This thesis deals with the investigations carried out by the author in the laboratory on the scientific validation of two Indian medicinal plants as potential antioxidant and anti ulcer agents.

1.1 TRADITIONAL SYSTEM OF MEDICINE

Herbal medicine have long been used therapeutically for a large number of human disorders and are still being used for many of the health needs of a large population of the world. The WHO has acknowledged the current usage of traditional and herbal medicine, which has been in existence for more than a hundred years, much before the development of modern medicine[1].

1.1.1 Current Scenario of Herbal Medicine World Wide

Natural products including herbal extractives are been used as prescription drugs in countries like Germany and France. Sales of herbal based products has steeply increased in the European Union with a steady growth in the years reaching to more than 20 billion from about 6 billion in early 1990s. Herbal drugs, which are sold through health food outlets in USA, have had a substantial growth in the last decade. European Union and USA markets are the export markets serviced by the $1 billion herbal drug business in India. Plant based crude drug export from India and other Asian countries have grown exponentially during the last decade[1].
1.1.2 Indian Scenario

The total number of plant species of all groups recorded from India is 45,000. The plant kingdom is by far the most efficient source of novel compounds to combat against various diseases, but many of them are still unexplored or confined in the various ethnic communities and remains to be validated scientifically. The vital ethnomedicinal leads and the available medicinal plant resources offer scope for developing latest therapeutic molecules[2].

1.2 PEPTIC ULCERS

1.2.1 Definition

Peptic ulcers are chronic and in most cases solitary lesions that develop in any part of the gastrointestinal tract which have been exposed to the aggressive action of acid-peptic juices[3]. The lesser curvature of the stomach and the first part of the duodenum are the most common sites of peptic ulcers and are called gastric ulcers and duodenal ulcers respectively.

1.2.2 Epidemiology

Peptic ulcer is one of the common diseases in human population. Due to rapid development and civilizational constraints including a stressed lifestyle, the incidences of peptic ulcer are increasing. The estimates of incidence of peptic ulcer vary ranging between 3–10%. An estimated 15,000 deaths occur each year due to Peptic ulcer disease
(PUD). Approximately four million people suffer from peptic ulcers in the United States and 3.6 lakh new cases are reported each year. About 1 lakh patients yearly are treated in hospitals and about 3000 people die each year due to PUD. The male to female ratio for duodenal ulcer is three to one and gastric ulcer about 1.5 to 2.1\cite{4}.

### 1.2.3 Etiology

Imbalances in mucosal defensive and offensive factors are the main causes of gastric ulcers. Treatment of gastric ulcers is usually focused on mitigating the aggressive effects of acid secretion. But currently a class of cytoprotective agents, which act by protecting the gastric mucosa from agents without effecting secretion of acid are of interest today\cite{5}.

In gastric or duodenal peptic ulcers, the mucosa has been attacked by digestive juices to such an extent that the subjacent connective tissue layer (submucosa) gets exposed. When the equilibrium between the corrosive hydrochloric acid and acid-neutralizing mucous, which forms a protective layer on the mucosal surface, is shifted in favor of hydrochloric acid, self digestion occurs.

Various hypotheses have been proposed to understand the biochemical changes taking place during ulcer generation. Increased gastric motility\cite{6}, vagal hyperactivity\cite{7}, mast cell degranulation\cite{8}, reduced flow of blood to the gastric mucosa\cite{9} and decreased prostaglandin levels during conditions involving stress are involved in
generation of gastric ulcers. Reactive oxygen species plays a role in experimental gastric damage induced by ischemia and reperfusion\textsuperscript{[10]}, hemorrhagic shock\textsuperscript{[11]} and ethanol administration\textsuperscript{[12]}. \textit{Helicobacter pylori}, a pathogen is known to be the most common cause of gastric ulcer in humans.

\subsection*{1.2.4 Gastric Cytoprotection}

‘Cytoprotection’ is a term introduced by Andre Robert in 1979 and refers to protection by prostaglandins against experimentally induced acute gastric lesions without affecting gastric secretion in rats. This term is now used in a broader sense to mean protection against gastric mucosal injury by a mechanism other than inhibition or neutralization of gastric acid.

\textbf{Mechanism of Cytoprotection}

The exact mechanism of action of cytoprotective agents has not been clearly established but various mechanisms have been suggested as follows\textsuperscript{[13]}.

\textbf{Increase in Mucous Secretion}

Several studies have suggested that gastric mucous is involved in protecting the gastric mucosa from causing extensive damage by forming a protective layer over the rapidly moving epithelial cells favoring a rapid re-epithelalization of the mucosa\textsuperscript{[14]}. 

Increase in Bicarbonate Secretion

Both acid and alkali secretion in the body is increased by vagal stimulation. Bicarbonate delivery to the surface epithelium is increased by the ‘alkaline tide’ during hydrogen ion secretion. The rate of bicarbonate secretion is only 4 to 12 percent of the maximal output of gastric acid. Hence, bicarbonate ions alone cannot lower sufficiently the hydrogen ion concentration but it can complement the action of mucous, forming what is known as the ‘mucous-bicarbonate barrier’\[^{15}\].

Strengthening of Gastric Mucosal Barrier

Hydrogen ions do not permeate through the apical membrane or tight junctions between epithelial cells and hence blocks the back movement of acid. This barrier was referred to as the ‘gastric mucosal barrier’. Prevention of hydrogen ions and other water-soluble ions\[^{16}\] are also brought about by surface-active phospholipids which form a hydrophobic lining on the luminal surface of the gastric epithelium.

Increase in Mucosal Blood Flow

The mucosal microcirculation is significant in maintaining oxygenation and supplying nutrients. The gastric vasculature is so designed that the secreting oxyntic cells, which is alkaline in nature, has ready access to the surface epithelial cells, which are located in the basal side. Thus rich blood supply can lead to adequate supply of bicarbonate, which can cause neutralization of hydrogen ions which have undergone back diffusion. In addition, the absorbed injurious
agent gets diluted within the sub-epithelial capillaries due to enhanced flow of blood.

**Decrease in Gastric Motility**

Formation of mucosal folds relates closely to muscle action, especially circular muscle. Reduced gastric motility may cause protection of the gastric mucosa through a levelling effect of the mucosal folds. This increases the overall surface area of the mucosa to be exposed to irritant substances, which leads to reduction in the quantity of the irritants on specific sites of the gastric mucosa \cite{17}.

**Increased Release of Endogenous Mediators of Gastric Cytoprotection - Prostaglandins**

Prostaglandins were the first endogenous compounds implicated in gastric cytoprotection. Increased mucosal blood flow induced by prostaglandins has been suggested to be responsible for their gastroprotective effect\cite{18}.

Various other mechanisms have also been postulated which include, dilution of noxious agents by prostaglandin stimulated mucous secretion, stimulation of basal bicarbonate secretion, decrease in gastric motility and stimulation of cyclic AMP. Prostaglandins also act by stimulating rapid resolution of disrupted surface epithelium\cite{19}.
**Sulfhydryls**

Amino acids like methionine, L-cysteine and drugs containing sulfhydryl groups exert a protective effect on gastric lesions induced by ethanol. However, drugs, which cause blockade of the sulfhydryl containing moieties, counteract the cytoprotective effect of prostaglandins. It has been postulated that endogenous sulfhydryls mediate cytoprotection. Synthesis of prostaglandin and their receptor actions is dependent on endogenous sulfhydryls[20].

**Epidermal Growth Factor**

This polypeptide, a potent inhibitor of gastric acid secretion is found in salivary glands as well as other sources like duodenal mucosa and pancreas. Non-antisecretory doses of this polypeptide have been reported to have a cytoprotective action. This effect is probably mediated through sulfhydryl group rather than prostaglandin or alkali secretion[21].

**Scavenging of Free Radicals**

Oxygen derived free radicals, mainly the superoxide radical is involved in ischemic gastric mucosal damage, which may cause lipid peroxidation (LPO), and damage to intracellular compounds. Antioxidants like vitamin E and selenium have shown protective effect on the gastric mucosa against stress ulcers and chemically induced lesions[22].
Reduction in the release of Gastric Vasoconstrictors

In addition to mast cell and vasoactive amines, leukotrienes induce gastric vasoconstriction and increases vascular permeability. Mucosal levels of leukotrienes are increased after exposure to ethanol. In addition, inhibition of synthesis of cysteinyl leukotrienes in the gastric mucosa protects against damage by noxious agents\textsuperscript{19}.

Stimulation of Cellular Growth and Repair

It is well known that reconstitution of the epithelial cells of the damaged mucosal surface takes place by cells, which migrate from the deeper gastric pits, which recovers the basal lamina, which is denuded. Following injury with agents like ethanol, aspirin and hypertonic saline mucosal re-epithelialization occurs within 30 min. For migration of cells during this repair process, an intact basal lamina is vital. The integrity of the basal lamina is maintained by a medium to high pH. Conversely, if the luminal pH is low (acid) re-epithelialization is hampered\textsuperscript{23}.

1.2.5 Free Radicals in Pathogenesis of Peptic Ulcer Disease

Reactive oxygen species have been found to have a significant role in the pathogenesis of acute gastric mucosal injury induced by experimental stress, NSAIDs and ethanol\textsuperscript{24}. Gastric-duodenal mucosal integrity is mainly dependant on Oxygen derived free radicals as they are responsible for maintaining ulceration.

Excess production of Reactive Oxygen Metabolite (ROM) is directly related to the severity of gastritis in \textit{Helicobacter pylori} infected
patients\textsuperscript{[25]}. Lipid peroxidation takes place through tissue injury induced by free radicals. Superoxide dismutase and Catalase are important antioxidant enzymes that protect the oxygen handling cells against the deleterious effects of oxygen-derived free radicals. When the free radical generation exceeds the ability of free radical scavenging enzymes to cause dismutation of the radicals, the gastric mucosa may be damaged\textsuperscript{[26]}. The oxygen radicals in the gastrointestinal tract may induce the suppression of a protective mechanism of the gastric mucosa inhibiting glucosamine synthetic activity, a possible cause of decreased mucosal protective capacity\textsuperscript{[27]}.

Iron/Copper binding capacity with ferritin, transferrin, lactoferrin, plasmin and albumin does not allow the existence of free metal ions that are known to intensify oxidative stress by promoting the generation of hydroxyl radical (OH\textsuperscript{-}), a very deleterious reactive oxygen species\textsuperscript{[28]}. Antioxidants like Vitamin E and selenium are shown to have a protective effect on the gastric mucosa against stress and chemically induced lesions.

The antioxidant enzymes contain metals (Cu, Zn, Mn, Fe or Se) at the catalytic site. These cofactors are essential for enzymatic activity and have the potential to limit the expression of enzyme activity\textsuperscript{[29]}. Cofactors for metals are necessary for maintaining antioxidant enzyme efficiency. For example, Selenium, copper and iron is required for increasing the efficiency of glutathione peroxidase, SOD and catalase respectively.
Role of Stress in Gastric Ulcers

Most individuals commonly experience stress. Pathogenesis of disorders like diabetes mellitus, peptic ulcer, mental depression, ulcerative colitis and infertility are associated with stress\[^{30}\]. Cellular homeostasis of aerobic organisms is disrupted during stressful conditions due to generation of reactive oxygen species. This free radical generation can cause reaction with most of the intracellular molecules\[^{31}\] leading to a process of lipid peroxidation as these radicals are extremely reactive\[^{32}\]. During stress ulceration, lesions rarely penetrate the muscularis mucosa and are therefore strictly erosions.

Stress induced ulcers are produced by subjecting the animal to various forms of stress, either in combination or singly by restraining the animals in small cage, producing 3\(^{rd}\) degree burns, shocks and cold environment\[^{33}\].

Forced swimming in cold water is a method of inducing stress in rats. Irrespective of the technique used, ulcer incidence and pathology are remarkably similar indicating that with the exception of burning, which probably acts via histamine release all other techniques act through a common mechanism\[^{34}\]. The cause of stress-induced ulcer is clearly not understood. The most likely increased components are acid concentrations, reduced mucosal blood flow, reduced mucous secretion and gastric epithelial cell turnover and activation of the hypothalamic pituitary - adrenal axis.
1.3 FREE RADICALS

No other discovery in the field of science had an impact on pathobiology with respect to the implication of free radicals in almost all the diseases within a discovery of 20 years back. Free radicals can be of two types depending upon its origin, namely reactive nitrogen species (RNS) and reactive oxygen species (ROS).

1.3.1 Free Radical Generation

During normal biochemical reactions in our body there is a generation of oxygen and nitrogen derived free radicals. When reactive oxygen and reactive nitrogen species exceed the total antioxidant activity it causes oxidative stress. It has been postulated that disorders in the aged such as cancer, diabetis, cardiovascular and neurodegenerative disorders involve oxygen free radicals (OFR) at some stage of their development[35].

It has been demonstrated that oxygen derived free radicals play a significant role in chronic and acute ulceration[36]. Different models of gastrointestinal mucosal injury such as colitis, ischemia[10], reperfusion[37], stress[38] and ethanol[12] induced ischemia/reperfusion have shown the involvement of neutrophils. Lipid peroxidation due to oxygen derived free radicals follows release of lysosomal enzymes due to damage of the cellular membranes which further aggravates tissue damage.
Another hypothesis is that hyaluronic acid present in the epithelial basement membrane is degraded by free radicals promoting mucosal damage. However, this damage induced by free radicals is taken care by the body's effective antioxidant system by a set of endogenous antioxidant enzymes such as superoxide dismutase, glutathione peroxidase, glucose oxidase and catalase. They help in maintaining the balance between generation of free radicals and its eradication.

1.3.2 Reactive Oxygen Species

**Superoxide Anions (O$_2^-$)**

The first reduction product of oxygen is superoxide anion which is a relatively non-reactive species and dismutates to H$_2$O$_2$. Intracellular enzymes like superoxide dismutase acts as catalyst in this reaction or may occur spontaneously. The most important source of O$_2^-$ is oxidative enzymes, among which NADPH/NADH oxidase and xanthine oxidase are the most effective.$^{[39]}$

**Hydrogen Peroxide (H$_2$O$_2$)**

Hydrogen peroxide belongs to the class of reactive oxygen metabolites which are most stable. It is not very reactive and is detected readily. It can be generated directly by divalent reduction of O$_2$ or indirectly by univalent reduction of O$_2^-$.

**Hydroxyl Radical (·OH)**

Hydroxyl radical is one of the most reactive radicals and is produced following the reaction of O$_2^-$ and H$_2$O$_2$ in the presence of
metallic ions such as Fe$^{2+}$. Lipid is very susceptible to ·OH attack and initiate lipid peroxidation (LPO). ·OH is the most potent among ROM’s, as it reacts with many macromolecules at a very high rate constant. Conformational changes including breakage of DNA strands are induced by ·OH, which further enhances protooncogenes expression.

**Reactive Nitrogen Species**

Nitric oxide rapidly undergoes addition, substitution, redox and chain terminating reactions. The target molecules of nitric oxide are intracellular thiol and metal containing proteins and low molecular weight thiols like glutathione and cysteine$^{[40]}$.

1.4 **ANTIOXIDANTS**

Cells under aerobic conditions are threatened to the insult of reactive oxygen metabolites (ROMs) that are efficiently taken care by the powerful antioxidant system in the human body. The term antioxidant can be defined as any substance that delays or inhibits oxidative damage to a target molecule. Antioxidant enzymes, together with the substances that are capable of either reducing ROMs or preventing their formation, forms buffers which have strong reducing properties which effects the capability of the cell to counteract the activation of ROM. All reducing agents thereby form protective mechanisms, which maintain the lowest possible levels of ROMs inside the cell. The first line of defense against O$_2^-$ and H$_2$O$_2$ mediated injury are antioxidant enzymes like SOD and CAT$^{[41]}$. 
Allopathic drugs used in peptic ulcer are directed against a single luminal agent\textsuperscript{[42]}. Hence, we need to identify effective anti ulcer drugs, which not only heal peptic ulcers but also effectively prevent their recurrence. With the new and potent anti ulcer drugs, healing of peptic ulcer is usually achieved within six to eight weeks in most patients\textsuperscript{[43]} and 89\% of gastric ulcer patients experience ulcer recurrence within one year of successful healing with conventional anti ulcer therapy\textsuperscript{[44]}. Allopathic drugs produce adverse effects like impotence, arrhythmia, gynacomastia etc. and cause drug interactions on chronic administration besides being expensive.