Review of Literature
Chapter III

REVIEW OF LITERATURE

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1.1 Introduction to Gastric Ulcer

Definition: Ulcers are erosions in the stomach lining or duodenum. Gastric ulcer is an ulcer in the stomach. Ulcers of the stomach and duodenum together are referred to as peptic ulcers.

Most ulcers are erosions of the first layer of the inner lining ie., the mucosa.

Sometimes, the ulcer extends through the muscularis mucosa and causes perforations of the intestinal lining. This can cause a medical emergency.

Fig R1: Peptic ulcer inside the stomach

Prevalence: The lifetime prevalence of peptic ulcer disease is approximately 5% to 10% (1).
1.2 Factors associated with the disease

Causes: Gastric ulcer disease is the end result of an imbalance between aggressive and defensive factors in the gastric mucosa. The causes for this disease can be various, including:

1) *H. pylori*- the major cause and responsible for 10-20% prevalence.

2) Nonsteroidal anti-inflammatory drugs (NSAIDs)- approximately 1,07,000 patients are affected annually for NSAID-related gastrointestinal (GI) complications, and at least 16,500 NSAID-related deaths occur each year among arthritis patients alone. (2)

3) Zollinger-Ellison syndrome, or, acid-secretory abnormalities- A large amount of excess acid is produced in response to the overproduction of the hormone gastrin, which in turn is caused by tumors on the pancreas or duodenum.

4) There is a small percentage of ulcers not related to *H. pylori* infection or NSAID use. These are classified as "idiopathic" and may be related to defective mucosal defense mechanisms, tobacco use, genetics, rapid gastric emptying, or psychological stress.

Fig: R2 ulceration due to hyperacidity

Ulcer due to hyperacidity
1.3 Gastric Physiology related to Peptic Ulcer

1.3.1 Acid-peptic Pathogenesis

The gastric mucosa possesses an extra-ordinary capacity to secrete acid. Parietal cells (Oxyntic cell), interspread along the course of mucosal glands of the body and the fundus of the stomach, secrete hydrochloric acid by a process involving Oxidative Phosphorylation. Parietal cells secrete hydrogen ions at a concentration about three million times \((3 \times 10^6)\) than that found in blood. The estimated concentration of hydrochloric acid secreted directly by parietal cells is approximately 160mM \((3)\). Each secreted hydrogen ion \((H^+)\) is accompanied by a chloride ion \((Cl^-)\). With each increase in hydrogen ion secretion, there is a reciprocal decrease in sodium ion \((Na^+)\) secretion. For each hydrogen ion secreted into the gastric venous circulation, accounting for the alkaline tide, a direct reflection of the magnitude of gastric hydrogen ion secretion could be seen. Bicarbonate is released from carbonic acid generated from carbon dioxide by parietal cell carbonic anhydrase. The final step in hydrogen ion secretion is accomplished by a proton pump mechanism, involving a specific hydrogen-potassium adenosine triphosphate \((H^+, K^+\text{-ATPase})\) located in the apical microvillus membrane and tubulo-vesicular apparatus of the parietal cell. This \(H^+, K^+\text{-ATPase}\) exchanges hydrogen for potassium across the microvillus membrane.

**FigR3:** Layers of gastric mucosa
1.3.2 Mechanism of gastric acid secretion

a) Role of Histamine - The gastric mucosa contains large amount of histamine. Histamine is contained in cytoplasmic granules of mast cells, which are nonepithelial (interstitial) in location, and enterochromaffin like cells (ECL), epithelial endocrine cells distributed singly in the oxyntic glands, often in direct contact with parietal cells. For many years views differed on the importance of histamine in stimulating gastric acid secretion.

b) Food: Stimulus for acid secretion - The major stimulus for gastric acid secretion is ingestion of food. Traditionally, regulation of gastric acid secretion has been classified into three phases: cephalic, gastric and intestinal. This classification is of some value in analyzing factors that participate in regulation of gastric acid secretion.

c) Role of pepsin - The proteolytic effects of pepsin in concert with the corrosive properties of secreted gastric acid contribute to the tissue injury that produces peptic ulcer apart from its beneficial effect of food protein digestion, if in excess.

d) Inhibition of gastric acid secretion - Gastric acid secretion can be inhibited by acid in the stomach or duodenum, by hyperglycemia, or by hypertonic fluids or fat in the duodenum. Reduction of the intragasic pH at 3.0 produces partial inhibition of gastrin release; further reduction of pH at 1.5 or below completely blocks release of gastrin in response to almost all stimuli.
1.3.3 Mucosal Defense

The mechanisms whereby the normal stomach and duodenum resist the corrosive effect of acid-pepsin (i.e., mucosal resistance to injury or mucosal defense) have not been defined completely. However, a variety of factors have been identified which contribute to and are considered to comprise mucosal defense.

a) Role of Gastric Mucus: Gastric mucin is important in mucosal defense and in preventing gastric ulceration. Gastric mucin is secreted by mucus cells of the gastric epithelium and gastric glands. Mucin secretion is stimulated by mechanical or chemical irritation and by cholinergic stimulation. Gastric mucin is present in 2 phases: in gastric juice as a soluble phase and as an insoluble mucus gel layer which coats the mucosal surface of the stomach. Normally the mucus gel is secreted constantly by gastric mucus epithelial cells and is continuously solubilized by pepsins secreted into gastric lumen. Gastric mucin is a large polymeric glyco-protein containing four sub-units connected by di-sulfide bridges.

b) Role of Bicarbonate: Non-parietal gastric epithelial cells secrete bicarbonate ions into the mucous gel, which creates a microenvironment with a substantial hydrogen ion gradient ranging from pH 1 to 2 in the luminal side of the gel layer to pH 6 to 7 in the zone in contact with gastric mucosal cells.
c) **Role of Prostaglandin:** Prostaglandins are abundant in gastric mucosa. Different types of Prostaglandins are responsible for offensive as well as defensive effect for gastric mucosa. Their role depends on their type and relative concentration in the mucosa.

1.3.4 **Helicobacter pylori** infection

*Helicobacter pylori* is a spiral-shaped bacterium measuring approximately 3.5 x 0.5 μm that lives in the stomach and duodenum, despite the highly acidic environment. It is believed to be transmitted orally by means of fecal matter ingested in tainted food or water. It can be cultured from the stools of infected persons, supporting a fecal-oral route. *Helicobacter pylori* infection correlates with socio-economic status also supporting the fecal-oral transmission route theory. It may also be transmitted by oral contact from bacteria transmitted into the mouth by belching or gastric reflex. This secondary theory is supported by detection of *H. pylori* in dental plaque from 30% of persons with gastric infection.

*H. pylori* takes advantage of the stomach’s own mucus for protection. Any acid that reaches the bacteria is converted to bicarbonate and ammonia by *H. pylori*’s urease enzyme.

\[
\text{urea + stomach acid + water} \rightarrow \text{bicarbonate + ammonia}
\]

\[
C=O\cdot2NH_2 + H^+ + 2H_2O \rightarrow HCO_3^- + 2NH_4^+
\]

Bicarbonate and ammonia, are strong bases that protects the bacteria because of their acid-neutralizing capability. The body’s innate immunity system
responds to the presence of H. pylori and sends infection-fighting cells to that area. However, the neutrophils cannot reach the H. pylori infected place as they cannot get through the stomach lining easily. Inflammation in the stomach tissue occurs as the neutrophils die and superoxide radicals on the stomach wall are released, resulting in tissue damage. The nutrients sent by the immune system to help the neutrophils, are utilized by H. pylori. Even if H. pylori itself does not cause a stomach ulcer, but it can very much cause the inflammation in the stomach lining as part of the immune response (4)

Treatment

Treatment often involves a combination of medications to kill the Helicobacter pylori bacteria, reduce acid levels, and protect the GI tract. This combination strategy allows your ulcer to heal and reduces the chance it will come back. Take all of your medications exactly as prescribed.

The medications may include one or more of the following:

- Antibiotics to kill Helicobacter pylori
- Acid blockers (like cimetidine, ranitidine, or famotidine)
- Proton pump inhibitors (such as omeprazole)
- Medications that protect the tissue lining (like sucralfate)
- Bismuth (may help protect the lining and kill the bacteria)
1.3.5 NSAID induced ulceration

Non-steroidal anti-inflammatory drugs are among the most frequently prescribed drugs worldwide used in the therapy for chronically painful patients. Their principal mode of action is to block prostaglandin production by binding and inhibiting cyclooxygenase (COX). While the result of this effect is mainly a reduction in inflammation and peripheral nociceptor sensitization, there is some evidence that NSAID's have a central analgesic action as well, though the exact mechanism remains unclear.

Though reasonably safe in most cases in prescribed dosages and for short durations, these drugs cause gastrointestinal toxicity in a large number of cases. They can affect all segments of the gastrointestinal tract. In the mouth, they cause oral ulceration, in esophagus, they can cause ulceration and stricture formation. In stomach and duodenum, they can cause ulcers, severe bleeding, perforation, and obstruction. Most cases of NSAID-induced gastrointestinal ulcers can heal spontaneously, even when the drug is continued. However, in some they can cause serious toxicity requiring hospital admission and aggressive management.

Mechanisms of NSAID-induced GI ulcerations

Ulceration due to NSAID is believed to occur as the result of a complex interplay of aggravating factors and protective factors. Most NSAIDs are weak organic acids and have low pKa. Therefore, they remain unionised in stomach and are absorbed appreciably from stomach. However, once they
breach the cell membranes of stomach cells and reach within, they encounter a basic pH. This causes so called “trapping” of the drugs inside the cell(8). This topical effect is considered an important mechanism of gastro-duodenal damage associated with their use. Even short-term (< 1 week) use of nonsteroidal anti-inflammatory drugs (NSAIDs) can precipitate ulcer-related bleeding (9). Presence of H. pylori infection aggravates the development of NSAID-induced ulcer. Thus, it can be understood to be the disease of the war between the factors favouring and those opposing the development of ulcers where the former win over the latter. Although NSAID use is primarily associated with upper GI problems, it is also associated with lower gastrointestinal symptoms such as haemorrhage, inflammation, perforation, and stricture formation. ARAMIS data suggested that risk of death from NSAID use is four times more than non-user (10,11). Recent studies have shown that use of multiple NSAIDs; non-use of anti-ulcer medication, and NSAID use in patients with previous history of peptic ulcers raises the possibility of developing GI ulcers by 14-17 fold(8). Even aspirin use for prophylactic reasons in low dosages is not free of gastrointestinal complications. All formulations of aspirin like buffered, enteric coated, and plain aspirin carry same amount of risk (9). The vascular integrity of the ulcer base is poor; therefore, ulcers bleed easily (8,9). Many more predisposing factors have been identified.

NSAID induced GI damage is mainly of three types:
a) Superficial damage such as mucosal haemorrhages and erosions.
b) silent ulcer- endoscopically documented non-symptomatic ulcers
c) symptomatic ulcers causing complications such as GI haemorrhage.

Pharmacology and metabolism of Indomethacin.

Indomethacin is a non steroidal anti-inflammatory drug first introduced in 1963(12) the structural formula of Indomethacin is that of a methylated indole derivative. Indomethacin inhibits COX enzymes and prevents the formation of prostaglandin PGE\(_2\), PGE\(_{24}\), PGD\(_2\), PGI\(_2\), and TxA\(_2\) from arachidonic acid (13).

Fig: R4 Biosynthesis of prostaglandins and thromboxanes via the cyclooxygenase pathway and the structural formula of Indomethacin.


Gastro-intestinal effects

Adverse effects of NSAIDs: NSAIDs have numerous side effects. During the long-term administration of nonsteroidal anti-inflammatory drugs (NSAIDs), approximately 3% of patients have gastric ulcers develop in each year. (14). Gastro-intestinal disturbances including ulceration are the commonest adverse responses to NSAIDs and carry the greatest risk of death. Other than that, significant renal impairment and an increased risk of post-operative haemorrhage. Asthma and allergic reactions are uncommon.

The degree of enzyme inhibition of many NSAIDs correlates with their capacity to erode gastric mucosa (15). The effects on gastric mucosa results from both a systemic and a direct local irritant action of NSAIDs (16). Indomethacin treatment has been shown to decrease the blood flow in the terminal ileum and block the autoregulation of intestinal oxygen consumption (18). It may also increase the risk of bowel necrosis after temporary intestinal ischaemia in infants.(19).

The mechanism underlying is not very clear till date. But partially it may be due to the inhibition of PG synthesis as speculated by Konturek et al.(20), Levine et al (21) and Pezzati et al(22). Additionally, as the general protective effects, including inhibition of gastric acid secretion, stimulation of bicarbonate secretion and synthesis of mucus, as also an increase in the hydrophobicity of the gastric mucosa by increasing phospholipids are
attributed to prostaglandins, the inhibition of PG synthesis with
indomethacin further compromises intestinal defence mechanisms (23).
It is presumed that ulceration is due to the cascade effect of reduced mucosal
prostaglandin synthesis that accompanies NSAID-induced inhibition of
cyclooxygenase. Inhibition of prostaglandin synthesis leads to decreased
epithelial secretion of mucus and carbonate, diminished mucosal blood flow,
reduced mucosal proliferation, and impaired resistance to peptic injury(24).

**Genetics of ulcer diseases**

Genetic factors appear to play a role in gastric ulcer pathogenesis(25). The
concordance for peptic ulcer among identical (i.e, monozygote) twins is
approximately 50% and is increased among nonidentical twins, although not
to the same degree(26). The lifetime prevalence of developing ulcer in the
first degree relatives of ulcer patients is about threefold greater than that in
the general population.

The inheritance of blood group “O” is associated with a modest (1.3 fold)
increase in duodenal ulcer incidence. Inheritance of a nonsecreting status of
blood group antigens in body fluids (e.g., saliva, gastric secretion) increases
the risk by about 1.5 folds, and the combined presence of blood group “O”
and nonsecretor status increases the risk of developing duodenal ulcer about
2.5 fold compared to the general population(27).

Human leucocyte antigen (HLA) subtypes (e.g., HLA-B5, HLA-B12) may be
identified as genetic markers for duodenal ulcer, but further study is needed.
Inheritance of peptic ulcer may be polygenic, in which, several genes plus environmental factors results in clinical diseases or could be caused by genetic heterogeneity, representing a group of distinct genetic and non-genetic diseases that produce identical clinical findings. The genetic components may be attenuated by other factors, like hyper-secretion of pepsinogen I, or infection of *H. pylori*.

1.4 Mechanism of Ulcer Formation

1.4.1 Inhibition of Prostaglandin Pathway

**Inflammatory Role of Prostaglandin**

Inflammation is a nonspecific response of the immune system to damaged cells (28). It is characterized by redness, pain, heat and swelling of tissue. Although there are many components to inflammation, only the prostaglandin component is substantially reduced by the action of an NSAID. Prostaglandin E2 (PGE2) is the form of prostaglandin most associated with inflammation (29). It dilates blood vessels, allowing more blood to flow through the affected tissue. The increased blood flow generates the heat and redness of inflammation. PGE2 prolongs pain, and may also stimulate the emigration of phagocytes through capillary walls(30). In an autoimmune disease, the body is inadvertently attacking its own cells through the inflammatory process as though they were foreign particles. So, by reducing prostaglandin, the swelling, heat and pain of inflammation is reduced. However, other parts of the inflammatory response, such as the
destruction of healthy tissue by phagocytes, continue unabated in an autoimmune disease.

**Cyclo-oxygenase inhibition**

A 71 kilodalton membrane bound glycoprotein enzyme catalyzes the production of prostaglandins from arachidonic acid. This enzyme was named as Cyclooxygenase(31), which is a part of a bifunctional enzyme called as Prostaglandin H Synthase, necessary to catalyze the first step of conversion of arachidonic acid to eicosanoids, a class of biologically active lipid molecules that includes prostaglandins, thromboxanes and leukotrienes. There are 2 types of COX isoenzymes: COX-1, and COX-2, and Indomethacin has an inhibitory effect on both of them(32). The general conception has been that COX-1 is expressed in most tissues and that PG's contributing to homeostatic functions are derived from it, whereas COX-2 is induced in inflammatory cells and is the enzyme which produces prostanoids mediators for inflammation. The anti-inflammatory action of Indomethacin has been thought to be related to COX-2 inhibition and the undesirable effects of the drug to COX-1 inhibition (33). There is evidence that COX-2 is expressed in many tissues, including DA and the kidney, where it has physiological functions(34, 35). There is also evidence suggesting an important COX-1 mediated component in inflammation (35). The two forms of cyclooxygenase have equal molecular weights and are very similar in structure. However, the attachment site of COX-1 is smaller than
the attachment site of COX-2, so it accepts a narrower range of structures as substrates (36). Cox-2 is encoded in a different gene that cox-1. A third form of cyclooxygenase, a ‘COX-3’, is now discovered, which exists in the brain(37).

Fig R5: The current Cox concept

Table R1: Comparison of COX-1 and COX-2

<table>
<thead>
<tr>
<th>Parameter</th>
<th>COX-1</th>
<th>COX-2</th>
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<tr>
<td>Regulation</td>
<td>Usually Constitutive</td>
<td>Inducible</td>
</tr>
<tr>
<td>Range of Induced Gene Expression</td>
<td>2 to 4-fold</td>
<td>10 to 80-fold</td>
</tr>
<tr>
<td>Rate of Gene Activation</td>
<td>24 hours</td>
<td>0.5 to 4 hours</td>
</tr>
<tr>
<td>Effect of Glucocorticosteroids</td>
<td>Little or None</td>
<td>Inhibits Expression</td>
</tr>
<tr>
<td>Relative Size of Active Site</td>
<td>Smaller</td>
<td>Larger</td>
</tr>
<tr>
<td>Rate of Arachidonic Acid Consumption</td>
<td>34 nmol/min/mg</td>
<td>39 nmol/min/mg</td>
</tr>
<tr>
<td>Effect of aspirin on COX activity</td>
<td>Inhibited</td>
<td>Not Affected</td>
</tr>
</tbody>
</table>
Action of NSAIDs on Cyclooxygenase

Some NSAIDs have worse side effects than others, although they have the same amount of anti-inflammatory action. This is due to the specificity each drug has towards each form of COX. Most NSAIDs inhibit COX-1 more than COX-2. The drugs with the greatest specificity to COX-1 also happen to be the drugs with the greatest side effects. For example, aspirin is about 160 times more specific to COX-1 than COX-2, and is also well known for its ulcerative potential.

In summary, NSAIDs block both forms of cyclooxygenase from converting arachidonic acid to prostaglandins. The exception is aspirin, which irreversibly acetylates cyclooxygenase (37). The benefit of an NSAID comes from its COX-2 blocking action, keeping COX-2 from forming prostaglandins involved in inflammation. The undesirable side effects of NSAIDs result from COX-1 blocking. Each NSAID affects COX-1 and COX-2 differently, and those that have the highest ratio of COX-1 to COX-2 specificity are also the drugs with the greatest number of side effects. The recent knowledge on cyclooxygenase specificity will lead to the development of new COX-2-specific NSAIDs.

The concept of the COX-2 to COX-1 ratio provides us with a mechanism to assess the balance of inhibition of the inducible COX-2. Analysis of these ratios and side effects of the older conventional non-steroidal anti-
inflammatories show that the lower the ratio, the lower the Cox-1 inhibition, and the lower the overall side effect profile.

Topical damage: Many NSAIDs are weak acids that are soluble at gastric pH and are lipid soluble (38). As undissociated, neutral compounds, they readily diffuse from stomach lumen to the mucosal cells, where the higher pH favors the dissociation of the H⁺ ion and trapping of the negatively charged acid moiety. The intracellular concentration of the ionized, acidic NSAIDs directly injures gastric mucosal cells, leading to back diffusion of H⁺ ion, a decreased transmucosal potential difference, and epithelial disruption (39). Other toxic mechanisms may include inhibition of mucosal prostaglandin secretion, reduction of mucus secretion, and interference with cell turnover. Though the topical effects can be largely prevented by administering enteric-coated NSAID formulations or pro-drugs, the failure of these agents to reduce the incidence of NSAID induced ulcers or complications imply that topical injury is not the most important component of NSAID induced injury. Indomethacin, the NSAID, enter the entero-hepatic circulation, resulting in the high intestinal concentrations of the active drug. This may contribute to the small intestine and colonic ulcerations, strictures and perforations.

Systemic effects: the clinically important effects of NSAIDs - the production of ulcers with an increased risk of significant complications - appear largely to be caused by their systemic actions. Inhibition of cyclooxygenase with a
resultant decrease in endogenous prostaglandins, especially \( \text{PGE_1}, \text{PGE_2} \) and \( \text{PGI_2} \), is thought to be the most important mechanism of action. NSAIDs inhibit plasma and mucosal prostaglandin production in humans. Mucosal prostaglandins play an important role in normal mucosal defense. Their actions are diverse and depend in part on the type of prostaglandin. The prostaglandin actions that are decreased by NSAID inhibition include normally increased mucus secretion, increased bicarbonate secretion, increased mucosal blood flow, inhibition of gastric acid secretion, cell proliferation and slowing of leukocyte rolling with adherence to endothelial linings. NSAIDs may thereby compromise mucosal mechanisms that prevent the development of gastro-duodenal ulceration. Platelet cyclooxygenase also is inhibited irreversibly by NSAIDs. The resulting decreased platelet aggregation and prolonged bleeding times may potentiate gastrointestinal bleeding from the upper and lower gastrointestinal tract. Hence a specific inhibitor of COX-2 should retain its anti-inflammatory properties and have reduced adverse GI and antiplatelet effects. This concept has led to the development of COX-2 specific NSAIDs. Even though all nonspecific NSAIDs inhibit both COX isoforms, their selectivity for COX-1 or COX-2 widely varies.
1.4.2 Increase in Cellular Cytokine levels

The healing response (i.e. curing of ulcer in the stomach wall) is largely coordinated by a variety of inflammatory mediators, cytokines and growth factors that are produced locally in the ulcerated portion. There has been accumulating evidence of the involvement of Prostaglandins, Cyclooxygenase-1&2, Nitric Oxide/ inducible Nitric Oxide Synthase, Interleukin-1, (interleukin-1β), Interleukin-8, Tumor Necrosis Factor–α(TNF-α). The cytokines are induced or increased by gastric ulceration and might contribute ulcer healing when decreased in amount. There is a crossover role of COX-2, NO / iNOS, cytokines with inflammatory process and ulcer healing along with NF-kB that up regulates the expression of healing promoting factors e.g. COX-2, iNOS and IL-1β (40,41,42).

References also show that the cytokines like IL-1β and TNF-α increases the neutrophil infiltration and thus, the oxidative burst occurs resulting in ulcer formation.

The inflammatory reaction requires de-novo synthesis of defined proteins, which include chemokines that serve to amplify and spread the primary pathogenic signals during the progression of peptic ulcer disease (43,44).

Tumour necrosis factor (TNF-α) is involved in non-steroidal anti-inflammatory drug induced gastropathy by modulating neutrophil infiltration. (45,46).
Nitric oxide (NO) is a mediator of gastrointestinal mucosal defense but, paradoxically, it also contributes to mucosal damage depending upon the concentration of NO (47).

It is seen that neuronal and endothelial nitric oxide synthase (NOS) isoforms produce low amounts of NO. In contrast, the inducible form of NOS (iNOS) produces NO in higher quantities (48). Piotrowsky et al showed that indomethacin induced gastric ulceration gives a 12-fold increase in gastric epithelial expression of iNOS activity compared with controls which is correlated with damage of epithelium (49). Whereas, Wallace and Miller showed that NO mediates a critical role in modulation of several components of mucosal defense, including increased gastric blood flow, reduced neutrophil adhesion, and increased mucus secretion (50).

Activated neutrophils play a critical role in indomethacin-induced gastric mucosal injury (51).

Interleukins are polypeptide molecules that have an important role in the immune system. These polypeptides are secreted by lymphocytes, spherical cells that mediate specificity of immune responses. Concentrating on interleukin 1, interleukin 2, and interleukin 3, each originate from different processes. Although as cytokines all are similar in function; they have different factors that affect their synthesis and production. Regulation and control of these three interleukins vary by the different producers, activators, and inhibitors or by which they are synthesized. Interleukin 1 and 2 are
similar in the way they are synthesized. Interleukin 3 has a simpler process of being produced. As important molecules that regulate other cells, it is important to understand the regulation of interleukins so as to create methods of controlling their production for medical purposes. For medical reasons, the control and regulation of interleukins can be beneficial for more specific applications of these molecules for diseases and for the immunological field since they play such a crucial part in the immune system.

Interleukine-1β also increases the inflammatory responses in indomethacin induced gastric ulcer (52).

**IL-8 production**

The gastric epithelial and vascular endothelial cells secrete chemokines, which have neutrophilic attractant properties in response to bacterial infection, such as IL-8. IL-8 shows potent chemotactic activity for neutrophils and induces expression of adhesion molecules, such as CD11b/CD18, and the production of reactive oxygen species. It is also known to be a chemotactic and an activating peptide for T lymphocytes. If defense mechanisms fail and chronic infection results, continued upregulation of IL-8 and activation of neutrophils and lymphocytes could lead to mucosal damage and increased free radical formation. In many human studies, increased IL-8 immunoreactivity and increased IL-8 mRNA expression in NSAID infected mucosa have been demonstrated (53,54).
In vitro studies have shown that the expression of IL-8 in gastric epithelial cells, such as MKN45 cells, MKN-28 cells, and AGS cells, is upregulated by inflammatory cytokines, such as tumor necrosis factor-α (TNF-α) and interleukin-1 (IL-1β), that are detected in the gastric mucosa with NSAID infection;

**INTRACELLULAR SIGNALING IN GASTRIC INFLAMMATION**

The intracellular mechanism for IL-8 production has recently been studied. The human IL-8 gene contains Nuclear translocation of NF-kB is followed by increased IL-8 messenger RNA and protein levels consistent with NF-kB upregulation of IL-8 gene transcription (55,56). Maeda et al. 2000, identified upstream mediators that regulate *H. pylori*-induced NF-kB-dependent IL-8 production. TNF-α-induced NF-kB activation is closely related to the resistance of rat gastric epithelial cells to TNF-α-mediated apoptosis [57]. This concept is supported by recent findings of Kanai et al. (58), who noted that transforming growth factor-α plays an antiapoptotic role in gastric mucosal cells.

Mucosa of the ulcer margin forms a characteristic "healing zone" (59,60,61,62). The epithelial cells lining glands of the ulcer margin undergo de-differentiation, express epidermal growth factor receptor (EGF-R) and actively proliferate (63). Proliferation is essential for ulcer healing because it supplies epithelial cells crucial for reepithelialization of the mucosal surface and reconstruction of gastric glands (64). 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 (65). Growth factors are the major stimuli for cell proliferation, division, migration and re-epithelialization (66,67,68). 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 Cox2, in a well synchronized spatial and temporal manner (69).

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

4. *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 (70) and that healing of the epithelial component of ulcers involves activation of the EGF-R - MAPK (Erk) signal transduction pathway (71,72). Non-steroidal anti-inflammatory drugs (NSAIDs) and *H. pylori* toxins interfere with the ulcer healing by inhibiting epithelial cell proliferation, migration and angiogenesis and by blocking growth factor-triggered signaling pathways (73,74).
1.4.3 Production of Reactive Oxygen Species (ROS)

Reactive oxygen species (ROS)

ROS can be defined literally as entities containing one or more oxygen atoms that meet the defining criteria for being chemically reactive. The defining criteria require identification of the molecular environment, but ROS generally is a more appropriate and useful term than oxy radicals, oxygen free radicals, and related terms, unless the more limited meaning of one of the later terms used is intended. ROS consist of radical and non-radical species. A radical is an atom or molecule that contains one or more unpaired electrons. A free radical is a radical that has moved out of the immediate molecular environment of its generation. Conversely, radicals that are retained within their sites of generation have been called “caged radicals” (75). In biological systems most of the free radicals are derived from oxygen. Another group of reactive species contain both oxygen and nitrogen and include physiologically important nitric oxide and toxic peroxynitrite. These
species are referred as reactive nitrogen species (RNS). Not all of these reactive species are radicals but in many cases the reactive non-radical species will end up as radicals, damaging biomolecules by one-electron oxidation. The danger of this type of reaction is that the oxidation products formed are radicals themselves, which are in many cases able to propagate the reaction, leading to extensive damages. Important ROS and RNS, their salient properties are listed in Table R2, and few of the ROS pertaining to this study are explained briefly below.

**Table R2**: Reactive oxygen and nitrogen species significant in human health.

<table>
<thead>
<tr>
<th>Name of the reactive species</th>
<th>Symbol</th>
<th>Half life at 37 °C</th>
<th>Remarks</th>
</tr>
</thead>
<tbody>
<tr>
<td>Superoxide</td>
<td>O₂⁻⁻</td>
<td>10⁴ sec</td>
<td>Not very reactive</td>
</tr>
<tr>
<td>Hydrogen peroxide</td>
<td>H₂O₂</td>
<td>Minutes</td>
<td>Not very reactive but yield potent species</td>
</tr>
<tr>
<td>Hydroxyl</td>
<td>'OH</td>
<td>10⁻⁹ sec</td>
<td>Very highly reactive</td>
</tr>
<tr>
<td>Hydroperoxyl</td>
<td>HO₂⁻⁻</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Alkoxyl</td>
<td>RO'</td>
<td>10⁻⁶ sec</td>
<td>Reactive</td>
</tr>
<tr>
<td>Peroxyl</td>
<td>ROO'</td>
<td>Sec</td>
<td>Reactive</td>
</tr>
<tr>
<td>Organic hydroperoxide</td>
<td>ROOH</td>
<td>Stable</td>
<td>Reacts with transient metal ions to yield reactive species</td>
</tr>
<tr>
<td>Singlet oxygen</td>
<td>O₂⁺</td>
<td>10⁻⁶ sec</td>
<td>Highly reactive</td>
</tr>
<tr>
<td>Ozone</td>
<td>O₃</td>
<td>Sec</td>
<td>Can react with biological molecules yielding O₂⁻⁻</td>
</tr>
<tr>
<td>Nitric oxide</td>
<td>NO'</td>
<td>Sec</td>
<td>Neurotransmitter and blood pressure regulator</td>
</tr>
<tr>
<td>Peroxynitrite</td>
<td>ONOO⁻⁻</td>
<td>10⁻⁶ sec</td>
<td>Highly reactive</td>
</tr>
<tr>
<td>Nitrogen dioxide</td>
<td>NO₂⁻⁻</td>
<td>Sec</td>
<td></td>
</tr>
<tr>
<td>Nitronium ion</td>
<td>NO₂⁺</td>
<td>Sec</td>
<td></td>
</tr>
</tbody>
</table>
Hydroxyl radicals: Hydroxyl radicals can be produced experimentally by various procedures, including exposure to radiation (76), or by decomposition of peroxynitrite (77). Because of its low half-life, the direct action of the hydroxyl radicals is confined to regions immediately in the vicinity of their formation. However, being the most aggressive member of the ROS family, it can bring about extensive damage to different types of molecules, including proteins, nucleic acids, and lipids. Hydroxyl radicals are extremely reactive oxidant, with a redox potential of roughly +1.35 V. Hydroxyl radicals reacts rapidly with DNA and can cause over 100 different types of DNA modifications (78). Additionally, hydroxyl radicals are known to activate certain oncogenes, such as K-ras, which may also play a role in its tumor-promoting activities (79). The action of hydroxyl radicals on proteins leads to extensive protein-protein cross-linking (80). The peroxidation of polyunsaturated fatty acids (PUFA) by hydroxyl radicals constitutes one of the most severe attacks on cellular integrity (81,82).

Superoxide radicals: The superoxide radicals are anionic radicals formed by the reduction of molecular oxygen through the acceptance of a single electron. Hydroperoxyl radicals, which are unstable at physiological pH, dissociate to superoxide radicals. The superoxide radicals do not cross cell membranes and, by itself, are not very reactive towards cell constituents. Although superoxide radicals are considerably less reactive than hydroxyl radicals, they are still quite capable of damaging DNA. Superoxide radicals
are fairly abundant and endogenous mitochondrial generation of gives them an intracellular concentration of about $1.0 \times 10^{11}$ M. Superoxide radicals appear to do most of the damages through the production of hydroxyl radicals, via the Haber-Weiss reaction (83).

*Hydrogen peroxide:* Hydrogen peroxide is produced by a variety of intracellular reactions, particularly oxidative electron transport in the mitochondria, and is normally present in cells at a concentration of roughly $1.0 \times 10^{38}$ M (84). Hydrogen peroxide appears to play a role in normal metabolism and is required for a number of cellular events such as thyroid hormone biosynthesis and the microbicidal activity of macrophages (85). By itself, it is relatively non-reactive toward DNA. Most of the hydrogen peroxide-mediated DNA damage is due to the production of hydroxyl radicals via events such as the Fenton reaction (86,87). The importance of hydrogen peroxide and superoxide radicals are realized by the fact that increased expression of both catalase and SOD, the enzymes responsible of detoxifying the designated ROS results in an increased life span in *Drosophila* species (88).

**Generation of ROS in living systems**

*Endogenous generation:* A number of intracellular sources of ROS have been identified (Table R2). The importance of each source in any specific order is unknown, and the relative role each plays in tissue injury seems certain to vary with the specific experimental conditions employed.
A. Phagocytes: Perhaps the best recognized biological sources of free radicals are phagocytic cells, e.g., neutrophils and monocytes. When activated to begin phagocytosis, these cells exhibit a marked increase in oxygen consumption. This "oxidative burst" of activated phagocytes was shown to involve the rapid reduction of oxygen to superoxide radicals (89). Subsequent work demonstrated that this reaction is catalyzed by a plasma membrane bound NADPH oxidase, with extracellular production of large amounts of ROS.

B. Mitochondrial electron transport system: The mitochondrial electron transport chain is a very efficient system ensuring complete oxidation of fuel molecules, but the very nature of the alternating one-electron oxidation-reduction reactions predisposes each electron carrier to side reactions with molecular oxygen (90). There exists a tendency for an electron to pass directly to oxygen (generating superoxide radicals) instead to the next electron carrier in the chain (91). Thus, it is commonly accepted that mitochondrial generation of superoxide radicals represents the major intracellular source of oxygen radicals under physiological conditions. About 1-2% of the total daily oxygen consumption goes to mitochondrial superoxide generation (92). The mitochondrial outer membrane enzyme monoamine oxidase catalyzes the oxidative deamination of biogenic amines, and is a large source of hydrogen peroxide that contributes to an increase in the steady state concentrations of reactive species within both the
mitochondrial matrix and cytosol (94).

c. **Soluble oxidase enzymes:** Xanthine oxidase, dopamine-β-hydroxylase, D-amino acid oxidase, urate oxidase, glucose oxidase, lipoxygenases, cyclooxygenases and fatty acyl CoA oxidase are some of the enzymes that can oxidize endogenous and exogenous substrates, and generate ROS.

D. **Autooxidation of substrates:** Autooxidation of some substrates like epinephrine and various chemicals inhaled/ingested generate ROS.

E. **Transition metals:** Endogenous redox active metal ions that form an integral part of normal function of life, notably iron and copper, can facilitate transfer of electrons to macromolecules like lipids, proteins and DNA. Metal ions also catalyze decomposition of existing organic hydroperoxides to generate reactive species.

**Exogenous generation:** Exogenous factors contributing to the generation of ROS are ionizing radiations, pollutants like industrial and cigarette smoke, certain drugs (doxorubicin, cyclophosphamide, 5-fluorouracil, methotrexate, and vincristine) and exposure to metal ions. A brief detail highlighting the importance of IR exposure and iron as sources of ROS generation is given below as these two are employed extensively in this study.

A. **Ionizing radiations:** Exposure of eukaryotic cells to ionizing radiation (IR) results in the immediate formation of free radicals that last a
matter of less than $10^{-14}$ sec (reviewed in Asmus, 1984). It has been assumed that the subsequent alterations in multiple intracellular processes following irradiation are due to the initial oxidative damage caused by these free radicals. It is clear that intracellular metabolic oxidation/reduction (redox) reactions are affected by this initial IR-induced free radical insult, and may remain perturbed for minutes, hours, or days and contribute to the activation of protective or damaging processes that affect the damaging effects of IR. It has long been recognized that the most critical target of IR passing through living tissues is the DNA (genetic material) that is present in the nucleus and mitochondria of most cells. IR damages DNA directly and/or indirectly. Since the volume of the DNA is very small compared with the total volume of the cell, the probability of direct damage occurring is relatively low. The indirect actions of radiation occur when it interacts with water molecules in the cell, resulting in the production of highly reactive free radicals, such as hydroxyl radicals, hydrogen atom and aqueous electron. The half-life of these free radicals is extremely short, in the order of $10^{-6}-10^{-10}$ seconds. However, they immediately react with any biomolecules in the vicinity and produce highly site-specific oxidative damage. Hydroxyl radicals cause an estimated 60%-70% of tissue damage induced by IR (77).

Among the bases in the DNA, guanine is the most susceptible target for oxidative reactions mediated by hydroxyl radicals and other free radicals. The resulting product, 8-hydroxy-2-deoxyguanosine (8-OHdG), is
an abundant and measurable lesion, and is reported to be a key biomarker of carcinogenesis (98,99). Free radicals also attack the sugar-phosphate backbone of DNA, resulting in the production of ssb and dsb, as well as DNA-DNA and DNA-protein cross-links (100,101). The single-strand breaks can be repaired quickly using the undamaged DNA strand as a template, whereas double-strand breaks are not easily repairable and are considered the primary lesion leading to radiation-induced mutagenesis, carcinogenesis, and cell death (102,103,104).

Besides DNA, radiation induced free radicals also interact with unsaturated sites in lipids, resulting in the production of hydroperoxides (105,106). The hydroperoxide residues change the hydrophobic interactions between adjacent chains of phospholipids, allowing easier penetration of water molecules, thereby altering the electric constant across the lipid bilayer, which in turn leads to changes in membrane permeability and lipid peroxidation (107,108). The basic mechanism of radiation induced lipid peroxidation, various factors, which determine the mode and magnitude of lipid peroxidation, and similarities between radiolytic and non-radiolytic lipid peroxidation were reviewed recently (109). Ionizing radiation also induces detectable changes in the structure and function of intrinsic proteins (110,111).

Radiation-induced damage may be repairable, but in some cases the repair is inaccurate (112,113,114,115), resulting in adverse health
effects within a short time of hours to weeks or delayed effects observable many months or years after exposure. Radiation-induced mutations in a germ cell can lead to heritable changes that may not be expressed for many generations. The manifestation of adverse health effects, of course, depends on the radiation dose, duration of exposure, differentiation and sensitivity of the tissues, and intrinsic antioxidant defense mechanism(s). Substantial evidence indicates that low doses of radiation, typically below 5 cGy, actually elicit adaptive responses and stimulate protective antioxidant defense processes (116,117,118,119).

B. Metal overload: It is widely accepted that major forms of oxidant induced damage involve catalytically active metals (120). Recent studies have shown that metals, including iron, copper, chromium, and vanadium undergo redox cycling, resulting in the production of ROS as superoxide radicals, hydrogen peroxide, and hydroxyl radicals. We restrict our discussions to iron due to its relevance in the current investigation. Iron overload and toxicity occurs due to failures in inherent defense systems or due to contaminated environments (121). Aust (122) has reviewed the relationship between iron, oxygen radicals, and tissue damage. The role of iron in the initiation of lipid peroxidation has also been reviewed (123,124). Production of ROS due to iron overload enhanced lipid peroxidation, DNA damage, and altered calcium and sulfhydryl homeostasis. Iron (and copper) play major role in generation of oxidants in a cell by catalyzing Fenton (Eq. 1)
and Haber-weiss reactions (Eq. 2) and arguably are kept under tight control in healthy personnel.

\[
\text{Fe(II) + H}_2\text{O}_2 \rightarrow ^*\text{OH} + \text{OH}^- + \text{Fe(III)} (\text{Eq. 1})
\]

\[
\text{O}_2^{-\ast} + \text{H}_2\text{O}_2 \rightarrow ^*\text{OH} + \text{OH}^- + \text{O}_2 \quad (\text{Eq. 2})
\]

The significance of such reactions in redox biology has been well appreciated (125,126,127,128). A historic account of all the pioneering works on transition metal involved biological reactions (109) and the rediscovery (129,130) of Haber-Weiss reaction have been reviewed (127). Cellular consequences of exposure to iron include enhanced radiosensitivity, mutation, chromosomal aberrations, oncogenesis, degenerative aging and death (131). A detailed account of mammalian defense systems, mechanisms of iron toxicity and diseases, and the therapies have been reviewed (132,133).

**Oxidative stress**

From the foregoing, the continuous generation of toxic ROS in cells is apparent. However, the excess ROS that is harmful to cells is kept under check by the cellular defense mechanisms. Firstly, intracellular antioxidants such as vitamin C, vitamin E and selenium quench ROS. Secondly, antioxidant enzymes such as glutathione-S-transferase catalyze the conjugation of ROS to glutathione. Other antioxidant enzymes catalase, superoxide dismutase and glutathione peroxidase act directly on certain ROS and neutralize them. This precarious balance is upset during several conditions that either led to overwhelming production of ROS or failure of
endogenous antioxidant mechanisms, although the former is observed to be more prevalent. ROS production and subsequent “oxidative stress” can lead to the damage of important biomolecules and contribute to the origin and pathogenesis of several diseases (discussed later in this chapter). The term, oxidative stress, coined by Prof. Sies, denotes an imbalance between the production of oxidants and the respective defense systems of an organism (134). Oxidants in a cell encompass mainly ROS. Oxidative stress was first demonstrated to damage living organisms in 1952 (135) when an increase in oxygen pressure was shown to cause chromosomal aberrations in pollen grains. Oxidative stress, due to an excess of ROS leads to oxidative damage to cellular constituents (Fig. R7).
Fig R7: Summary of the events in a cell that occur under oxidative stress conditions

- Inhibition of ATP synthesis
- NAD(H) depletion
- Rises in intracellular free iron
- Damage to DNA
- Damage to lipids
- Damage to proteins
- Inhibition of ATP synthesis
- Membrane blebbing
- Metal ion release into surrounding tissues, injury to adjacent cells
ROS mediated damages to biomolecules

DNA damage: ROS mediated reactions can cause structural alterations in DNA (nicking, base-pair mutations, rearrangements, deletions, insertions, and sequence amplification). The endogenous reactions that are likely to contribute to ongoing DNA damage are oxidation, methylation, depurination, and deamination (136,137). Methylation of cytosines in DNA is important for the regulation of gene expression, and normal methylation patterns can be altered during carcinogenesis (138). Conversion of guanine to 8-hydroxyguanine, a frequent result of ROS attack (139,140,141) has been found to alter the enzyme-catalyzed methylation of adjacent cytosines (128), thus providing a link between oxidative DNA damage and altered methylation patterns. The chemistry of DNA damage by several ROS has been well characterized in vitro (140,141,142,143), although specific information about the changes produced by peroxyl, alkoxy, ozone and several of the RNS is lacking. Different ROS affect DNA in different ways; for example, hydrogen peroxide does not react with DNA bases at all (129,130), whereas hydroxyl radicals generate a multiplicity of products from all four DNA bases, and this pattern seems to be a diagnostic "fingerprint" of hydroxyl attack (139). By contrast, singlet oxygen selectively attacks guanine (143,144). The most commonly produced base lesion, and the one most often measured as an index of oxidative DNA damage, is 8-hydroxyguanine. It is sometimes measured as the nucleoside, 8-OH-Gua (137,145). These assay
methods have been reviewed in detail (139,140,145,146).

Damage to DNA by ROS/RNS seems to occur naturally; low steady-state levels of base damage products have been detected in nuclear DNA from human cells and tissues (137,145,146,147,148). The pattern of damage to the purine and pyrimidine bases suggests that at least some of the damage occurs due to attack by hydroxyl radicals suggesting that hydroxyl radicals is formed in the nucleus in vitro (146). ROS/RNS can also damage mitochondrial DNA, and such damage has been suggested to be important in several human diseases, and in the aging process (149,150). Mitochondria are often said to be the most important intracellular source of ROS, but it is difficult to unambiguously confirm this postulate (134). However, it seems very likely that the mitochondrial electron transport chain generates ROS in vivo (151,152,153), and that mitochondrial DNA is damaged by them.

DNA damage can be repaired by the action of a series of enzymes (154). However, DNA from human cells and tissues contains low levels of DNA base damage products (137,146,155 156,157,158), suggesting that these enzymes do not achieve complete removal of modified bases, perhaps because they operate at close to maximum capacity in vivo. DNA damage by ROS/RNS can cause multiple lesions, including single and double strand breaks, apurinic/apyrimidinic sites and modified pyrimidines and purines. Repair of these lesions occurs primarily by base excision repair, although nucleotide excision repair may also be involved. A repair system
for the abasic apurinic/apyrimidinic sites produced by spontaneous depurination also exists.

Cellular system is flooded with oxidative agents, which can activate the neutrophils. In activated neutrophils, NADPH oxidase in cell membranes becomes activated, and an electron transfer takes place from NADPH in cells to oxygen inside and outside cells, and the oxygen molecules that receive an electron become superoxide radicals (O$_2^{•-}$), which is rapidly converted to hydrogen peroxides (H$_2$O$_2$) by spontaneous dismutation or enzymatic superoxide dismutase (SOD), and hydroxyl radicals (• OH), which are formed nonenzymatically in the presence of Fe$^{2+}$ as a secondary reaction. In neutrophils, myeloperoxidase also results in the formation of the potent oxidant hypochlo-rous acid (HOCl) from H$_2$O$_2$ in the presence of chloride ions.

**Lipid peroxidation:** Polyunsaturated fatty acids (PUFA) have a propensity to oxidize, resulting in the formation of alkanes, aldehydes, alcohols, and hydroperoxides among other products. This propensity arises from the fact that bis-allylic methylene hydrogens are more susceptible to hydrogen abstraction by the oxidizing radicals than are the methylene hydrogens from fully saturated lipids. Lipid peroxidation (LPO) reactions are generally free radical-driven chain reactions in which one radical can induce the oxidation of a comparatively large number of substrate molecules (159). Such a chain
reaction is initiated by the abstraction of a hydrogen atom from a methylene group (LH, the carbon framed by the double-bonds in a so-called bisallylic double-bond) of a PUFA residue. Monounsaturated and saturated fatty acids are much less reactive and do not usually participate in LPO. This initiation is usually performed by a radical (R*) of sufficient reactivity:

\[ \text{LH} + \text{R}^* \rightarrow \text{L}^* + \text{RH} \quad \text{(Eq. 3)} \]

The rapid reaction of the resonance forms with oxygen 'fixes' the double bonds in a conjugated arrangement to form peroxyl radicals at positions +2 and -2 with respect to the carbon atom from which the original abstraction occurred (Scheme R1). For example, oxidation of linolenic acid by hydrogen abstraction at carbon-11 can result in peroxyl radicals at both the 9- and 13-position. Molecular oxygen rapidly adds to the carbon centered radical (L*) formed in this process, yielding lipid peroxyl radical (LOO*):

\[ \text{L}^* + \text{O}_2 \rightarrow \text{LOO}^* \quad \text{(Eq. 4)} \]

The lipid peroxyl radical is the central species of the lipid peroxidation chain reaction. This radical can abstract bis-allylic hydrogen from an adjacent fatty acid to form a lipid hydroperoxide, and a second lipid radical (Eq. 5), which subsequently reacts with oxygen to regenerate a peroxyl radical (Eq. 6).

\[ \text{LOO}^* + \text{LH} \rightarrow \text{LOOH} + \text{L}^* \quad \text{(Eq. 5)} \]
\[ \text{L}^* + \text{O}_2 \rightarrow \text{LOO}^* \quad \text{(Eq. 6)} \]

These reactions are the chain-propagation steps of lipid
peroxidation. Eq. 5 is rate limiting and so the rate of the propagation reaction is proportional to the concentration of lipid peroxyl radicals. Consequently any reaction that alters the concentration of peroxyl radicals will affect the rate of lipid peroxidation. The concentration of lipid peroxyl radical can be altered by a number of factors.

**Fig R8: Generation of lipid peroxy radical in PUFA.**

In the presence of transition metal ions, LOOH can give rise to the generation of radicals capable of reinitiating LPO by redox cycling of these metal ions. These metal ions have redox transitions with potentials of a magnitude that
allows the catalytic decomposition of hydroperoxides. The redox couples of importance to biological systems are Cu(I)/Cu(II) and Fe(II)/Fe(III). The one electron redox cycle results in the formation of peroxyl and alkoxy radicals (160), the latter rearrange and react with oxygen to form peroxyl radicals (161). By this mechanism, transition metal ions increase the concentration of peroxyl radicals and accelerate lipid peroxidation. As lipid peroxidation generates LOOH, the effect of transition metal ions is autocatalytic. This behavior is also observed during copper catalyzed oxidation of low-density lipoprotein (162,163).

\[
\text{LOOH} + \text{M}^{n+} \rightarrow \text{LO}^* + \text{Me}^{(n-1)+}, \quad \text{(Eq. 7)}
\]

\[
\text{LOOH} + \text{M}^{(n-1)+} \rightarrow \text{LOO}^* + \text{M}^{n+} \quad \text{(Eq. 8)}
\]

Where M represents the metal ion in question.

LOOH in the presence or absence of catalytic metal ions also give rise to a large variety of products, including short and long chain aldehydes, and phospholipid and cholesterol ester core aldehydes, many of which can be used to assess the degree of LPO in a system (164). In the absence of any additional reactions, the LPO chain reaction will terminate when two lipid radicals react to form non-radical products. These reactions decrease the level of peroxyl radicals and slow the rate of lipid oxidation. The mechanism and products of such reactions are complex and only partially understood. It has been reported that endogenous chemiluminescence is associated with such reactions, perhaps indicating the
formation of singlet state oxygen (165).

As demonstrated in Eq. 5, lipid peroxyl radicals propagate the chain reaction of LPO by abstracting a hydrogen atom from an unsaturated fatty acid. It follows that any compound that can donate a hydrogen atom to the peroxyl radical should be able to break, or at least divert, the chain of reactions. Compounds that donate hydrogen to leave a relatively inert radical product are referred to as chain-breaking antioxidants. The most well-known and well-studied chain-breaking antioxidants are the phenolic compounds, such as the natural tocopherols (166).

The endotoxin LPS is known to induce septic shock by free radical damage. It enhances the formation of ROS and lipid peroxidation products, such as superoxide anions and peroxides as well as their secondary product MDA. The primary form of the free oxygen radical is superoxide, which is formed through the activity of NADPH oxidase or Xanthine oxidase and is a source for another active form H₂O₂. The combination of these two active forms form a more active form of free radical, the hydroxyl radical.

Lipid peroxidation mediated by oxygen free radicals is believed to be an important cause of cell membrane destruction and cell damage. Cell membranes, which contain phospholipids that are rich in polyunsaturated fatty acids (PUFAs), are readily attacked by reactive oxygen species, producing fatty acid radicals and lipid hydroperoxides (169,170).
The most obvious consequence of membrane lipid peroxidation is the perturbation of various cellular and organellar membrane functions, including transport processes, maintenance of ion and metabolite gradients, receptor-mediated signal transduction, etc. Lipid peroxides are unstable compounds that tend to degrade rapidly to a variety of products, such as short-chain alkanes and aldehydes. Accumulation of lipid per-oxidation products in the NSAID-infected gastric mucosa provides evidence of increased oxidative stress. Although the thiobarbituric acid (TBA) test is not specific for lipid peroxides, it is one of the oldest and most frequently used methods for measuring the peroxidation of fatty acid, membranes, and food products. It is the easiest method to use, and it can be applied to crude biological samples. It has been shown that the levels of TBA-reactive substances are higher in patients (171). TBA-reactive substances are also correlated with chemi-luminescence levels (172), an index of ROS production, and with myeloperoxidase activity, an index of neutrophil accumulation (173). These data suggest that NSAID is likely to be a cause of increased ROS generation and damage rather than merely associated with these events, and also that the correlation between myeloperoxidase activity and the levels of TBA-reactive substances is further evidence that TBA-reactive substances reflect ROS-mediated lipid peroxidation.
**Protein oxidation:** Among the various types of macromolecular oxidative damages that occur during oxidative stress, oxidative modifications of intracellular proteins have been suggested to play a key role in the origin of senescence-associated losses in physiological functions because oxidized proteins often lose catalytic function and undergo selective degradation (174, 175). Oxidative damage to a specific protein, especially at the active site, can induce a progressive loss of a particular biochemical function. Pioneering studies have documented the relevance of protein oxidative damage in the aging process and in the etiology of certain pathological conditions (176). Several types of ROS-induced protein modifications have been demonstrated (174, 177), including the loss of sulfhydryl (–SH) groups, formation of carbonyls, disulfide crosslinks, methionine sulfoxide, dityrosine crosslinks, nitrotyrosine, glyoxidation and lipid peroxidation adducts, among others. Loss of protein –SH groups can be induced by a wide array of ROS and is one of the most immediate responses to an elevation in the level of oxidative stress. Functional consequences of –SH loss include protein misfolding, catalytic inactivation, decreased antioxidative capacity, and loss of certain specific functions, such as binding of heavy metals and sulfur-containing amino acids by albumin, among others (177). Age-associated losses in protein –SH content have been reported in a variety of tissues and species, including homogenates of brain, heart, skeletal muscle, and kidney of rodents and houseflies (178, 179). Caloric restriction attenuates –SH loss,
whereas hyperoxia has an opposite effect (179,180). Another oxidative modification in proteins is the formation of dityrosine crosslinks, which apparently arises following reaction between two tyrosyl radicals, generated by peroxidases and other heme proteins. Dityrosine cross-linking of proteins has been found to increase with age in mouse skeletal muscle and heart, but not in the brain or liver; caloric restriction attenuates these increases (181). There is a large body of evidence implicating oxidative damage to proteins in the pathogenesis of both normal aging and neurodegenerative illnesses. Oxidative damage is selective in inactivating particular proteins preferentially. This is true of both protein carbonyls and protein nitration. This leads to inactivation of enzymatic activity and kinase signaling pathways.

It is by and large now clear that oxidative stress occurs due to inadvertent ROS generation and depletion of cellular antioxidant levels. This leads to various pathogenic conditions that can be reversed or prevented by external supplementation of antioxidants. A broader definition of an antioxidant is any substance that when present at low concentrations compared with those of an oxidizable substrate significantly delays or prevents oxidation of that substrate. There have been several classifications of antioxidant molecules based on solubility (water/lipid soluble), chemical nature (vitamins, trace elements, proteins, polyphenols, polysaccharide), source (endogenous, exogenous/dietary), mechanism/mode of action (enzymatic, non-enzymatic;
direct, indirect; sacrificial, interceptive; metal ion chelator, ROS scavenger) and molecular weight (low, high molecular weight). Hence a comprehensive classification of antioxidants is difficult. A general view of antioxidants present in a cell is provided in Fig R2. Although have been reported from several sources, plants remain the single major contributors of antioxidants. Functionally antioxidants could act by one or more of the following mechanisms:

A. Prevention of generation of ROS
B. Scavenging of ROS thereby averting oxidation of biological targets.
C. Chemical repair of oxidized targets
D. Up regulation of endogenous defense and repair mechanisms

Fig R9: Overview of antioxidant defense system.
Following are some of the important features about antioxidants.

**Nutraceutical antioxidants and health:** In recent years, many studies have shown that diets containing high content of phytochemicals can provide protection against various diseases. Approximately 90% of all cancer cases correlate with environmental factors, including one's dietary habits, and one-third of all cancer deaths are avoidable by changing dietary habits only (182,183). These discoveries have rapidly amplified the consumer awareness of the potential benefits of naturally occurring compounds from plants in health promotion and maintenance, and researches in nutraceuticals and functional foods and natural health products have become the hot topics in recent years (184,185,186). The term "nutraceutical" was coined from "nutrition" and "pharmaceutical" in 1989 by Stephen DeFelice. According to him, nutraceutical can be defined as, "a food (or part of a food) that provides medical or health benefits, including the prevention and/or treatment of a disease" (187). The protective effects of fruits, vegetables and spices and herbs were found not only for cancer (188,189,190,191), but also other chronic diseases such as cardiovascular diseases (192,193,194,195).

Antioxidants such as vitamins C and E are essential for the protection against ROS. However, the majority of the antioxidant activity of botanical sources may be from compounds such as phenolic acids and flavonoids, rather than from vitamin C, E or β-carotene (196,197). Intake of
controlled diets rich in fruits and vegetables increases the antioxidant
capacity of plasma significantly. This increase could not be explained by the
increase in the plasma α-tocopherol or carotenoid concentration (198).

Antioxidant phytochemicals are, therefore, the focus of many recent studies.
The antioxidant activity of these compounds is predominantly determined
by their structures, in particular the electron delocalization over an aromatic
nucleus for the phenolics. When these compounds react with free radicals, it
is the delocalization of the gained electron over the antioxidant, and the
stabilization by the resonance effect of the aromatic nucleus, that prevents
the continuation of the free radical chain reaction. This is often called radical
scavenging. In addition, antioxidant phytochemicals also inhibit oxidation
through a variety of mechanisms (199,200,201,202). Synthetic antioxidants
such as butylated hydroxyanisole (BHA) and butylated hydroxytoluene
(BHT) only tend to have one mode of action, i.e. via free radical scavenging,
and are not able to sequester metal ions (203).

Polyphenolics, the major class of antioxidant phytochemicals is a highly
inclusive term that covers many different subgroups of phenolic acids and
flavonoids. More than 5000 polyphenolics, including over 2000 flavonoids
have been identified, and the number is still growing (204). Polyphenolics
vary in structures: hydroxybenzoic acids and hydroxycinnamic acids have a
single-ring structure, while flavonoids can be further classified into
anthocyanins, flavan-3-ols, flavones, flavanones and flavonols. Some of the
flavonoids such as flavan-3-ols can be found as dimers, trimers and even polymers. Many of the phenolics are often associated with sugar moieties that further complicate the phenolic profiles of plants (205). Polyphenols are antioxidants, because of their high redox potentials, which allow them to act as reducing agents, hydrogen donors and singlet oxygen quenchers (206). In addition, many of them show metal chelating potential (208). The antioxidant activity of the dietary polyphenolics is considered to be much greater than that of the essential vitamins, therefore contributing significantly to the health benefits of vegetarian diets.

Prooxidant properties of antioxidants: In some cases dietary antioxidants have been shown to act also as prooxidants in systems containing redox-active metals. In the presence of oxygen, transition metals such as copper and iron catalyze the redox cycling of phenolics, leading to the formation of ROS and phenoxy radicals that can damage DNA, lipids, and other biological molecules (209,210,211). The beneficial effects of antioxidants in cancer therapy have often been linked to their reducing capacities and ROS-scavenging capabilities. Interestingly, a recent review suggests that antioxidants may exert modulatory actions in cells through actions at protein kinase and lipid kinase signaling pathways (212). The antioxidant concentrations achieved in vivo can mediate receptor or enzyme activity. By inhibiting or stimulating various signaling pathways (e.g., tyrosine kinases, protein kinase C, and mitogen-activated protein kinase), antioxidants could
affect cellular function. The phenoxyl or semiquinone radicals derived from antioxidants also inhibit cell proliferation signaling.

The chemopreventive properties of antioxidants are generally believed to reflect their ability to scavenge endogenous ROS. However, the prooxidant action of plant-derived phenolics rather than their antioxidant action may be an important mechanism for their anticancer and apoptosis-inducing properties, as ROS can mediate apoptotic DNA fragmentation (213,214). The antioxidant properties of dietary phenolics may only partly explain their antitumor promotion effects. For example ellagic acid is ten times more potent than tannic acid an antioxidant(215).

1.5 Treatment of Gastric Ulcer

1) Stomach Self-Protection - Mucosal protection is the maintenance of the integrity of the gastrointestinal mucosa in the presence of endogenous aggressive factors, such as gastric acid, and exogenous aggressive factors, such as non-steroidal anti-inflammatory drugs. Components of mucosal defense are: Components of the mucosal barrier, Mucus gel formation and secretion, Bicarbonate secretion, Mucosal blood flow, Restricted routes of hydrogen ion permeation, Epithelial regeneration.

Mucus provides a protective barrier, shielding the mucosa from Hydrogen ions as well as abrasion. Bicarbonate ions serve to neutralise gastric acid. Mucosal blood flow provides the mucosa with energy in the form of
nutrients and removes excess Hydrogen ions. Finally, mucosal protection depends on rapid regeneration of epithelial tissue.

Certain Prostaglandins, particularly those of the E series, have a number of actions that protect the gastro-duodenal mucosa against numerous aggressive factors, thereby maintain mucosal integrity.

❖ The first line of defense is a layer of mucous, which contains bicarbonate to neutralize stomach acid.

❖ Next, cell membranes on the stomach wall contain lipids, which repel water-soluble ions such as hydrogen.

❖ Finally, any ions that do penetrate the surface layer are removed by the underlying blood flow. The blood flow is also essential for developing the cap of mucus, and for maintaining the continually shed alkaline layer of fibrin and cellular debris beneath the mucous.

Fig R10: Self- defense of Gastric mucosa

In the absence of mucous, acid attacks the first layer of stomach tissue. Then the inner lining is destroyed faster and an ulcer develops. With enough
acidity and continued absence of mucous, the ulcer grows deep enough to reach the underlying blood flow. The stomach wall gets perforated, allowing stomach acid and pepsin directly act on the tissue underneath. If the acid reaches an artery, death may occur.

In terms of the molecular mechanism, the ROS amount in the gastric mucosa gets reduced, reducing the level of cellular cytokines like IL-1β and TNF-α and increasing the protective IL-8 and Smad-4, as well as it enhances the activity of the Epidermal Growth Factor (EGF), which is known to heal the tissue damage.

1.5.1 Available Synthetic Drugs

Treatment:

- **H2-antagonists** - The H2-antagonists inhibit acid secretion by blocking H2-receptors on the gastric parietal cell.

- **Prostaglandin analogue** - Misoprostol (Cytotec) is a synthetic prostaglandin E1 analogue that enhances GI mucosal protection. It inhibits gastric acid secretion, stimulates mucus and bicarbonate secretion from the GI mucosa and increases mucosal blood flow.

Misoprostol is approved for use in the treatment and prevention of NSAID-induced gastric and duodenal ulceration. Misoprostol differs structurally from naturally occurring PGE1, allowing it to become metabolized. When metabolized it acts systemically to stimulate mucous production. To a lesser extent, misoprostol acts locally on the stomach wall. (216)
Misoprostol has not been shown to aid in the healing of existing NSAID-induced ulcers, but it does prevent them.

**Fig R11: Misoprostol Chemical Structure**

![Misoprostol Chemical Structure](image)

*Fig R11: Misoprostol Chemical Structure*

**Misoprostol** Consists of approximately equal amounts of two diastereomers of prostaglandin El analogue. Formula: C22H38O5 Molecular wt.: 382.5

Misoprostol is more likely to cause side effects such as diarrhea, abdominal pain and bloating. These side effects normally disappear within one or two weeks. Studies have shown that misoprostol reduces the incidence of gastric ulcers from 20% to 2% in persons taking NSAIDs for one year. It may be that the 2% of people who still develop a gastric ulcer while taking an NSAID and misoprostol are infected with H. pylori, and would benefit from antibiotics. Since misoprostol is a synthetic prostaglandin of type El, it can be expected to have an effect in other areas of the body where regulatory prostaglandins are created. In addition to the stomach, two areas where misoprostol has an effect are the kidneys and uterus. Normal prostaglandins in the kidneys are released to compensate for renal vasoconstriction. The
prostaglandins PGE2 and PGI2 stimulate vasodilatation (217). Use of NSAIDs reduces these prostaglandins by blocking constitutive cyclooxygenase. It follows that the addition of a synthetic prostaglandin such as misoprostol may help protect against renal impairment in chronic NSAID users (218, 219, 220).

Natural prostaglandins also induce uterine contractions during labor. It is for this reason that misoprostol has found a novel application in the area of chemical abortion. Package warnings are very clear that misoprostol is not to be taken by pregnant women. Uptake of Misoprostol in woman's pregnancy may continue with a greatly increased risk of birth defects (221).

Misoprostol is not effective at treating dyspepsia associated with NSAIDs compared to proton-pump inhibitors (PPIs) and H2-antagonists, which are proven treatments for dyspepsia.

- **Proton pump inhibitors**- PPIs decrease gastric acid secretion through inhibition of H+, K+ and ATPase, the proton-pump of the parietal cell, and are the most effective inhibitors of gastric acid secretion available. Using once-daily dosing regimens, healing rates are 80-100 per cent for duodenal ulcer and 70-85 per cent for gastric ulcer. They are safe and well tolerated, with headache and diarrhoea the most frequently reported side-effects at a rate that does not differ significantly from placebo.

- **COX-2 specific inhibitors**- There have been two large, prospective trials of the COX-
inhibitors celecoxib (Celebrex, the CLASS study) and the now-withdrawn rofecoxib (the VIGOR study) in patients with osteoarthritis and rheumatoid arthritis. They suggest that COX-2 inhibitors are associated with a lower risk of both symptomatic and complicated (upper GI bleeding, perforation or obstruction) gastric and duodenal ulceration compared to nonselective NSAIDs.

However, although data are limited, it appears that low-dose aspirin may reduce or eliminate any GI protective benefit of the COX-2 inhibitors. Furthermore, although COX-2 inhibitors appear to be associated with a lower incidence of GI ulcers compared to nonselective NSAIDs, there may be an increased incidence in the rate of myocardial infarction. This is because all nonselective NSAIDs, but not COX-2 inhibitors, inhibit the platelet production of thromboxane.

Thus, in older patients, the GI protective effects of COX-2 inhibitors may be outweighed by the cardiac benefits of nonselective NSAIDs.

**Future directions**

Increasing use of NSAIDs and with many upcoming uses like prevention of malignancies, stroke, pre-eclampsia, Alzheimer’s disease, and many other illnesses, it is imperative that these drugs are made safer and more tolerable. Development of COX-2 selective inhibitors is an important therapeutic advance in this regard, but they too are not entirely free from the problem of
GI ulceration. Preventive strategies like use of PPIs or misoprostol are a welcome move, but it mandates the use of a 2nd drug. Moreover, PPIs can reduce NSAID absorption from GI tract; they can reduce the gastric acid output to almost nil. Therefore, newer ways to modify the drugs have been developed.

✓ **Nitro-aspirins (NO-aspirins)**

Nitric oxide shares most of the muco-protective properties of prostaglandins. Therefore, several NSAIDs like flurbiprofen, naproxan, and diclofenac have been combined with nitric oxide moieties like glycercyltrinitrate or s-nitroglutathione. In experimental models, these have shown markedly reduced gastrotoxicity (222).

It has been found that glycercyl trinitrate, a supplier of nitric oxide (NO), speeds the healing of ulcers caused by acetic acid in animals (223, 224). It is thought that NO helps heal ulcers by increasing blood flow in the stomach wall. NSAIDs are being modified to release nitric oxide, and in animal models, drugs such as nitronaproxen (NO + naproxen) and nitrofenac (NO + diclofenac) have aided healing of existing ulcers. It appears that despite suppressing cyclooxygenase-1 activity, NO-releasing NSAIDs are capable of accelerating gastric tissue repair (225, 226, 227, 228).

✓ **Zwitterionic phospholipids**
The rationale of combining NSAIDs with phospholipids is that the combination prevents the interaction of hydrophobic portion of cells to the drugs. This can help in reducing damage. One such combination of acetylsalicylic acid and dipalmitophosphatidylcholine retains analgesic and anti-inflammatory effects while exhibiting more antipyretic effects. Its tendency to cause GI damage is substantially reduced(229).

✓ Chiral NSAIDs

Attempts are being made to purify some of the commonly used drugs such as ibuprofen that exist as recemic mixtures. This is done following realization that GI damage is caused by one of the isoforms, while the other one is safer25. Experiments in mice show that the S isoform leads to usual mucosal damage, while R has substantially less propensity to do so.

✓ Trefoil peptides

These are a family of cysteine-containing protective peptides normally secreted in GI tract. Oral administration of these peptides has been shown to abrogate the GI damage produced by indomethacin(230).

✓ A newer non-steroidal drug with novel mechanism of action: locofelone

Locofelone is a dual action, competitive, COX-2 cyclooxygenase and 5-lipoxygenase blocker. The rationale of its development represents the simple application of pharmacological principles in therapeutics. It is well known that cyclooxygenase enzyme has two isoforms upon which NSAIDs
act, i.e., COX-1 and COX-2. Conventional NSAIDs and COX-2 selective inhibitors act mainly on COX-2. Blockade of COX-2 enzyme by COX-2 inhibitors leaves the COX-1 isoform unchecked and this contributes to enhanced thrombogenicity. Blockade of both COX-1 and COX-2 leads to increased formation of products of 5-lipoxygenase pathways and hence gastric damage. Locofelone acts to inhibit both isoforms (COX-1, COX-2) and 5-lipoxygenase. Its improved safety profile compared to other NSAIDs is believed to be due to its unique mechanism of action(231).

1.6 Introduction of Natural Products as Therapeutic Agents

Need for a Novel Drug: Due to all these side effects, the need to formulate a drug with a better prospect was needed. According to our ancient literature, there are a wide number of plants having anti-ulcerogenic and antioxidant properties. Since, natural products are mostly devoid of side effects, they can prove themselves to be an efficient drug with low cost, safely and efficacy.

1.7 Rationale of the Current Work

Several plant parts are known to have very good effect on human body. The importance of usage of ethnomedicines is increasing nowadays as they have less or no side effects, low cost and are, often easily accessible to the common people. Many naturally occurring agents have shown chemopreventive (antioxidant) and chemotherapeutic (anticancer) potential in a variety of bioassay systems and animal models. Epidemiological studies have reported
decreased incidence of several pathogenic diseases in populations consuming diets rich in antioxidants. In view of these there is a growing interest on antioxidants derived from medicinal and edible plants/herbs, as these can be used in preventive medicine. An effective and acceptable chemopreventive or chemotherapeutic agent should have certain properties: (i) no toxic effects in normal and healthy cells, (ii) high efficacy against multiple cancers, (iii) capability of oral consumption, (iv) known mechanism of action, (v) low cost, and (vi) acceptance by the human population. Given the importance of our traditional remedies towards various diseases it is imperative to explore the great Indian biodiversity for novel nutraceuticals. Almost half of the pharmaceuticals are originated from plant products. Some of them are leaves of *Piper betel*, rhizhome of *Zingiber officinalis*, fruits of *Emblica officinalis*, *Terminalia chebula* and *Terminalia belerica*.

*Piper betel*: This plant belongs to the family Peperaceae and is known by the common names betel and pan. The *Piper betel* plant is widely growing in the tropical humid climate of South East Asia and its leaves, with a strong pungent and aromatic flavor are widely consumed as a mouth freshener. The leaves are credited with wound healing, digestive and pancreatic lipase stimulant activities in the traditional medicine, which has also been proved with experimental animals. In fact, mention of this plant against various diseases can be traced in the ancient Vedic literature. Earlier, our
collaborators have reported gastrocytoprotective properties of the leaf extract also exhibit on experimentally induced gastric lesions and rationalized the activity in terms of its antioxidant property (232). In addition, its antimicrobial (233), antifungal, anti-inflammatory (234) activities are also reported.

*Terminalia chebula:* This plant belongs to the family Combretaceae and is known by the common names Indian gall nut and Chebulic myrobalan. In ayurveda it is known as haritaki, harar, hardh and har and the fruits and bark are reported to possess various medicinal properties. This is one of the herbs in ayurvedic combination of three herbs called "Triphala". The plant is known to possess anticancer (235), antimitogenic (236), antibacterial (237) and wound healing (238) properties. Very recently the aqueous extract from this plant was reported to possess antioxidative and radioprotective properties (239,240).

*Terminalia bellerica:* This plant belongs to the family Combretaceae and is known by the common names beleric myrobalan, bibhitaki, bhaira, bahira, bilhitak, bahera, vibhidhaka. The fruit is used in the treatment of piles, dropsy, leprosy, diarrhoea, biliousness, dyslepsia and headache (241). This plant is known to possess anti-HIV, anti-malarial and anti-fungal compounds (242). Antimutagenic effects of molecules isolated from the fruits of this plant have been reported (243,244). Although not many reports are present on the beneficial effects of individual principles from this
plant, it has been used in conjunction with other ayurvedic plants. Beneficial effects of such combinations have been reported previously (245,246). Crude extracts and isolated compounds from a related plant *Terminalia arjuna* has been reported for antimutagenic (247) and antigenotoxic (248,249) potentials. *Emblica officinalis*: This plant belongs to the family Euphorbiaceae and is known by the common name Indian gooseberry. In ayurveda it is referred to as amla and amlaki. This plant has been reported to possess anticancer (250), antiulcer (251), anti-diabetic (252) and hepatoprotective (253) properties. Recently antioxidant properties of this plant have been reported (254). Earlier, we also reported gastrocytoprotective properties of the fruit extract on experimentally induced gastric lesions and rationalized the activity in terms of its antioxidant property.(255,256)

To this end we also comprehended an urgent need to discover novel prophylactic/therapeutic molecules and undertook this investigation with the following objective:

1. To evaluate the antioxidant potential of plants of medicinal importance. The plants chosen for the study were *Piper betle*, *Terminalia bellerica*, *Terminalia chebula*, *Emblica officinalis*. Different extracts of these plants were evaluated for their ROS scavenging properties and evaluated to protect biological targets against oxidative damages induced by iron and IR.

2. To evaluate their ulcer healing property and their effect on modulation of biochemical and immunological property.
Overall, the studies were aimed at screening various natural compounds/extracts as some suitable synthetic congeners for the target bioactivities using a series of in vitro assays. Based on the in vitro results, some of the promising test samples were subsequently used for studies using suitable in vivo models. These led to several new findings as briefed earlier. While the novel findings with some of the plant extracts could be correlated with their chemical compositions, new lead compounds with cytotoxicity or iron-chelating properties are some of the important contributions in the field of antioxidants.

For the present work these plant-parts were extracted in a suitable solvent. The antioxidant properties of these extracts were screened, the best among them were screened for the anti-ulcerogentic and radio-protective properties. The immunological potential of the better ones were seen. Throughout, attempts have been made to rationalize the activities in terms of the chemical structures of the test compounds. Hence, the best were taken for the isolation of active components. The crude extract was passed through a column chromatography to get the different fractions. With the obtained fractions again a screening was done for antioxidation capacity. Based on the results, the active-most fractions were chosen and anti-ulcer as well as immunological parameters were seen with them. The most active fractions were purified by gel filtration and preparative TLC and were taken for spectrosocopical analyses of their chemical structures.