tract and its direct contact with the mucosa can induce numerous metabolic and functional changes, leads to marked mucosal damage which can result in acute and chronic broad spectrum gastrointestinal bleeding
and ulcers (Bode & Bode, 1997). The pathogenesis of ethanol-induced gastric lesions is complex and may interact directly with the gastric mucosa or it may act through a general mechanism affecting the release of hormones and the regulation of nerve functions involved in acid secretion (Bode & Bode, 1997; Chari et al. 1993).

Alcohol can contribute peptic ulcer through topical stimulation of the parietal cells with an increase in cAMP production and histamine release. The effects of alcohol upon gastric motility and emptying have been extensively studied. Ethanol causes pyloric relaxation, which may facilitate the gastric emptying, could favor duodenogastric reflux and lead to ulcer (Dinoso et al., 1972). Oxidative stress and depletion of non-protein sulphhydrals concentration, modulation of NO system and reduction of gastric MBF, the decreased formation of prostaglandins frequently underlie the development of gastric lesions (Arafa & Sayed-Ahmed, 2003; Bode et al. 1996). On the other hand, alcohol-dependent increase in the production of leukotrienes also contribute to the development of alcohol-induced mucosal damage (Bode and Bode, 1997). A number of studies demonstrated that, increased oxidative stress and depletion of antioxidants have been considered as crucial step in alcohol induced mucosal damage. Ethanol treatment induces intracellular oxidative stress, causes mitochondrial permeability transition and mitochondrial depolarization, which precede cell death in gastric mucosal cells (Hirokawa 1998; La Casa et al., 2000; Siddaraju et al., 2009) (Fig. 1-10)

![Diagram](image)

**Figure 1-10:** Pathophysiology of gastric mucosal damage by alcohol
1.5.3.3. Stress

Stress and psychosocial factors can cause ulcers in certain patients (Peters and Richardson, 1983). This usually occurs in the setting of multiple organ failure or critical illness, such as in the patients with extensive burns or head trauma. This may result from a combination of mucosal ischemia and increased acid secretion. Currently, no study has demonstrated a causal relationship between psychological stress and PUD. Psychological stress however, remains significant because it can influence perception and reporting of dyspeptic symptoms. The generation of free radicals is mainly implicated as a causative mechanism in stress induced ulcers (Das et al., 1997).

1.6. Complications of peptic ulcer

Many investigators reported the complications of peptic ulcer if it is left untreated. Fig. 1-11 depicts the network of top 20 life threatening diseases caused due to PUD (http://www.malacards.org/card/peptic_ulcer). It leads to four major complications like hemorrhage, perforation, penetration and obstruction (Milosavljevic et al., 2011). Hemorrhage is the most frequent PUD complication and its incidence is increasing in comparison to other complications. If ulcers become larger and extend deeper into the digestive tract lining, they may damage large blood vessels resulting in sudden serious bleeding into the intestinal tract.

![Graphical network of the top 20 diseases related to peptic ulcer](http://www.malacards.org/card/peptic_ulcer)

**Figure 1-11:** Graphical network of the top 20 diseases related to peptic ulcer

Perforation occurs when an ulcer spreads through the wall of the stomach or intestine into the abdominal cavity. Though it is a much less frequent complication, still it is a significant problem. When perforation occurs, partially digested food, bacteria,
enzymes from the digestive tract may enter into the belly cavity and cause inflammation that leads to peritonitis. In severe peptic ulcer condition, ulcer continues into adjacent vital organs such as the liver and pancreas by the phenomenon called penetration. Untreated ulcer can also lead to gastric obstructions resulting in narrowing of pyloric canal by scarring and swelling of gastric antrum and duodenum.

1.7. Diagnosis of peptic ulcer

The most common symptoms of PUD are collectively known as dyspepsia. However, PUD can occur without dyspepsia or any other gastrointestinal symptoms, especially when they are caused by NSAIDs. The most common other PUD symptoms are abdominal pain, heartburn and regurgitation. Sometimes, ulcers can cause hidden bleeding; patients may experience symptoms of anemia, including fatigue and shortness of breath.

Peptic ulcers are always suspected in patients with persistent dyspepsia. It is estimated that, only 15 - 25% of those having dyspepsia actually anchor PUD. It can be diagnosed by using two important techniques (NIH); invasive and noninvasive techniques.

1.7.1. Invasive techniques

If a patient has any alarm symptoms, the doctor prescribes an endoscopy or upper gastrointestinal (GI) series, which provides the most sensitive and specific approach for examining the upper GI tract. Endoscopy facilitates photographic documentation of a mucosal defects and tissue biopsy to rule out malignancy or H. pylori. Followed by the urease test for the biopsy sample, which helps in diagnosing H. pylori with >90-95% of sensitivity and specificity.

1.7.2. Noninvasive techniques

If a patient has symptoms of PUD, physicians ask the patient to stop use of NSAIDs. In noninvasive test, examining one of the three samples blood, breath or stool can detect the presence of H. pylori with high degree of accuracy.
1.7.2.1. **Blood test** is used to measure antibodies to *H. pylori* and the results are available in a short period with 80 - 90% of accuracy. One such important test that can be used extensively is enzyme-linked immunosorbent assay (ELISA).

1.7.2.2. **Urea breath test (UBT)** is simple test called the carbon isotope-urea breath test (UBT) can identify up to 99% of people who have *H. pylori*. Up to 2 weeks before the test, the patient must stop taking any antibiotics, bismuth-containing medications such as Pepto-Bismol and proton pump inhibitors (PPIs). As part of the test, the patient swallows a special substance containing *urea* that has been treated with carbon atoms. If *H. pylori* are present, the bacteria convert the urea into carbon dioxide, which is detected and recorded in the patient’s exhaled breath after 10 minutes.

1.7.2.3. **Stool antigen test** to detect the genetic traces of *H. pylori* in the feces appears to be as accurate as the breath test for initially detecting the bacteria and for detecting recurrences after antibiotic therapy.

1.8. **Treatment of peptic ulcer**

Most peptic ulcers heal if gastric acid production is adequately suppressed. The rationale behind the treatment of peptic ulcer disease includes the reduction of hostile factors and augmentation of protective factors. Based on the cause of PUD, different types of treatments are available which includes the use of antibiotics, proton pump inhibitors, Histamine (H-2) blockers, antacids and cytoprotective agents (Fig. 1-12).

1.8.1. **Antibiotics**

Treatment of *H. pylori* associated peptic ulcer includes the eradication of infection by the use of antibiotics. No single drug cures *H. pylori* infection because niche of *H. pylori* residence is at low pH. Combination therapies employing one proton pump inhibitor (e.g., omeprazole) and two or three antibiotics (e.g., amoxicillin, clarithromycin, or tetracycline) have been used as preferred treatments (Ulmer *et al.*, 2003).
Most of the treatment regime includes the use of proton pump inhibitors to decrease the production of stomach acid, which allows the tissues damaged by the infection to heal. However, the multiple therapy regimens have not been very effective in a clinical setting because *H. pylori* likely to develop resistance (Cameron *et al.*, 2004). Moreover, this treatment may disrupt the natural population of commensal microorganisms in the gastrointestinal tract, leading to undesired side effects.

1.8.2. Proton pump inhibitors (PPIs)

Proton pump inhibitors reduce stomach acid by blocking the action of cells parts that produce acid. Clinically used proton pump inhibitors include Omeprazole, Lansoprazole, Dextansoprazole, Esomeprazole, Pantoprazole and Rabeprazole. The inner surface of the stomach is formed into numerous gastric pits from which acid is secreted. Parietal cells, lining the gastric pit secrets hydrochloric acid. The proton pumps present in the lumenocyte of the parietal cells are responsible for acid secretion. The proton pump actively transport proton into the stomach lumen with the exchange of potassium to the parietal cell with the hydrolysis of ATP. The PPIs are the drugs, which reduce acid secretion of the stomach. Drug binds irreversibly to the proton pump and prevents the active transport of protons; this dramatically decreases the acid secretion of the stomach.
(Sachs et al., 1978) (Fig. 1-13). In general, safety of this class of drugs has been excellent. However, in the past several years, epidemiologic studies have indicated possible risks (Diarrhea, constipations and headache) that are biologically plausible (Waldum et al., 2005; Ryan and Madanick, 2011).

Figure 1-13: Gastric acid is secreted by parietal cells of the stomach in response to stimuli such as the presence of food in the stomach or intestine, the taste, smell, sight or thought of food. Such stimuli result in the activation of histamine, acetylcholine or gastrin receptors (the $H_2$, $M_3$ and CCK2 receptors, respectively) located in the basolateral membrane of the parietal cell, which initiates signal transduction pathways that converge on the activation of the $H^+\cdot K^+$-ATPase — the final step of acid secretion. Inhibition of this proton pump has the advantage that it will reduce acid secretion independently of how secretion is stimulated, in contrast to other pharmacological approaches to the regulation of acid secretion; for example, the inhibition of acid secretion by $H_2$ receptor antagonists can be overcome by food-induced stimulation of acid secretion via gastrin or acetylcholine receptors (Ref: Lars Olbe et al., 2003).

1.8.3. Histamine $H_2$ blockers

Histamine $H_2$-receptor blockers or antagonists decrease the gastric acid production by blocking the $H_2$ receptor on the parietal cell (Figure 1-8). $H_2$ blockers were the standard treatment for peptic ulcers until the development of antibiotics against $H. pylori$. These drugs can not cure ulcers but relieves pain and encourage healing. Examples for commonly available $H_2$ blockers include cimetidine, ranitidine, famotidine and nizatidine. This group of compounds is relatively safe with few side effects like rash, headache, mild temporary diarrhea, fever, muscle pain, confusion etc.

1.8.4. Antacids

Antacids are effective in accelerating healing of duodenal and gastric ulcers. The ulcer healing action of antacids was thought to be due to the neutralization of gastric luminal acid (Tarnawski et al., 1995). Antacids can provide symptom relief but generally are not used to heal ulcer. However, antacids (eg: aluminum hydroxide, calcium
carbonate, sodium bicarbonate etc.) have to be taken in relatively large doses 1-3 hours after meals and at bedtime. However, they cause side effects like nausea, headache, weakness, loss of appetite, constipation or diarrhea.

1.8.5. Cytoprotective agents

Cytoprotective agents (e.g., sucralfate, bismuth subsalicylate, misoprostol) protect the tissues that line stomach and small intestine thus helping in rapid healing of ulcers. Although exact mechanism is unclear, these drugs appear to stimulate prostaglandin synthesis, promote improved mucosal integrity, inhibition of oxidative stress and enhance epithelial regeneration.

1.9. Etiology of PUD and Reactive oxygen species (ROS)

Metabolic pathway of an aerobic organism is continuously exposed to several degradative stresses like Reactive Oxygen Species (ROS) and free radicals (hydroxyl radicals, superoxide anions, hydrogen peroxide etc.) because of an aerobic metabolism (Johnson, 2002). Over production of these reactive species above the capability of naturally produced antioxidants extensively cause the oxidative damage to the biomolecules; proteins, lipids, carbohydrates and nucleic acids leading to protein oxidation, lipid peroxidation, depolymerization of polysaccharides, DNA modifications/strand breaks, etc. (Droge, 2002; Southorn and Powis, 1988) contributing to the pathogenesis of oxidative stress related diseases; cancer, ageing, heart failure etc. including PUD (Maxwell, 1995; Halliwell and Gutteridge, 2007; Papas, 1999; Tandon et al., 2004; Wojtaszek, 1997). Free radicals or ROS are highly toxic chemical species and evidence suggests that they play an important role in the pathophysiology of ischemic injury in the stomach as well as in the small intestine (Itoh and Guth, 1985, Granger et al., 1981).

To counter the destructive process of ROS, all aerobic organisms developed an extensive defensive mechanisms (Enzymatic and non-enzymatic) that limit molecular damage caused by ROS (Sies, 1993). In the illn ess state, oxidative stress of the stomach may occur and result in an elevation of mucosal lipid peroxide that are generated from the reaction of oxyradicals and cellular polyunsaturated fatty acids (PUFA). Various
antioxidant enzymes and molecules with different functions play their respective roles in the defense network *in vivo*. The enzymatic and non-enzymatic antioxidant defenses include superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (CAT), glutathione reductase (GR), ascorbic acid (vitamin C), \( \alpha \)-tocopherol (vitamin E), glutathione (GSH), b-carotene and vitamin A. If the array of these defensive networks becomes unable to maintain cellular homeostasis under some circumstances, the exogenous supply of antioxidants is an obligatory to restore the homeostasis.

1.10. Antioxidants

*Antioxidants, the amazing molecules that fight dangerous free radicals which prevent the oxidation of biomolecules and guard the cells, even slow the aging process.* (Richard, 1998). Oxidation is a chemical reaction that transfers an electron from one substance to oxidizing agent and results in the formation of free radicals and eventually cause cell damage and alters the cellular homeostasis.

The free radical scavenging antioxidants function by scavenging active free radicals before they attack biologically essential molecules by donating hydrogen atom (Fig. 1-14) to give a stable form.

![Figure 1-14: Schematic representation of antioxidant mechanism](image)

1.11. Drawbacks of the current therapy

A modest approach to control ulceration is via stimulation of gastric mucin synthesis, enhancement of antioxidant levels in the stomach, scavenging of ROS, inhibition of \( H^+ \)-\( K^+ \)-ATPase and *H. pylori* growth (Bandyopadhyay et al., 2002). Since ulcer is a multi-step disease, the anti-ulcer drugs available in the market such as proton
pump blockers, histamine receptor blockers, mucosal protectants and antimicrobials; failed to exert multi-step anti-ulcer effect as a single entity and also they have been shown to pose adverse side effects as discussed in previous section (1.8) (Carcanague et al. 2002).

The participation of ROS in the etiology and pathologies of gastrointestinal inflammation and gastric ulcer has been reported earlier (Repetto et al., 2002). Thus, drugs with multiple mechanisms may be beneficial (Barry 1991). Efforts were made to find a suitable agent for the treatment of gastric ulcer in natural products of plants and animals origin. Thus, there is a critical need to search an indigenous drug with fewer side effects to have a better and safer alternative for the treatment of PUD. In this context, extensive studies and research has been undertaken which mainly focuses on search of anti-ulcer agents of plant origin. Due to the lack of side effects compared to synthetic drugs, approximately 60% of the world’s population relies entirely on such natural medications.

1.12. Herbs/phytochemicals in treating ulcer

Each herb has a packet of vibration that specifically matches a vibration in the quantum mechanical body. All the body organs, for example, the liver, stomach and heart are built up from a specific sequence of vibrations at the quantum level. In the case of a malfunction, some disruption of the proper sequence in these vibrations is fault. According to Ayurveda, an herb exists with this exact same sequence and when applied, it can help restore the organ’s function (Quantum publications, 1995).

The use of phytoconstituents as drug therapy to treat major ailments has proved to be clinically effective, less relatively toxic than the existing drugs and also reduces the offensive factors serving as a tool in the prevention of peptic ulcer (Jainu et al., 2006). The chemical constituents present in the herbal medicine or plant are a part of the physiological functions of living flora and hence they are believed to have better compatibility with human body (Kamboj, 2000).

Generally, anti-ulcer properties have been attributed to phenolics (Sun-Sook et al., 2005; Reyes-Chilpa et al., 2006; Siddaraju et al., 2009), tannins (Jesus et al., 2012)
and occasionally to polysaccharides (Ye et al., 2003; Gao et al., 2004; Srikanta et al., 2010) of plant extracts. Thus, many plants are known to have beneficial therapeutic effects as noted in the traditional Indian system of medicine, Ayurveda. Therefore, development of agents, which reduces the free radical-mediated damage acts as enhancers of repair, recovery process, as well as modifiers of immune system (biological-response modifiers) and has been investigated to rescue the organism from injury.

1.13. Need of the current study

Based on the literature, it is clear that ulcer incidences are increasing and current therapeutics are not satisfactory. In this scenario, there are certain leads in the potential alternatives from plants and dietary sources, but precise components responsible for potential ulcer prevention and their mechanism of action is not clear. In the current thesis, particular attention has been paid to evaluate potential antioxidant, safety aspects and anti-ulcer property of *Mesua ferrea* Linn.


*Mesua ferrea* L. a tropical Asiatic tree of the family Clusiaceae grown in wet tropical climate of India, Sri Lanka, Southern Nepal, Burma, Thailand, Philippines, Malaysia and Sumatra. *M. ferrea* is commonly called as Naga kesara (Sanskrit), Naga sampige (Kannada) and Cobra saffron (English) commonly found in Western Ghats in India up to an altitude of 1500 m, is rich with polyphenols and flavonoids (Ghani, 2003).

1.14.1. Taxonomical description

It is a medium sized to hefty evergreen tree with reddish brown bark which peels off in thin flakes. Leaves are simple, opposite, thick, lanceolate, coriaceous, covered with waxy bloom underneath, and red when young, acute or acuminate and with inconspicuous nerves. Flowers are white, very fragrant, auxiliary or terminal, solitary or in pairs. Stamens are numerous, golden yellow, much shorter than the petals. Fruits are ovoid with a conical point surrounded by the enlarged sepals. Seeds are 1-4 in number, angular, dark brown and smooth (Warrier et al., 1995).
Scientific Classification:

Kingdom : Plantae
Division : Magnoliophyta
Class : Magnoliopsida
Order : Malpighiales
Family : Clusiaceae
Subfamily : Kielmeyeroideae
Tribe : Calophylleae
Genus : Mesua
Species : M. ferrea

1.14.1. Ethnomedicinal importance of M. ferrea L.

The plant is used in inflammation and septic conditions (Rai et al., 2000). The tribal’s of Assam were using this plant for its antiseptic, purgative, blood purifier, worm control, tonic properties (Parukutty et al., 1984). Leaves are used in the form of poultice, which is applied to head in severe colds. Bark and roots in decoction is a better tonic and are useful in gastritis and bronchitis. The ashes of leaves are used to treat sore eyes. Stem bark consist of astringent alkaloid which is used in treating cough, dysentery, fever, headache, heart trouble, piles, rheumatism, skin diseases, snake bite, sour throat, thirst, scorpion sting and ulcer either in the form of decoction, tincture or infusion (Keshavamurthy, 1994; Sahni, 1998; Husain et al., 1992; Joy et al., 1992; Nadkarni, 1976; Santamaria, 1978). Dried flowers powdered and mixed with ghee, or a paste made of flowers with addition of butter and sugar, are given in bleeding piles as well as dysentery with mucus. They are also useful in thirst, irritability of the stomach, excessive perspiration, cough with much expectoration, dyspepsia, indigestion etc. Both leaves and flowers are stomachic, expectorant and astringent. Fixed oil expressed from seeds is used as application for sores, scabies, wounds, etc. and as an embrocation in rheumatism.

Kernels are used to poultice wounds and in skin eruptions (Burkill, 1966; Kumar et al., 2006). The aerial parts are Chorionic villus sampling (CVS) active, spasmolytic, diuretic, (Husain et al., 1992; Joy et al., 1992), abortificient (Nath et al., 1992) and used in fever, dyspepsia, renal disorders and in cosmetics (Kumar et al., 2006).
Figure 1-15: *Mesua ferrea* Linn. (A) Habitat of *M. ferrea* L. (B) Flower, (C) Stem bark and (D) powdered stem bark of *M. ferrea* L.
1.14.2. Pharmacological activities of *M. ferrea* L.

The research is going on with a view to provide the scientific evidence for the ethnomedicinal claim and for their clinical application. *Mesua* is a large genus consisting of about 48 species but the extensive research work has been carried out only on *M. ferrea* L. The study of Adewale et al., (2011) concluded that the seed kernel oil has a remarkable disinfection potential. The alcoholic extracts of *M. ferrea* L. stem bark, seeds, flowers have showed potent antioxidant and hepatoprotective activity (Rajesh et al., 2013; Garg et al., 2009; Makchuchit et al., 2010; Surveswaran et al., 2007). n-Hexane, ethyl acetate and methanol extracts of *M. ferrea* leaves (125 and 250 mg/kg) exhibited significant analgesic activity in acetic acid induced writhing response in mouse (Hassan et al., 2006). The aqueous extract of *M. ferrea* leaves was screened for its activity against fibroblast cell lysis after *Heterometrus laoticus* scorpion venom treatment. The extract was evaluated against viability of fibroblast cells after 30 min. treatment with mock control or with plant extracts preincubated with *H. laoticus* venom. Viability of fibroblast cells after 30 min. treatment with mock control or with extract showed efficiency in protecting against venom induced lysis (Uawonggul et al., 2006).

The ethanol extract of *M. ferrea* was evaluated against human cholangio carcinoma (CL-6), human laryngeal (Hep-2), and human hepatocarcinoma (HepG2) cell lines in vitro. The extract showed promising activity against cholangio carcinoma (CL-6), with survival of less than 50% at the concentration of 50 μg ml⁻¹. There was potent cytotoxic activity against Hep-2 and HepG2 shown by Mahavorasirikul et al., (2010). The cytotoxicity of methanol extract was evaluated against Ehrlich Ascites carcinoma cell lines in mice and showed significant tumor growth inhibition (Rana et al., 2004).

The ethanol extract of *M. ferrea* flowers was evaluated for anticonvulsant activity at 3 different dose levels (200, 400 and 600 mg/kg p.o.) by Maximum electroshock seizure (MES) test using albino mice. The study showed that *M. ferrea* flowers significantly increased the onset time and decreased the duration of seizures by electroconvulsive shock (Tiwari et al., 2012). *M. ferrea* seed extracts (petroleum ether, ethyl acetate and alcohol) were evaluated in formaldehyde and Complete Freund's Adjuvant (CFA)-induced arthritis in rats. The results indicate that *M. ferrea* protects rats...
against formaldehyde and CFA induced arthritis (Jalalpure et al., 2011). *Mesua ferrea* L. crude extract and isolated phytoconstituents showed *in vitro* and *in vivo* anti-microbial efficiency against different microbial strains (Chakraborty et al., 1959; Mazumder et al., 2004; Verotta et al., 2004; Ali et al., 2004; Parekh et al., 2007; Parekh et al., 2008; Adewale et al., 2012; Jayanthi et al., 2011).

Mesuol isolated from *M. ferrea* seed oil was evaluated for immunomodulatory activity in experimental animals by using specific and non-specific immune response models. The study indicated that mesuol has potent immunomodulatory effect (Chahar et al., 2012). Mesuaxanthone A and mesuaxanthone B from *M. ferrea* were evaluated using albino rats by carrageenan induced-hind paw oedema, cotton pellet implantation and granuloma pouch tests and xanthones have been found to produce significant anti-inflammatory activity. Xanthones from *M. ferrea* were also screened for anti-ulcer activity by pyloric ligation method in albino rats. The control animals showed extensive ulceration, haemorrhage and perforation, while the xanthones pre-treated animals exhibited only scattered areas of hyperemia and occasional haemorrhagic spots (Gopalakrishnan et al., 1980).

### 1.14.3. Phytochemistry of *M. ferrea* L.

Several workers have carried out survey of Nagakesara. *M. ferrea* is the only species that has been chemically studied from the genus Mesua (Kirtikar, 1935; Rao et al., 1981). Phytochemical studies have revealed plants from this genus found to be rich in many classes of secondary metabolites including phenylcoumarins, xanthones and triterpenoids (Chow et al., 1968; Bandaranayake et al., 1975; Raju et al., 1976). Boorsma (1904) has reported the presence of an essential oil and two bitter substances in the flowers (See Chakraborty et al., 1959). Subramanyam *et al* (1975) have isolated mesuanic acid, a new carboxylic acid, from the acetone extract of *M. ferrea* stamens. Subramanyam and Subba Rao (1969) had earlier isolated mammeisin and mesuol as the main phenolic components from two different samples of seed oil. Dutt *et al* (1940), Chakraborty and Bose (1960) and Chakraborty and Das (1966) isolated two crystalline antibiotic principles mesuol and mesuone from the seed kernel oil. 4-Phenylcoumarins like mesuol, mesuagin, mammeisin, mammeigin and mesuone were isolated from the
seed oil of *M. ferrea*. The trunk bark and the heartwood yielded 4-alkylcoumarins ferruols A and B, a lupeol-type triterpenoid guttiferol, mesuaxanthones A and B, erraxanthone 1,7-dihydroxyxanthone, 1,5-dihydroxy-3-methoxyxanthone, 1,6-trihydroxyxanthone, 1,5-dihydroxyxanthone, 1-hydroxy-7-methoxyxanthone and β-sitosterol. Stamens give α and β-amyrin, β-sitosterol, biflavonoids- mesuaferrones A and B, mesuanic acid, 1,5-dihydroxyxanthone, euxanthone 7-methyl ether and β-sitosterol. Other isolated constituents were mesuaferrol, leuco anthocyanidin, mesuone, euxanthone, etc. Presence of xanthone derivative and essential oil had also been reported from various parts of the plant (Sharma et al., 2002; Chow et al., 1968; Govindchari et al., 1967). Two new yellow pigments, meauxanthone A and memaxanthone B have been isolated from the heartwood extracts of *M. ferrea* (Govindchari et al., 1967). The stamens, which yield the drug Nagakeshara contain mesuferrone-A and B, mesuaferrol, mesuonic acid, α, and β-amyrin (Handa et al., 1992).

### 1.15. Scope of the present investigation

PUD is a common global problem with increasing incidence and most prevalent disease of the modern society. It is a heterogeneous group of disorder attributed to several factors encountered during day-to-day life, such as stress, alcohol, exposure to bacterial infection and the use of NSAIDS. Mucosal damage, an initial step in the ulcer development has been correlated with oxidative stress by ROS generation and hyper-secretion of HCl through H⁺-K⁺-ATPase action (Phull, Green, & Jacyna, 1995). Thus, a modest approach to control ulceration may be by scavenging ROS in the stomach and inhibiting H⁺-K⁺-ATPase, a proton pump to prevent acid secretion in the parietal cells of gastric mucosa. Although several drugs are being used to treat PUD, they produce adverse side effects on human health.

*M. ferrea* is being used in India and several parts of the world for its potential medicinal and several other properties. The plant is known for its antioxidant, analgesic, anti-inflammatory, antitumor, immune-stimulant, antimicrobial and several other activities. It is an ingredient of several *Ayurvedic* and *Unani* formulations. The phytochemical screening confirms the presence of phenyl coumarins, xanthones, triterpenoids, fats and flavonoids as main constituents of the plant. Apart from medicinal
uses it is also being used commercially in polymer industry, painting, as firewood and preparation of nanoparticles. Traditionally this plant bark has been used to treat ulcer and stomachic disorders (Keshava Murthy, 1994). Therefore, further studies are required to prove the potential of this plant as well as the isolated phytoconstituents. The present study is a preliminary approach to evaluate the in vitro antioxidant, safety aspects and anti-ulcer property of *M. ferrea* L., which can throw light on the medicinal property of *M. ferrea* L. to treat gastritis.

1.16. Objectives of the work

Keeping in view of deleterious effects of the PUD and medicinal importance of *Mesua ferrea* Linn., the following objectives were proposed;

1. To evaluate in vitro antioxidant and protective effects of *Mesua ferrea* Linn. bark extracts on induced oxidative damage.

2. To assess safety of *Mesua ferrea* Linn., through acute, sub-chronic and chronic administration in rats.

3. To determine the modulatory effect of *Mesua ferrea* Linn. in stress induced ulcers in comparison with known antiulcer drug, a possible mechanism of action.

4. To isolate, screen free radical scavenging and H⁺-K⁺-ATPase inhibition property of phyto-constituents of *Mesua ferrea* Linn.

5. To screen virtually the phyto-chemicals as probable anti-*Helicobacter pylori* drug.

1.17. Organization of thesis

The thesis is organized as follows:

Chapter 1 has already provided an introduction and detailed review regarding peptic ulcer, causes, need of the present study, *M. ferrea* L. and scope of the present investigation. Each of the resultant chapters begins with a chapter outline, detailing the main objective of the study, the workflow as well as the major results and conclusions.
Chapter 2 discusses the detailed *in vitro* antioxidant and protective activity of *M. ferrea* L. stem bark extract against induced oxidative stress in erythrocytes, hemoglobin and DNA.

Chapter 3 outlines the safety aspects of *M. ferrea* L. in male rodents by acute, sub-chronic and chronic oral administration of ethanol extract *M. ferrea* (MEE).

Chapter 4 discusses the anti-ulcer property and possible mechanism of action of MEE in ethanol and swim stress-induced gastric ulcer.

Chapter 5 discusses isolation, characterization, free radical scavenging activity and H⁺-K⁺-ATPase inhibition property of phytoconstituents from *M. ferrea* stem bark.

Chapter 6 discusses the virtual screening of a set of phytochemicals as probable anti-*Helicobacter pylori* drug.
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CHAPTER OUTLINE

**Objective:** To evaluate *in vitro* antioxidant and protective effects of *Mesua ferrea* Linn. bark extracts on induced oxidative damage.

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**Work flow**

1. Collection of bark
2. Preparation of MCE and MEE
3. Qualitative and Quantitative analysis of MCE and MEE
4. Determination of *in vitro* antioxidant activity
5. Protective effect of MCE and MEE against induced oxidative stress

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**Results:** (a) Qualitative and quantitative analysis of MCE and MEE revealed the presence of significant antioxidant molecules, (b) MCE and MEE showed remarkable antioxidant and free radical scavenging activity, (c) MCE and MEE showed protective activity to erythrocytes, hemoglobin and DNA in hostile conditions of H₂O₂, (d) MCE and MEE showed protective activity against oxidative stress by acting as antioxidant and electro catalyst.

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**Conclusion:** Overall result of the investigation accentuates the antioxidant and protective activity against induced oxidative damage in erythrocytes, DNA and Hb of *M. ferrea*. The study has gathered substantial evidence of *M. ferrea* L. antioxidant property and also confirms the popular use of plant in antioxidant medicine. Thus, *M. ferrea* L. can be a valuable source of antioxidant agent in pharmaceuticals.