Introduction
For more than a century, peptic ulcer disease (PUD) has been a major health problem throughout the world as it affects millions of people each year. It is characterized by symptoms of burning epigastric pain, vomiting and gastrointestinal hemorrhage. Clinically, it is defined as disruption of gastroduodenal mucosal integrity of stomach or duodenum leading to a local defect of excavation due to active inflammation. The major forms of peptic ulcer include gastric and duodenal ulcer, both of which are often chronic in nature (Valle, 2005).

Epidemiologically, peptic ulcer represents a global disorder because of its widespread occurrence with high morbidity, mortality and economic loss. Approximately 10% of adults suffer annually from peptic ulcer disease and 5,000,000 new cases along with 4,000,000 cases of recurrence are reported each year. An estimated 15,000 deaths per year occur as a consequence of complicated PUD (Valle, 2005). The financial impact of this common disorder has been substantial, with an estimated burden on direct and indirect health care costs of ~ $10 billion per year in the United States (Valle, 2005).

Such a high global incidence rate, simpler to severe pathophysiological effects and uniform occurrence across all ages, races and ethnicity, makes peptic ulcer an important target that continues to arrest the attention of both clinicians and researchers; hence there is a need for major therapeutic strategy. Important advances have occurred in last two decades that have improved our understanding for this disease as well as our approach towards its treatment.

1.1 Pathophysiology of gastric ulcers

The pathophysiology of peptic ulcer has centered on an imbalance between aggressive and protective factors in the stomach (Chan and Leung, 2002). Major breakthrough occurred when different factors that lead to integrity and disruption of gastric mucosa were unveiled.

Gastric mucosal integrity is maintained through a balance between aggressive and defensive factors (Figure 1.1). The former include acid, pepsin, Helicobacter pylori (H pylori), nonsteroidal anti-inflammatory drugs (NSAIDs), smoking, alcohol,
ischemia and corticosteroid use whereas later include mucus, bicarbonate, blood flow, prostaglandins and growth factors. Among these aggressive factors, acid and pepsin confers the contributory role in ulcerogenesis (Hoogerwerf and Pasricha, 2001).

Figure 1.1: Different offensive and defensive factors whose imbalance results into causation of ulcer while the normal balance results into mucosal reconstruction

An increase in offensive factors results into causation of ulcers. Most of the ulcer therapies acts on decreasing these factors

An increase in defensive factors avoids ulcer/ causes ulcer healing by causing mucosal reconstruction. Recent target of ulcer therapies

The ability of gastric mucosa to resist injury by endogenous secretions (acid and pepsin) and by ingested irritants (alcohol and NSAIDs) is attributed to a number of factors, which are collectively termed as mucosal defence (Wallace, 2001). These lines of defense act finally for ulcer healing process that cause the repair and reconstruction of mucosal architecture through growth and redevelopment of gastric
glands, growth of new blood vessels (angiogenesis) and re-innervation of the mucosa by extrinsic and intrinsic nerves (Wallace and Ma, 2001).

1.2 Ulcer healing

Ulcer healing, a genetically programmed repair process, is an active and complicated array of different mechanisms that involves filling of mucosal defect with proliferating and migrating epithelial cells and connective component so as to reconstruct the mucosal architecture (Perini et al., 2003; Tarnawski et al., 2005). Ulcer healing is considered to be a spontaneous process, where either the precipitating cause of ulceration gets removed or there occurs a readjustment of the gastric milieu to a state that favours healing. Healing requires angiogenesis in the granulation tissue at the base of the ulcer, together with replication of epithelial cells at the ulcer margins and subsequent re-establishment of glandular architecture (Wallace, 2005).

Ulcer healing is a cumulative effect of several physiological and constitutive processes that occurs in tandem and requires a high level of co-ordination and regulation. There occurs a two-tier strategy that forms the basis of both normal as well as drug mediated ulcer healing. First involves decrease of acid concentration in the lumen of the stomach while second one involves strengthening of mucosal defense system and tissue repair mechanism.

1.3 Role of Prostaglandins in ulcer healing

A high degree of coordination and regulation during complex sequence of ulcer healing is carried out by different factors. Among them prostaglandins (PGs) and growth factors have received much attention in recent years (Szabo and Vincze, 2000; Berenguer et al., 2002). The gastric mucosa contains abundant level of PGs mainly PGE_{2} and PGI_{2}, which provide their effect by stimulating mucosal bicarbonate and mucus secretion, increasing blood flow, preventing the disruption of mucosal barrier, accelerating cell proliferation, stimulating cellular ionic transport processes, retarding cAMP production, promoting formation of surface phospholipids, maintaining gastric mucosal sulfhydryl compounds, stabilizing cellular lysosomes and cell membrane (Robert, 1979; Miller, 1983; Isenberg et al., 1985; Scheiman, 1996; Valle, 2005).
However, these PGs exhibit a dual character in their functioning because along with being a strong ulcer healing agent, PGs are also a well known mediators in different features of inflammation- pain (dolor), swelling (tumor), erythema (rubor), warmth (calor) and loss of function (function laesa). This duality of PGs in their functioning is well established when on one side inhibition of their synthesis by aspirin and NSAIDs causes anti-inflammatory, analgesic and antipyretic effect while on the other hand it produces the side effects of gastric ulceration, bleeding and renal dysfunction. The therapeutic and adverse side effects of these agents are due to the inhibition of prostanoid synthesis.

1.4 Cyclo-oxygenase Enzymes (COX)

The duality of PGs as a mediator of physiologic and pathologic function was clarified when two different isoforms of Cyclooxygenase enzyme (COX) were identified. COX is the key enzyme responsible for the synthesis of PGs via arachidonic acid, exist in two isoforms- COX-1 and COX-2. According to the classical COX hypothesis, COX-1 considered to be a classical COX enzyme and is constitutively expressed; while COX-2 is expressed as an inducible enzyme in most cells (Smith and Dewitt, 1996). Nevertheless, there is also a constitutive expression of COX-2 in the kidney, brain, and female reproductive system (Dubois et al., 1998). PGs, which are products of COX-1, exert different physiological or “housekeeping” effects while the products of COX-2 are essentially involved in inflammation (Seibert et al., 1994). The molecular identification of two isoforms of COX have led to the development of selective COX-2 inhibitors as new NSAIDs drugs that are devoid of gastric toxicity (Xie et al., 1991; Kargman et al., 1996; Pairet and Engelhardt, 1996). Selective COX-2 inhibitors such as celecoxib and rofecoxib are endowed with significantly improved gastric tolerability associated with a therapeutic efficacy comparable to traditional NSAIDs (Hawkey, 1999; Laine, 2002).

However, recent advances reveal that the classical COX hypothesis is oversimplistic. There emerges a broad consensus on the fact that COX-2 plays more complex and wider biological role than mere involvement in inflammation and pain (Peskar, 2001). Important among them are healing process in various gastrointestinal
pathologies (Schmassmann et al., 1998; Fidalgo et al., 2004) and some regulatory effect in renal system (Harding et al., 1997). Further, Gilory et al. (1999) has shown that COX-2 produces PGs that exerts anti-inflammatory action while Shigeta et al. (1998) and Brzozowski et al. (2001) have highlighted their role in gastric ulcer healing.

These observation brought a change in the classical COX hypothesis and have strengthen the view that COX inhibition is not associated with gastric damage in normal mucosa, but it can be detrimental when gastric mucosal defense is impaired. Further support to this hypothesis was provided by Mizuno et al. (1997) who have demonstrated that COX-2 is strongly expressed at the margin of healing ulcers in mice and Brzozowski et al. (2001) that have reported that administration of selective COX-2 inhibitors delay healing of gastric ulcers. All these findings mark a shift in our understanding of ulcer healing mechanisms. COX-2 enzyme and PG synthesis starts appearing to be an important target for the treatment modalities of gastric ulcers and continues to arrest the attention of researchers.

1.5 Current therapeutic strategies

Anti-ulcer drugs mainly focuser on decreasing the acid secretion and/or strengthening the mucosal defence system. The current treatment strategies of gastric ulcer include following categories of drugs each of which act through a different mechanism. 1) Acid neutralizing/inhibitory drugs: This category of drugs includes: (a) $H_2$ receptor antagonists (cimetidine, ranitidine and famotidine) (b) Proton pump inhibitors (omeprazole, lansoprazole and rabeprazole) (c) Antacid 2) Cytoprotective agents (sucralfate) 3) Prostaglandin analog (misoprostol) 4) Miscellaneous drugs (anticholinergic drugs and rabepimide)

Keeping these observations in our consideration, we have drafted present study to explore the role of COX-2 enzyme in normal and drug-mediated ulcer healing mechanism. We have made an attempt to systematically compare the ulcer healing effect of four drugs each of which is known to achieve its function through a different mechanism: omeprazole is a proton pump inhibitor, misoprostol a PG analog whereas sucralfate acts like a cytoprotective agent and ranitidine as a $H_2$ receptor antagonist.
The comparisons have been made in light of a broader hypothesis, which assumes that the most efficient and powerful ulcer healing drug is the one that apart from its known acting mechanism, elevates the expression level of COX-2 enzyme most efficiently, which eventually leads to better and faster repair process. Since the study focused on the expression level of COX-2 enzyme, we have also incorporated an additional drug, COX-2 selective NSAIDs- celecoxib as a negative control. Differential expression of COX-2 enzyme is carried out both at transcript (mRNA) and protein level for each category of drug.

Choice of drugs was based on the fact that all the studied five drugs acts through different mechanisms and have wider clinical consumption while the ulcer healing model used is acetic acid induced chronic gastric ulcer model. The model was first reported by Okabe et al. (1987) which is an ideal in-vivo model to study healing mechanism because it produces well-characterized ulcers resembling human ulcers and also exhibits a benign spontaneous ulcer healing.

To justify our hypothesis and to offer a more explainable conclusion, we have also compared other healing parameters for each category of drug. These include protective anti-oxidants enzymes, PGE\textsubscript{2} levels, expression level of different growth factors, estimation of mucus content in term of carbohydrate: protein ratio, total DNA content and myeloperoxidase (MPO) activity as a marker of neutrophil infiltration. Effect of the drugs on ulcer damaging factors like free and total acidity and pepsin estimation was also carried out along with estimation of proton pump inhibition as a possible reason of decrease in acid synthesis. The extent of ulcer healing was also complemented with the complete histopathological examination of ulcerated tissues.

The study along with establishing the role of COX-2 enzyme in the process of both natural and drug mediated ulcer healing, will also be helpful in providing necessary information for the development of better ulcer therapeutic modalities that strengthens the defensive mucosal system and targets mainly the repair and reconstruction of mucosal architecture in addition of inducing a fall in the levels of offensive factors.