REVIEW OF LITERATURE
2. REVIEW OF LITERATURE:

2.1 Family Liliaceae

The liliaceae is a family of about 240 genera and 4000 species widely distributed over most of the world and abundant in warm temperature and tropical regions. Members of this family are mostly perennial herbs. The rootstalk is a rhizome, bulb, corm or tuber, and the stems are erect or climbing, and often are modified into fleshy subterranean storage organ. These plants have basal or cauline leaves, which are alternate or whorled sometimes functionally, replaced by cladodes. Occasionally the leaves are fleshy or with prickly margins, and fibrous. Venation is mostly parallel. The flowers are usually bisexual and regular, axillary or terminal, solitary, umbelled, racemose, panicled; bracts usually small or spathelike when the flowers are in umbels. The perianth is coloured and often herbaceous. Segments are 6, 2 seriate, imbricate in bud. Stamens are 6, rarely 3 or fewer, hypogynous or adnate to the perianth lobes. Filaments are free or connate; anthers oblong or linear, often dorsifixed. Carpels 3, connate in a sessile, 3-quetrous, 3-celled ovary; ovules 4 or more in each cell. The fruit is a capsule or berry, usually 3 celled. Seeds are 1 or more usually albuminous.

Economically family liliaceae is important for the large number of ornamentals that it provides. In addition, asparagus, onion and garlic are important food crops. Red squill is an important rodenticide. As far as drugs are concerned, the family yields Aloe, which is used as a cathartic; Squill, which yields cardiotonic glycosides that are useful in medicine; and Varatrum, which yields the hypotensive alkaloids protovaratrines A and B.

Saponins and sapogenins are the major type of plant constituents found in the liliaceae, having been detected in or isolated from about
29 of 65 genera examined. Alkaloids have been detected in about 12 of 65 genera of the liliaceae. It is interesting to note that species in which alkaloids are present have not yielded saponins or sapogenins, and species from which saponins or sapogenins have been isolated, appear to be devoid of alkaloids.

2.2 Genus *Chlorophytum* Ker- Grawl.\textsuperscript{18-21,34-47} 

*(Family liliaceae)*

This genus of short rhizomatous herbs is widely distributed in the tropical and subtropical region of the world. The roots of the genus are thick, fleshy and tuber-like. The leaves are radical and clustered. Flowers are laxly racemned on a simple or branching leafless scape.

Fruits are of a coriaceous, truncate or emarginated. Seeds are broad and usually compressed. Embryo is often curved and large. About 40 species have been identified of the genus *Chlorophytum* in the tropical and subtropical region.

*Chlorophytum arundinaceum* Baker.\textsuperscript{18-21,36-39,44,46,47}

It is a small pretty, perennial herb often with rhizomes and tuberous roots. It is found from Bihar to Manipur, Orissa, Andhra Pradesh, Karnataka and Tamilnadu, eastern Himalaya, sikkim, chota nagpur and burma, ascending up to 1200m and also recorded from maharastra. The roots are fleshy and leaves are lanceolate to oblanceolate.

The leaves and flowers are eaten by the tribals. The plant is a substitute of onion. The tubers constitute of the drug, which is commercially known as safed musli.
**Chlorophytum tuberosum** Baker\(^{18,20,21,36-45}\)

It is a herb with short root stock, with cylindrical root fibres. Tubers are ellipsoid and hanging from root fibres. The tender leaves are eaten as a vegetable tonic\(^{21}\). The tubers, taken out and dried, known as safed-musli are said to be used as tonic. It is distributed throughout the Deccan peninsula and Central part of India.

**Chlorophytum laxum** R. Br. Prodr\(^{18,36,37,40,41}\)

It is an herb with distinct grass like leaves and flexuous scape, found up to an altitude of 1800m throughout Deccan peninsula. The leaves are eaten by the tribal people of Western Ghats. A paste of the plant is applied externally to swelling to remove inflammation\(^{18}\).

**Chlorophytum borivilianum** Sant. & Fernandez\(^{18}\)

It is found in Gujarat, Maharashtra and Goa and leaves are eaten by the tribal people of Western Ghats.

**Chlorophytum undulatum** Wall. ex Hook. F.\(^{18,37,38}\)

It is found in Sikkim, West Bengal and Manipur and is reported to contain a sapogenin (m.p.175-180 °). It is an herb with cylindric root fibres. Leaves are falcately recurved. A paste of roots mixed with mustard oil and applied in joint pains\(^{18}\).

**Chlorophytum attenuatum** Baker\(^{18,20,36,37,40,41}\)

It is found in Western ghats from Canara southwards to Coimbtore. The root-fibres are fleshy and cylindric and rarely tuberous. Leaves are 6-9 membranous, linear, acute and slightly narrowed at the base.

**Chlorophytum breviscapum** Dalz.\(^{20,35,37,38,40,41,48}\)

It consists root fibres with oblong tubers pendulous from them. Leaves are 6-9 and membranous type. It is found in Sikkim, Himalaya and West peninsula. It is also known as Bhimpal in growing areas.
Chlorophytum heyneanum Wall\textsuperscript{36,37}

It is found in Daccan peninsula at Nilghri hills at the altitude of 6000 ft. It consists tuberous root fibres. The leaves are ob lanceolate, 12-18 in numbers and 1-2 inch in size.

\textbf{Chlorophytum glaucum Dalz.}\textsuperscript{35-41}

It is found in Concan. Roots are spherical and depressed with fibrous tuber. Leaves are usually recurved, narrowly ob lanceolate 12-18 in numbers and 1-2 inch in size.

\textbf{Chlorophytum orchidastrum Lindl.}\textsuperscript{36,40,41}

It is found in Konkan, Cheeta hill and Belgaum. Whole plant is found with 3 ft. height. The root fibres are tuberous. Leaves are 6-9 membranous, elliptic-lanceolate, acute, strongly nerv ed, shining in both sides and narrowed at the base.

\textbf{Chlorophytum Khasianum Hook. f.} \textsuperscript{37,41}

It is found at an altitude of 3-6000 ft. on Khasia hills. Leaves are 10-24 linear and flat.

\textbf{Chlorophytum malabaricum Baker.}\textsuperscript{36,37}

It is found in Western ghats, from Canara southwards. The root-fibres are cylindrical and fleshy. Leaves are 6-12 and usually narrowed from the sheathing base to the apex.

\textbf{Chlorophytum nimmonii Dalz.}\textsuperscript{35}

It is found in the ghats opposite to Bombay and Malwan. Leaves are flat, broad lanceolate, long attenuated towards the base and very long.

\textbf{Chlorophytum parviflorum Dalz.}\textsuperscript{35}

It is found in rocky places near the sea at the Malwan district. The tubers are oblong, pendulous from the fibres. Leaves are erect, grass like, linear-folded.
**Chlorophytum anthericoideum** Dalz.\textsuperscript{35}  
It is found in Malwan district. Roots are many and tuberous. Leaves are radical, ensiform, slightly folded with waved margins.

**Chlorophytum comosum** (Thumb.) Jacq.\textsuperscript{18}  
It is found in South Africa. It is an herb with nice green leaves cultivated as ornamental plant in Indian gardens. In Africa, an infusion of the tuber is given as a purgative to children and to women after childbirth. It is reported to absorb formaldehyde vapors and used as biological air purification system in space station\textsuperscript{18}.

### 2.3 **Chlorophytum arundinaceum** Baker\textsuperscript{18,19,22,36-40}  
**Family** : Liliaceae  

**Varnacular Names**\textsuperscript{18,19,20,22,23}  
- **Hindi** : Biskandri, Safed musli  
- **Gujarati** : Safed musli  
- **Oriya** : Bharat batuli, Banadhau  
- **Sanskrit** : Sweta-musli, Musli

### 2.3a **Plant Description**\textsuperscript{18,19,22,36-39}  
It is a small pretty, perennial herb often with rhizomes and tuberous roots.  
Leaves: Suberect, narrow lanceolate, many nerved  
**Size** : 6-24" inch long (including the petiole), 1-2.5" inch wide.\textsuperscript{3}  
**Shape**: Lanceolate or oblanceolate  
**Apex** : Acute or acuminate  
**Base** : Narrowed in to broad petiole
Scape: Naked, 6-20 inch, stout
Flowers: Errect, raceme 3-8 inch
Bracts: $\frac{1}{4}$ -1/2 inch or lower longer pedicels $\frac{1}{4}$ -1/2 inch joined in middle.
Perianth: $\frac{1}{4}$ -1/2 inch lanceolate, white
Fruits: Capsule 1/3 inch broad, two lobed at the tip and base
Ovary: 3-Celled, ovules 4 or more in each cell.
Seeds: Broad, usually compressed, rugose, not angular, similar to onion seed.
Root: Slender, fleshy, cylindrical, 5-7 cm. in length, tapering on both the ends and white to buff in color. External surface shows longitudinal furrows.

2.3b THERAPEUTIC USES

*C. arundinaceum* is used for various therapeutic applications in Ayurveda, Unani and Allopathy. The plant is used as a substitute of onion. It has ability to cure many physical illness and weakness. The tubers constitute of the plant is commercially known as Safed Musli$^{18-26,49}$. The drug is considered a valuable nervine and general tonic for strength and vigor$^{18-26,49}$. It is responsible for improving general immunity. Root powder fried in Ghee is chewed in aphthae (ulcer)$^{18,21,22}$. A decoction of root with turmeric (Curcuma longa, Zingibaraceae) is given in rheumatism$^{18,22}$. Among tribal women in central Orissa, an extract of the roots crushed in rice water is taken for dysmenorrhoea$^{22}$. In Ayurveda the root is used for treating sprue, piles, blood disorders and as an aphrodisiac and rejuvenator$^{22,24-26}$. It has spermatogenic property and helpful in curing impotency. It is found very effective in increasing male potency$^{22,24,25}$. It cures many natal and postnatal problems. In literature it is mentioned to have ability to cure arthritis and diabetes. Leaves and flowers are edible$^{22}$. 
Review of literature

2.3c Phytochemical Profiles

Safed musli holds an important position in traditional system of medicine in India.

Bordia et al (1995)\textsuperscript{27} reported that the dried roots contain 42% carbohydrates, 8-9% proteins, 3-4% fibers and 2-17% saponins.

The saponin is actually responsible for the medicinal properties and "Higher the saponin content, higher will be the medicinal properties."

The quality of saponin depends on many factors. Generally roots collected from forest are rich in saponin as compared to cultivated plants.

Gupta et al (1979)\textsuperscript{28} isolated new galactoglucon from the fruit of \textit{C. arundinaceum}. This new polysaccharide consists of repeated units of D-galactose and D-glucose.

Tandon et al (1992)\textsuperscript{29} have isolated three different types of chemical components, which include lipids like docosanoate of pyranoside and pentacosyl (0.01428\%), lignoceric acid and triacontanoic acid; alkanes with 5 or more carbon like nonacosane and tetracosane and steroids like stigmasterol and stigmasterol-3-o-\textbeta-d-glucoside from the methanolic extract of the roots of \textit{C. arundinaceum}. Besides, they also isolated one new compound that is characterised as 4-hydroxy-8, 11-oxidoheneicosanol.

Tandon and Shukla (1992)\textsuperscript{30} have isolated sapogenins like gitogenin and neogitogenin (0.00034\%); tokorogenin (0.00045\%) and steroid like stigmasterol (0.00051\%) from the roots of \textit{C. arundinaceum}. 

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Tandon and Shukla (1993)\textsuperscript{49} have isolated a bibenzyle xyloside from the root of \textit{C. arundinaceum}. The structure of this new xyloside is 2',4,4'-trihydroxy-2-xylopyranosyl-bibenzyl (0.00285\%).

Tandon and Shukla (1996)\textsuperscript{50} have further reported arundinoside-B (0.00257\%), a new acylated glucoside from \textit{C. arundinaceum}. The structure of which has been elucidated as: 1-O- (\(\beta\)-D-3-benzoyl-glucopyranosyl)-octacosanol.

Tandon and Shukla (1997)\textsuperscript{51} have isolated a new spirostane saponin based on tokorogenin named as arundinoside-A (0.00171\%) from the roots of \textit{C. arundinaceum}.

Rashid and Hussain (1999)\textsuperscript{52} have done pharmacognostical study of \textit{C. arundinaceum} and a controversial drug \textit{Asparagus adscendens} that is also referred as “musli sufed”. Both these genera are found to be quite different in their chemical constituents and their actions.
2.4 Free Radicals and Antioxidants:

Free radicals are chemical species possessing an unpaired electron that can be considered as fragments of molecules, which are generally very reactive. They are formed continuously in the cells either as accidental by-products of metabolism or deliberately during, for example, phagocytosis. The most important free radicals in biological systems are derivatives of oxygen derived by its reduction. Reactive oxygen species (ROS) is a collective term, which is used by biologists to include not only oxygen radicals such as superoxide radical (O\textsuperscript{2−}) and hydroxyl radical (OH\textsuperscript{−}) but also some derivatives of oxygen that do not contain unpaired electron such as singlet oxygen (\textsuperscript{1}O\textsubscript{2}), hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}), thyl (RS, a sulphur-centered radical), trichloromethyl (CCl\textsubscript{3}), a carbon-centered radical) and nitric oxide (NO) in which the unpaired electron is delocalized between both atoms are also amongst the degenerative free radicals. The reduction may be mediated by normal metabolic pathways, exogenous compounds, or arise from exposure to electromagnetic radiation. The free radicals thus formed can nick DNA, damage essential enzymes and structural proteins, and provoke chain reactions, such as uncontrolled lipid peroxidation or autoxidation reactions. There is growing evidence that free radical damage is involved in the development of many diseases including atherosclerosis, cancer, Parkinson's disease and other neurodegenerative disorders, inflammatory bowel disease and lung disease. Antioxidants are believed to protect against certain diseases by preventing the deleterious effects of free radical-mediated processes in cell membranes and by reducing the susceptibility of tissues to oxidative stress.

2.4a Sources of Free Radicals:

Free radicals are normal products of cellular aerobic metabolism. Superoxide (O\textsuperscript{2−}) and hydroxyl (OH\textsuperscript{−}) species are predominant cellular free radicals. Hydrogen peroxide (H\textsubscript{2}O\textsubscript{2}) and peroxynitrite (ONOO\textsuperscript{−})
although not they free radicals, contribute importantly to cellular redox state. The major sources of ROS are mitochondrial oxidative metabolism, enzymatic reactions involving mixed function oxidation and autoxidation of small molecules. Mixed function oxidation reactions occur in the cytoplasm, plasma and nuclear membrane, endoplasmic reticulum and peroxisomes\textsuperscript{60}. Superoxide (O$_2^-$) is formed by leakage of high-energy electron along the mitochondrial electron chain and by a variety of cytosolic and membrane bound enzymes, including xanthine oxidase, the NADPH-cytochrome P-450 reductase, phospholipase A$_2$ and heme proteins such as hemoglobin and cytochrome P-450\textsuperscript{61}. Hydrogen peroxide (H$_2$O$_2$) also is produced along the electron transport chain, as well as through autoxidation of small molecules and dismutation of O$_2^-$ by superoxide dismutase. Once formed within the biological milieu, O$_2^-$ can undergo a variety of chemical and metabolic reaction yielding other ROS. These reactions include dismutation to H$_2$O$_2$ and protonation to form the hydroperoxy radical (HO$_2^-$). H$_2$O$_2$ may also arise directly from O$_2$ by the two-electron reduction catalyzed by a variety of enzymes such as monoamine oxidase\textsuperscript{62}.

One-electron reduction of oxygen produces superoxide radical (O$_2^-$)

\[
\text{O}_2 + \text{e}^- \rightarrow \text{O}_2^-
\]

A two-electron reduction of oxygen yields hydrogen peroxide (H$_2$O$_2$),

\[
\text{O}_2 + 2\text{e}^- + 2\text{H}^+ \rightarrow \text{H}_2\text{O}_2
\]

Hydrogen peroxide is an important compound in free radical biochemistry because it can rather easily breakdown, particularly in the presence of transition metal ions, to produce the most reactive and damaging of the oxygen free radicals, the hydroxyl radical (OH): (Fenton type reaction)\textsuperscript{63}.
Fenton reaction can be augmented by the reduction of ferric (Fe$^{3+}$) by O$_2^{-}$, regenerating Fe$^{2+}$. The net result is the production of OH$^-$ as in the iron catalyzed Haber-Weiss type of reaction$^{64}$.

\[
\begin{align*}
    \text{O}_2^{-} + \text{Fe}^{3+} & \rightarrow \text{Fe}^{2+} + \text{O}_2 \\
    \text{O}_2^{-} + \text{H}_2\text{O}_2 & \rightarrow \text{O}_2 + \text{OH} + \text{OH}^- \\
\end{align*}
\]

OH is also formed by the decomposition of ONOO$. The formation of ONOO does not require transition metals and is formed in cell when O$_2^{-}$ reacts with nitric oxide radical (NO) in a radical addition reaction.

\[
\begin{align*}
    \text{O}_2^{-} + \text{NO}^- & \rightarrow \text{ONOO}^- \\
    \text{ONOO} + \text{H}^+ & \rightarrow \text{OH}, \text{NO}_2, \text{NO}_2^+ \\
\end{align*}
\]

The OH is highly reactive and hence no enzyme systems involving it as a substrate exist.

### 2.4b Damaging Reactions of Free Radicals:

Normally a balance between oxidative events and antioxidative forces maintains the status quo within living cells. A variety of enzymes help to maintain cells in a reduced state despite the presence of aerobic environment. When normal balance is upset, the tissue is considered to be under oxidative stress.

All the major classes of biomolecules may be attacked by free radicals but lipids are probably the most susceptible. Cells are rich sources of polyunsaturated fatty acids (PUFAs), which are readily attacked by oxidizing radicals. The oxidative destruction of PUFAs, known as lipid peroxidation, is particularly damaging because it proceeds as a self-perpetuating chain-reaction$^{55}$. Oxidation of the PUFA generates a fatty
capable of attacking many amino acid residues. Proteins often bind transition metal ions, making them a target for attack by site-specific hydroxyl radicals55.

Thus oxidation of lipids, proteins and DNA can result in:
- Loss of membrane integrity and function
- Inactivation of enzymes
- Modification of Lipoproteins
- Chemical alteration of DNA

2.4c Antioxidant Defenses:

(A) Primary Defense:

(I) Enzymes:

a. Superoxide Dismutases (SODs):
SODs remove superoxide by accelerating its conversion to hydrogen peroxide. The SODs have a variety of prosthetic groups, allowing their classification into several groups. The prevalent enzyme is the CuZnSOD, is largely present in liver and certain areas of brain62. The Cu atom is necessary for the catalytic activity of the enzyme whilst the Zn atom imparts stability65. Eukaryotic MnSOD, mainly tetramer, is predominantly localized within mitochondria, protecting it against superoxide produced during electron transport66. CuSOD is found in most tissues but is maximally present in blood plasma.

b. Catalase:
Hydrogen peroxide generated by superoxide dismutase is converted to water and oxygen by the enzyme catalase55.

\[ 2H_2O_2 \rightarrow 2H_2O + O_2 \]
It is found in most aerobic cells tested. The catalase activity of eukaryotic cells is localized within the peroxisomes, organelles that contain many of the H₂O₂ generating enzymes present in aerobic cells.

**c. Selenium-dependent Glutathione Peroxidase (SeGSHPx):**
This protein is a member of a family of peroxidases that is found in the cytosol of most cells and is active towards both hydrogen peroxide and, if first cleaved form membrane phospholipids by a phospholipase, fatty acid hydroperoxides.

\[
\text{ROOH} + 2\text{GSH} \rightarrow \text{ROH} + \text{GSSG}
\]

SeGSHPx is a tetrameric protein containing four atoms of selenium bound as selenocysteine moieties, which confer the catalytic activity.

**(II) Small Molecules:**

**a. Glutathione:**
Glutathione is a tripeptide of glutamate, cysteine and glycine. It is found in most aerobic animals often in high (mM) concentration. As an antioxidant it reacts with ROS in a number of ways: As a reductant, it reduces species such as H₂O₂ to water by the enzyme glutathione peroxidase.
Reacted directly with free radicals such as O₂⁻, OH⁻ and RO⁻ by a radical transfer process.
Reacted with electrophiles to form covalent adducts.
These reactions are catalyzed by a group of enzymes, the glutathione transferases.

**b. Vitamin C:**
This water-soluble molecule is found both intra and extracellularly in most biological systems. It is a good scavenger of ROS. It is essential for recycling of alpha-tocopherol. However, in the presence of transition metal ions ascorbate can become pro-oxidant, acting as a reducing agent.
and generating $O_2'$, $H_2O_2'$ and $OH^*$ radical. Normally, since such metal ions are available in limited amounts in vivo, the antioxidant property of ascorbate predominates.

c. Uric Acid:
Uric acid in plasma possesses strong radical scavenging property. It has been shown to protect human blood plasma ascorbate from oxidation.\(^7^0\)

d. Taurine:
This β-amino acid has been identified in most eukaryotic cells and is also found extracellularly in a variety of body fluids. It reacts directly with ROS such as HOCL, to form less reactive species.\(^7^1\)

(B) Secondary Defense:
(I) Anti-lipid Peroxidation Systems:
These principles are present within the biological membranes and whose activity either directly interrupts initiation and/or removes peroxidized membrane components.

a. GSH Peroxidases:
GSH peroxidases include glutathione transferases and peroxidation inhibitory protein. Glutathione transferases do not metabolize $H_2O_2$ but show specificity only for low molecular weight organic hydroperoxides.

b. Tocopherols:
The tocopherols are a family of naturally occurring chroman derivatives found within biological membranes. The most commonly encountered tocopherols are alpha-tocopherol (vitamin E)\(^6^6\). It inhibits lipid peroxidation by scavenging peroxyl radicals, which are intermediates in the chain reaction:

$$LOO' + a\text{-tocopherol}-OH \rightarrow LOOH + a\text{-tocopherol}-O'$$
Other functions attributed to vitamin E are enhancement of vitamin A utilization, inhibition of prostaglandin production, and stimulation of an essential factor in steroid metabolism.

c. Carotenoids:
Carotenoid pigments, such as beta-carotene, are powerful quenchers of singlet oxygen\textsuperscript{67} and is found to be accumulated in the membranes of certain tissues e.g. ocular retina.

d. Bilirubin:
This lipid soluble product of hemoprotein catabolism, generally considered as a tissue toxin if accumulated to high concentrations, has recently been proposed as a chain-breaking antioxidant of physiological relevance\textsuperscript{72}.

(C) Others
Besides these biomolecules several diet supplements containing vitamins, natural compounds like carotenoid pigments, flavonoids, procyanidins, gallic acid, silymarin, curcuminoids, terpenoids, organic acids, alkaloids etc are known to have significant antioxidant potential\textsuperscript{73-78}. 
2.5 Stomach

The stomach is a J-shaped, dilated portion of the elementary tract directly inferior to the diaphragm in the epigastria, umbilical and left hypochondriac regions of the abdomen, which forms a receptacle for the food after its passage down the oesophagus. The stomach has anterior and posterior surfaces and upper and lower curved borders. The upper concave border is called the lesser curvature and the lower convex border is the greater curvature.

Stomach has two orifices namely cardiac and pyloric. The cardiac orifice is the most fixed part of the stomach while pylorus is very mobile due to its peritoneal covering.

Stomach mainly divided into four different parts. The cardia at its upper end, 1 to 4 cm wide that guards the oesophageal orifice, also known as cardiac sphincter. Dome shaped upper part called the fundus to the left of the cardia. Large central portion of the stomach called body, inferior to fundus. The region of the stomach that connects to the duodenum is the pylorus.

The lower part of fundic area is separated from the pylorus by a sharp angle on the lesser curvature, called the incisura angularis. The junction of the pyloric and fundic area is not sharply demarcated and is frequently known as the transitional zone.

Pylorus has three parts, the pyloric antrum, which connects to the body of the stomach and the pyloric canal, which leads into the duodenum. The pylorus communicates with the duodenum of the small intestine via a sphincter called the pyloric sphincter.
Fig.: 1  External and internal anatomy of stomach.

(a) Anterior view of regions of stomach

(b) Frontal section of the internal surface
2.5a Histology of Stomach

The stomach consists of the four layers from outside to inwards includes serosal coat, muscular coat, submucosal layer and mucus membrane.

The mucosal surface is ridged by the rugal folds created by contraction of the muscularis mucosa especially prominent in the body of the stomach and less obvious in the antrum.

These rugae are longitudinal at the lesser curvature but irregular in the outer region. They are of temporary characteristics and will disappear during distension.

From outside to inwards the structural layers are

**Serosal layer**

It is simple squamous epithelium and areolar connective tissue that covers the stomach and is part of visceral peritoneum.

**Muscular layer**

It is composed of three rather than two layers of smooth muscles unlike rest of the GI tract.

1. Outer longitudinal layer
2. Middle circular layer: Three muscles condense and unite with deepest fibers of longitudinal muscles to form pyloric sphincter.
3. Inner oblique layer: The fibers are like U shaped loop covering both the walls across the greater curvature but do not reach the lesser curvature.

This arrangement of smooth muscles fibers allows the stomach to churn food, break it into small particles, mix it with gastric juice and pass it to the duodenum.
**Submucosal layer**
This layer of the stomach is composed of areolar connective tissues, which connects mucosa to muscularis. It contains plexus of nerves, large blood vessels and lymphatics in loose areolar tissue. These layers contain fat cells and are rich in mast cells, lymphoid wandering cells and leukocytes.

**Mucous membrane**
The mucosa is a thick layer. Its surface is smooth, soft, velvety and reddish-brown in color but pink in pyloric region. Total surface area is 800 cm². The mucosa layer is composed of a *surface epithelium*, *lamina propria* and *muscularis mucosa*.

1. *Surface epithelium*
When viewed microscopically, the internal surface of the stomach wall appears honey combed by small somewhat irregular gastric pits (foveola), polygonal or slit like funnel shaped depressions about 0.2 mm in diameter. The base of each gastric pit receives several gastric glands, which extends deep into the lamina propria as far as the muscularis mucosa. The total number of these glands is estimated as 35 million. On the basis of differences in the glands and pits there are three different zones of glands are recognized:

(a) Cardiac glands
They are confined to small area near the cardiac orifice. Some are simple tubular glands while others are compound-branched tubular glands. In these types of glands, mucus-secreting cells are predominant while parietal and zygomatic cells are few in numbers. Functions of these glands are unknown but they may produce lysozyme.
(b) Gastric glands (Main or Fundic glands)
They lie in the fundus and main body of the stomach. They occupy the largest area of the stomach and produce most of the enzymes and acid secreted by the mucosa of the stomach. Pits are relatively short, occupying about one quarter of the mucosal thickness, while the simple branched tubular glands are long straight. It gives 3-7 opening into each gastric pit.

The wall of gland consists of 5 different types of cells.
❖ Chief (peptic or zygomatic) cells that secret pepsinogen.
❖ Parietal (oxyntic) cells that secret mucus.
❖ Mucus neck cells that secret acid.
❖ Stem cells from which other cells of the glands are derived.
❖ Enteroendocrine cells (Argentaffin cells), secretes a number of biogenic amines and polypeptides important in the control of motility and glandular secretion. They include G-cells, D-cells and EC cells.

The glands can be differentiated in to three different regions:
❖ The isthmus, base of the gastric pit
❖ The neck
❖ The base

(c) Pyloric glands
They are found in the pyloric antrum and canal which extends more proximally on the lesser than the greater curvature. They show deep pots extending to half the thickness of the mucosa. They consist of mainly mucus secreting cells, parietal cells being few and chief cells mainly absent. Enteroendocrine cells are also numerous.
Gastric juice is the combined secretions of mucous cells, parietal cells, and chief cells.
2. Lamina propria
It is found between the glands, these form a connective tissue framework and contains lymphoid tissue complex. Periglandular vascular plexus is also present and is thought to be important in the maintenance of the mucosal environment, including removal of bicarbonate produced in the tissues as a counter part to acid secretion. Neural plexus are also present.

3. Muscularis mucosa
This is a thin stratum of smooth muscle fibers lying external to the layer of glands, its fibers are arranged as inner circular and outer longitudinal layers, with a third external circular layer in places.

Epithelial Cells of Gastric Glands
1. Chief Cells (Peptic / Zymogenic Cells)
The chief cells are the source of the digestive enzyme pepsin and renin. The chief cells are usually basal in position. They are cuboidal in shape and nuclei are rounded and open faced. These cells are found predominantly in the base of the gastric pit and they are abundant in the corpus of the stomach.

A typical chief cell is characterised by a luminal surface with short microvilli covered by a thin coating of glycoprotein. Characteristically they contain zymogen granules that store pepsinogen and are usually most numerous in the apical cytoplasm. They contain secretory granules and because of the abundant cytoplasmic RNA they are strongly basophilic in nature. These granules formed in the Golgi apparatus and are released into the gland lumen by exocytosis.

Chief cells secrete pepsinogen, which is inactivate in nature and converted to active form pepsin in the acidic medium of the stomach. The function of pepsin is to hydrolyze the protein to smaller peptides
2. *Parietal Cells (Oxyntic Cells)*

The parietal or oxyntic cells are source of gastric acid and of intrinsic factor. They are large, oval and strongly eosinophilic with centrally placed nuclei and appear to bulge in to the surrounding lamina propria. They are scattered singly and in small groups between other cells types from the isthmus to the base of gastric glands, but more numerous in the neck and isthmus region.

The cytoplasm contains numerous mitochondria with prominent cristae. The parietal cell has unique ultra structure. The luminal side of the cell is deeply imaginated to form canaliculi with numerous irregular microvillai. Within the cytoplasm facing these channels, there are myriads of the fine tubules (tubulo-vesicular system) directed towards the canalicular surface.

The membrane lining the microvilli have a high concentration of H\(^+\) / K\(^+\) ATPase antiport channels which actively secretes H\(^+\) ions into the lumen and Cl\(^-\) ions following along the elecrogenic gradient.

In the secretory state, the number of intracellular canaliculi is increased and they become externalized, resulting in the formation of long microvillai, which open to the lumen within a larger microvillar surface. At the end of stimulated secretion, the process is reversed with withdrawal of the microvillar membrane from the surface into the cytoplasm to reconstitute the tubulovesicular membranes\(^80\).

The golgi body and granular endoplasmic reticulum responsible for the synthesis and secretion of intrinsic factor a glycoprotein which is required for the absorption of the Vit-B\(_{12}\).
3. **Mucous Cells (Surface Epithelial Cells)**

Entire inner surface of the stomach has a continuous layer of the mucous cells that secrete large quantity of the viscid and alkaline mucus that is remains insoluble. It coats the entire surface and providing a major shall of protection (pH 6 to 7) from luminal pH 1 to 2. It also lubricates the food and facilitates its transport.

Even slightest contact with food or any type of irritation of the mucosa directly stimulates the mucous cells to secret thick and viscid mucus. There are two types of mucous cells present within stomach:

(a) Surface mucous cells that cover the free surface of the glandular stomach.
(b) Neck mucous cells that secret mucus and resembles intestinal goblet cells.

The main difference between these two is that neck cells stains more deeply with alcian blue at low pH then do surface cells, indicate the presence of more acidic staining sited (mucin) in these cells.

4. **Stem Cells**

They are present in the gastric gland. They are relatively undifferentiated cells from which the other types of cells are derived. They are few in numbers and situated in the isthmus region of the gland and bases of the gastric pits.

These cells constantly replace gastric epithelium leading to the formation of mucous neck, surface cells and oxyntic cells and also possibly the enteroendocrine cells. All these cells have limited lifespans. The replacement period for surface mucous neck cell about one week while other cell types appear to live much longer.
5. **Enteroendocrine Cells.**

These cells produce histamine, a key factor in gastric acid secretion. These cells occur in all types of gastric gland but more frequently in the body and fundus. They are columnar in shape with irregular nuclei surrounded by granular cytoplasm, which can be stained strongly with silver salts (hence also known as 'Argentaffin Cells'). Many of the cells can be stained by potassium dichromate and have been called 'Enterochromaffin Cells'.

The function of these cells is to secrete number of biogenic amines and polypeptides that are important in the control of motility and glandular secretion. These cells include G-cells (secreting Gastrin); D-cells (secreting Somatostatin) and EC-cells.

### 2.5b Control of Gastro-Intestinal Functions:

#### 2.5b (A) Neuronal control of GI function.

The gastrointestinal tract has a nervous system of its own called the enteric nervous system.

It is composed of two plexuses:

1. **Myenteric plexus or Auerbach’s or Outer plexus.**
   
   It lies between the longitudinal and circular muscular layers. It mainly controls the gastrointestinal movements.

2. **Submucosal plexus or Meissner’s plexus or Inner plexus.**
   
   It lies in the submucosal. It controls mainly the gastrointestinal secretion and local blood flow.

The sympathetic and parasympathetic fibers connects with both the myenteric and submucosal plexuses, although the enteric nervous system can function on its own, independent of these autonomic
nerves stimulation of parasympathetic and sympathetic system can further activate or inhibit gastrointestinal functions.

1. Myenteric plexus
It is concerned with controlling motor activity along the length of the gut. When stimulates its effects are:
- Increased tonic contraction or tone of the gut wall.
- Increased intensity of the rhythmical contraction.
- Increased velocity of conduction of excitatory waves along the gut wall, causing more rapid movement of the peristaltic waves.

2. Submucosal plexus
In contrast to myenteric plexus, it has inhibitory control over gastrointestinal functions. Many sensory signals originate from the gastrointestinal epithelium and are then integrated in the submucosal plexus to help control local contraction of the submucosal muscle that cause various degrees of in folding of the stomach mucosa.

2.5b (B) Autonomic control of the gastro-intestinal functions

1. Parasympathetic innervations
It is supplied by vagus nerves. The postganglionic neurons of the parasympathetic system are located in the myenteric and submucosal plexus. The parasympathetic nerve endings secrete acetylcholine. Stimulation of this system enhances the gi-motility and stimulates gastric secretion.

2. Sympathetic innervations
The sympathetic fibres supplies to the gi-tract are originated in the spinal cord between the segments T-5 to L-2. The postganglionic fibres of this system terminate principally on neurons in enteric nervous system. It secretes nor-adrenaline and stimulation of sympathetic nervous system inhibits activity in the gi-tract, effects opposite to those of parasympathetic system.
2.5b (C) Hormonal control of gastro-intestinal functions

The hormones of the gi-tract include both endocrine secretion and paracrine secretions. The endocrine secretions are mainly peptides synthesized by endocrine cells in the mucosa and the most important is gastrin. The gastrin cells also called G-cells present in the pyloric glands, secretes this hormone. Gastrin is absorbed into the blood and carried into the oxyntic glands in the body of the stomach and stimulates parietal cells prominently and peptic cells to lesser extent. Thus it increases the secretion of acid and pepsin.

Other hormones of this group, especially gastric inhibitory peptides (GIP), are important in giving and anticipatory signal to the pancreatic islets. GIP has a mild effect in decreasing motor activity of the stomach. Therefore it slow downs the emptying of gastric contents into the duodenum when the upper small intestine is already oversupplied with food products.

Table: 1. Summary of the hormones of GI-tract with their site of occurrence and actions.

<table>
<thead>
<tr>
<th>Hormone</th>
<th>Site of occurrence</th>
<th>Action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bombesin</td>
<td>Nerves and endocrine cells throughout GIT</td>
<td>-Release gastrin,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Increase gastric secretion,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Contracts sphincters and relaxes the muscles of GIT.</td>
</tr>
<tr>
<td>Cholecystokinin</td>
<td>Mucosal cells of duodenum and jejunum</td>
<td>-Increases pancreatic secretion,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Contracts gall bladder,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>-Inhibit gastric motility.</td>
</tr>
<tr>
<td>Gastric inhibitory peptide (GIP)</td>
<td>Mucosa of duodenum and jejunum</td>
<td>-Decreases gastric emptying.</td>
</tr>
<tr>
<td>Motilin</td>
<td>Duodenum</td>
<td>Stimulates intestinal peristalsis.</td>
</tr>
<tr>
<td>------------</td>
<td>----------------------------------------------</td>
<td>-----------------------------------</td>
</tr>
<tr>
<td>Neurotensin</td>
<td>Mucosa of ileum</td>
<td>Potent mast cells secretogogue,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Contracts fundus of stomach and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>relaxes duodenum.</td>
</tr>
<tr>
<td>Pancreatic polypeptide</td>
<td>Special endocrine hormone in islets.</td>
<td>Stimulated basal acid secretion,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases gastric motility.</td>
</tr>
<tr>
<td>Secretin</td>
<td>Mucosal cells of duodenum</td>
<td>Inhibits gastric secretion and</td>
</tr>
<tr>
<td></td>
<td></td>
<td>motility,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Stimulates pancreatic and mucus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>secretion and flow of bile.</td>
</tr>
<tr>
<td>Somatostatin</td>
<td>- Gastric mucosa,</td>
<td>Contracts ileum and smooth</td>
</tr>
<tr>
<td></td>
<td>- Pancreatic islets,</td>
<td>muscles.</td>
</tr>
<tr>
<td></td>
<td>- Nerves of GIT</td>
<td></td>
</tr>
<tr>
<td>Vasoactive Intestinal Polypeptides (VIP)</td>
<td>Releases from nerve endings and endocrine throughout GIT</td>
<td>Vasodilator and relaxes smooth</td>
</tr>
<tr>
<td></td>
<td></td>
<td>muscles of GIT,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Increases GIT secretions.</td>
</tr>
<tr>
<td>Gastrin</td>
<td>Stomach and Duodenum</td>
<td>Increases gastric secretion,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>motility and relaxes the pyloric</td>
</tr>
<tr>
<td></td>
<td></td>
<td>sphincter.</td>
</tr>
<tr>
<td>Substance P</td>
<td>In special endocrine cells in GIT and in nerve cells</td>
<td>Contracts ileal smooth muscles.</td>
</tr>
<tr>
<td>Enterogastrone</td>
<td>Endocrine secretion of intestinal mucosa</td>
<td>Delays gastric emptying and inhibit acid secretion.</td>
</tr>
<tr>
<td>Urogastrone</td>
<td>Male human urine</td>
<td>Delays gastric emptying and inhibits acid secretion.</td>
</tr>
</tbody>
</table>
The paracrine secretion or local hormones are released particularly in the mucosa. These hormones act on nearby cells and the most important of these in the stomach is the histamine. Similar to gastrin, histamine stimulates acid secretion by the parietal cells. Many of these hormones that are released by endocrine cells are also released by neurons in the tract and function as neurotransmitter.

2.5c Physiology of Gastric Acid Secretion:

Gastric juice contains many substances. The six most important of these are hydrogen ion, pepsin, mucus, bicarbonate, intrinsic factor and water. Hydrochloric acid and the enzyme pepsin participate in the digestion of proteins. Mucus lubricates ingested solids. Mucus and bicarbonate probably protect the mucosal lining against digestion by acid and pepsin. The intrinsic factor is required for normal absorption of digested cyanocobalamine (Vit-B12). Each day the stomach secretes about 2.5 L of gastric juice in an adult.

Gastric acid secretion

Parietal cells are stimulated by three secretogogue like acetylcholine a neurotransmitter released from the vagus nerve, the paracrine substance histamine and the hormone gastrin. Although parietal cell have receptors for all these secretogogue, the finding that specific H2-blockers not only antagonize the histamine-induced secretion but also acetylcholine and gastrin stimulated secretions which indicate that acetylcholine and gastrin may stimulate parietal cells indirectly by releasing histamine from paracrine cells.

Role of Ach

About half of the nerve signals to the stomach that cause gastric secretion originate in the dorsal motor nuclei of the vagi and pass via the vagus nerve, first to the enteric nervous system of the stomach.
wall and then to the gastric glands. All the secretory nerves release acetylcholine as the neurotransmitter at their endings on the glandular cells. Acetylcholine is released from neurons and stimulates specific muscarinic receptors, type M₁ and M₂, on the surface of the parietal cells and on the surface of the histamine containing cells. Signal transduction mechanism in the parietal cell involves a rise in cytosolic calcium and is dependent on an adequate extracellular calcium concentration⁸⁰.

**Role of Histamine**

Histamine is a paracrine agent because it diffuses from its release site to the parietal cells (rather than travelling within mast the blood circulation as does a hormone). It is released from mast cells located within the corpus or from enterochromaffin cells. A small amount of histamine is formed continuously in the gastric mucosa, either in response to acid in the stomach or for other reasons. The small amount alone cannot be able to releases large amount of acid but in the presence of acetylcholine and gastrin it will largely enhance acid secretion.

It acts on H₂ receptors and H₂ antagonists like cimetidine, ranitidine etc can block it. An intracellular mechanism involves increase in intracellular cAMP, which in turn stimulate protein kinase and it will finally increase hydrogen ion production and secretion. When an appropriate antihistaminic drug like cimetidine blocks the actin of histamine, neither acetylcholine or gastrin can cause significant amount of acid secretion. Thus, histamine is a necessary co-factor for exciting significant acid secretion.

**Role of Gastrin**

Gastrin is a hormone, which is released from G-cells also called 'gastrin cells' present in pyloric region of the stomach. Gastrin is not a
single substance but a family of straight chain peptides. In man they are generally found in three different forms.

- G-34 contain 34 amino acids known as big-gastrin
- G-17 contain 17 amino acids known as little-gastrin
- G-14 contain 14 amino acids known as mini-gastrin

Along these, G-17 appears to be the main form of gastrin involved in the control of gastric secretion. G-17 and G-14 have plasma half lives of 2-7 min. and G-34 has half life of 40 min. The plasma concentration is measured by radioimmunoassay. The normal fasting concentration being 50-100 pg / ml\(^{82}\).

The main action of gastrin is stimulation of the secretion of acid by parietal cells. This action is mediated partly by direct effect on parietal cells and partly by histamine mediated. Gastrin is absorbed into the blood and carried to the oxyntic glands in the body of the stomach where it stimulates the parietal cells very strongly and also peptic cells to a lower extent.

Release of gastrin is controlled by neuronal mediators, blood born mediators and the direct effects of the stomach contents. Small peptides, amino acids and calcium directly stimulate G-cells to release gastrin. The putative neurotransmitter for gastrin release is a peptide named gastrin-releasing peptide (GRP). Milk calcium salts also stimulate gastrin release. Therefore there is inappropriate to use calcium-containing salts as antacids.

Somatostatin inhibits the release of gastrin from the G-cells probably acting on a paracrine substance. In addition, the nerve of the gastric mucosa contain vasoactive intestinal peptide (VIP), gastrin releasing peptides (GRP) and bombesine mediates the release of gastrin, an action that is blocked by \(\beta\)-adrenoceptor antagonists. Gastrin secretion is inhibited when the pH of the gastric contents falls to 2.5
The cephalic, gastric, and intestinal phases of gastric digestion. Reflexes initiated by sensory receptors in the head, stomach, and small intestine constitute the cephalic, gastric, and intestinal phases of gastric digestion, respectively.

During the cephalic and gastric phases, digestion in the stomach is stimulated whereas during the intestinal phase, gastric juice secretion and gastric peristalsis are inhibited.
or lower. This effect is mediated through cholinergic nerves and is inhibited by atropine\textsuperscript{83}.

An excessive secretion of gastrin resulting in excessive secretion of acid that is seen with tumors of gastrin secreting cells, gastrinomas, the complex of signs and symptoms being called the Zollinger Ellison Syndrome\textsuperscript{83}.

**Regulation of Gastric Acid Secretion:**

*Basal Secretion*

Basal gastric secretion takes place in the fasting state. This secretion shows a circadian rhythm with high rates in the evening and low rates in the morning. It is mainly dependent on the vagus nerve and basal secretion is markedly reduced following abdominal vagotomy.

*Prandial Secretion*

Acid secretion in the prandial state include excitatory and neurohormonal mechanism that turn acid secretion on and off as the meal transport from the oral cavity to small intestine.

Gastric secretion can be classified in to three phases known as cephalic phase, gastric phase and intestinal phase according to the location of the afferent stimuli initiating the response.

1. *Cephalic Phase*

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

This stimulates afferent neural signals to the brainstem, which in term stimulates vagal efferent fibres to the oxyntic and antral gland portion of the stomach. These efferent neuronal fibres are primarily
cholinergic and it stimulates secretory activity in the cells of the stomach mucosa.

Activation of vagus nerve can stimulate HCl and pepsinogen secretion by two mechanisms:
1. Direct vagal stimulation of the gastric parietal and chief cells (primary mechanism)
2. Vagal stimulation of gastric secretion by the G-cells which in turn stimulates the parietal and chief cells to secrete HCl and pepsinogen respectively.

Sham feeding experiment in dogs reveal that the acid secretory response and its duration are relate to the duration of feeding while in human cephalic phase is stimulated by having subjects visualize and smell of an appetizing meal as chew and split out the oral contents. This cephalic phase is also triggered by hypoglycemia.

Acetylcholine, histamine and gastrin have a synergistic effect, which is greater than the sum of the individual response. The parasympathetic fibres also innervate smooth muscle of the stomach to promote gastric motility. Emotions such as anger fear and anxiety may slow down digestion in the stomach because they stimulate the sympathetic nervous system that inhibits gastric activity.

The cephalic phase stimulation of gastric secretion continues into the first 30 min. of a meal but gradually declines in importance, as the next phase becomes predominant.
3. Gastric Phase

The excitatory pathway of this phase is triggered by the presence of food in the stomach that influences gastric acid secretion through mechanical distension and chemical mechanism. Distention of the stomach stimulates acid secretion by the activation of both long vagovagal reflexes as well as short cholinergic intra gastric reflexes.

The peristaltic waves mix the food with gastric juice and when they become strong enough, a small quantity of chyme about 10 to 15 ml, passed through the pyloric sphincter to duodenum. As the pH of stomach chyme returns to low level and the stomach walls are not distended because chyme has passed into the small intestine. This negative feed back cycle turns down the secretion of gastric juice.

Distension of the stomach and the presence of partially digested proteins stimulate G-cells in the mucosa of the pyloric antrum to secrete hormone gastrin. This gastrin stimulates growth of the glands and secretion of large amount of gastric juice. It is also increases the motility of the stomach and relaxes the pyloric sphincter and ileocecal sphincter.

Gastrin secretion is inhibited when the pH of gastric juice drops below 2 and stimulated when pH rises. This negative feed back mechanism helps to provide and optimal low pH for the functioning of pepsin, killing of microbes and denaturation of proteins in the stomach. Other chemical substances that stimulate acid secretion include caffeine, calcium salts and beverages like coca-cola, coffee, milk, bear and wine also stimulate acid secretion.

4. Intestinal Phase

This intestinal phase of gastric regulation refers to the inhibition of gastric activity when chyme enters into the small intestine. The presence of food in the upper portion of small intestine particularly in
the duodenum can cause the stomach to secrete small amounts of gastric juice. This is partly because of the release of small amount of gastrin that is also released by the duodenal mucosa in response to distension or chemical stimuli. However several intestinal factors can also inhibit gastric secretion and these often completely override the excitatory factors. These inhibitory factors are of two types: (a) Hormonal factors (b) Neuronal factors.

(a) Hormonal factors
When chyme containing fatty acids and glucose leaves the stomach and enters into the small intestine, it starts secretion of various gut peptides like gastric inhibitory peptide (GIP), neurotensin, glucagone, vasoactive intestinal polypeptide (VIP) and cholecystokinin (CCK). The humoral agents or agents that inhibit gastric acid secretion are referred to as enterogastrone. This inhibitory peptide includes GIP, neurotensin and peptide YY (NYY).

GIP is released by fat. However in humans, GIP is only a weak inhibitor of acid secretion\textsuperscript{84}. This would suggest that at least in humans, GIP is not a major enterogastrone. Peptide YY is released by fat in the distal small intestine and proximal colon. Furthermore, PYY inhibits only meal stimulated acid secretion but does not inhibit pentagastrin or histamine stimulated secretion. Therefore it is unlikely that PYY plays a major role as an enterogastrone.

The most likely current candidate as an enterogastrone is neurotensin. It is also released by fat in the distal small intestine and inhibits gastric acid secretion. It is very potent inhibitors in the innervated stomach. Following vagotomy the inhibitory effect of neurotensin is abolished. In humans there is direct correlation between the plasma response to neurotensin and the inhibition of pentagastrin stimulated acid secretion.
(b) Neuronal factors
The presence of food in the small intestine during the intestinal phase also initiates a neural reflex called the 'entero gastric reflex'. Nerve impulses carried to the medulla from the duodenum return to the stomach and inhibit gastric secretion and motility. These impulses ultimately inhibit parasympathetic stimulation and stimulate sympathetic activity. Stimuli that initiate this reflex are distention of duodenum and the presence of fatty acids and glucose in chyme in the duodenum.

2.5d Mediators in Gastric Mucosa:

2.5d (A) Neuronal mediators in the gastric mucosa
(a) Cholinergic non-cholinergic processes

The direct effect of cholinergic stimulation on the gastric microcirculation may be obscured by concurrent induction of acid secretion and hence on accompanying vasodilation. However, an increase in mucosal blood flow following vagal stimulation has been observed to precede the secretion of acids, indicating a direct vasodilation action\textsuperscript{85,86}.

The gastric vasodilation induced by vagal stimulation can be blocked by hexamethonium and reduced but not abolished by atropine in doses sufficient to abolish the response to acetylcholine\textsuperscript{85,87,88}. This could suggest the release of vasodilator mediators other than acetylcholine acting on non-muscarinic sites following vagal stimulation. Local Non-Adrenergic Non-Cholinergic (NANC) neuronal processes within the gastric mucosa modulator its ability to withstand noxious challenge.

Thus local infusion through the left gastric artery of the neurotoxin, tetrodotoxin that did not itself induce gastric mucosal injury,
substantially potentates the hemorrhagic damage following local administration of PAF\textsuperscript{89}. In contrast, pretreatment with atropine or the adrenoceptor antagonists like phentolamine and propranolol did not augment such mucosal injury, suggesting the involvement of a NANC neuronal pathway\textsuperscript{89}. In further study local infusion of tetrodotoxin also potentates mucosal injury induced by intra arterial administration of a vasoconstrictor thromboxane mimetic\textsuperscript{90} as well as that brought about by intra-gastric application of acidified ethanol\textsuperscript{91,92} again indicating the involvement of a local neuronal mechanism in the regulation of mucosal integrity.

(b) Sensory neurons
The release of vasodilator neuropeptides from afferent sensory neurons through ad local reflex has been suggested to be a protective mechanism in the gastric mucosa. Much of the early evidences for such a role of sensory neurons come from studies with capsaicin pretreatment, a pungent extract of red peppers, which can deplete primary afferent sensory neurons of their neuropeptides content and cause their functional ablation\textsuperscript{91-94}. Thus capsaicin pretreatment which damage following a number of proulcerogenic procedures including acid distention and pylorus ligation as well as challenge with indomethacin, ethanol and PAF\textsuperscript{89,95-97}.

(c) Cacitonin gene related peptide (CGRP)
The predominant neuropeptide localized by immuno-histochemical techniques in capsaicin sensitive neurons in the rat stomach is CGRP\textsuperscript{94} and such neurons are found in close proximity to the submucosal microvasculature\textsuperscript{93,98,99}.

The neuropeptide occurs predominantly in the form of a \(\alpha\)-CGRP in the sensory neurons innervating gastro intestinal tissue\textsuperscript{100}. Intragastric instillation of capsaicin to stimulate mucosal sensory neurons induces acute gastric mucosal vasodilation\textsuperscript{92,101}. This acute
hyperemia involves the release of CGRP from spinal sensory neurons innervating the gastric mucosa, since it was inhibited by concurrent infusion of the CGRP receptor antagonist\textsuperscript{102}.

Subcutaneous or intra arterial administration of α-CGRP inhibited the gastric injury induced by intra gastric instillation of aspirin or ethanol\textsuperscript{103,104}. Local infusion of CGRP also prevented the vascular and hemorrhagic infusion of endothelin-1\textsuperscript{105}. Intravenous administration of antisecretory doses of α-CGRP did not alter rat mucosal blood flow\textsuperscript{106} whereas intravenous administration of higher doses of CGRP increases blood flow in the rat and rabbit stomach\textsuperscript{107}.

Thus CGRP has the profile to actions compatible with its proposed role as an endogenous vasoactive mediator involved in the regulation of gastric blood flow and integrity.

(d) Neuropeptide Y

In gastrointestinal tract, NPY like immuno-reactivity has been found in sympathetic nerves associated with blood vessels\textsuperscript{98,108} and in enteric neurons originating from the myenteric and sub mucosal plexus\textsuperscript{99,106,109}.

In addition peptides that are structurally similar to NPY including pancreatic polypeptide (PP) and polypeptide YY (PYY) are also found in the gut\textsuperscript{110,111}. Systemic administration of NPY stimulates duodenal alkaline secretion, through vagal non-cholinergic neuronal mechanism\textsuperscript{112}. The release of immuno reactive NPY from the rat isolated stomach can be provoked by acetylcholine, probably through nicotinic receptor stimulation of intrinsic ganglia\textsuperscript{113} while intracerebro ventricular administration of NPY stimulates both gastric acid and pepsin secretion through vagally mediated process\textsuperscript{114}.
2.5d (B) Endothelin Derived Factor

(a) Endothelin 1

The vascular endothelium cells synthesize a 21-residue peptide known as endothelin-1 (ET-1) which can exert vasoconstrictor actions in the perfused vasculature of the rat isolated stomach\textsuperscript{115, 116} and in vivo\textsuperscript{117, 118}. Furthermore intravenous infusion of ET-1 augments mucosal damage induced by intragastric instillation of ethanol or acid\textsuperscript{115, 119}.

Atropine, 5-lipoxygenase inhibitor and PAF-receptor blocker was not inhibited the ET-1 induced mucosal damage\textsuperscript{115, 120} showing that no involvement of adrenergic or cholinergic mechanism, local release of vasoconstrictor leukotriene and PAF in ET-1 induced vasoconstriction. Furthermore capsaicin pretreatment of morphine administration substantially elevated mucosal damage provoked by ET-1 while close of arterial infusion of CGRP inhibited the damage\textsuperscript{105}.

This finding suggests an interaction between the vascular effects of ET-1 and those of sensory neuropeptides and prostaglandins. The Endothelin level in gastric vein blood and NOS activity in gastric mucosa increased up to irritation\textsuperscript{121}.

(b) Nitric oxide

Endothelial cells also release another highly labile humoral vasodilator substance originally known as endothelium derived relaxing factor (EDRF), which mediates the vascular relaxation induced by agents known as acetylcholine and bradykinin\textsuperscript{122, 123}. It is known that nitric oxide (NO) accounts for the biological properties of EDRF\textsuperscript{124, 125}.

NO is a prime mediator of the blood flow changes associated with acid secretion whereas inhibition of NO biosynthesis appeared to have no direct acute effect on the stimulation of acid secretion. Endogenous
NO interacts with sensor neuropeptides like substance P and CGRP modulates the gastric mucosal integrity\textsuperscript{126}.

(c) Epidermal growth factor
Epidermal growth factor involved in maintenance of gastric mucosa integrity, cytoprotection and ulcer healing\textsuperscript{127}. Gastric ulceration stimulates epithelial cell proliferation and over expression of epidermal growth factor (EDF) and EDF-receptor (EDF-R) in the mucosa bordering necrosis. Experimental gastric ulcer healing involves activation of EGF-R-ERG signal transduction pathway.
2.6 Peptic Ulcer Disease:

2.6a Types of Peptic Ulcer Disease:

Peptic ulceration (both gastric and duodenal), reflux esophagitis (in which gastric juice causes damage to esophagus) and the Zollinger Ellison Syndrome (a condition which is due to a gastrin producing tumour) are the principal pathophysiological conditions of upper gastrointestinal tract. The term ‘peptic ulcer’ refers to ulcers that occur as a result of the action of pepsin and acid on the mucous lining. Peptic ulcer represents a major health problem affecting large populations in all geographical regions, both in terms of morbidity and mortality. Although acid is thought to be the most important cause of ulcerogenesis, mucosal defense is also considered to a dominant factor.

Peptic ulcers occur in the first few centimeters of the duodenum. In addition, peptic ulcers frequently occur along the lesser curvature of the antral end of the stomach or, more rarely, in the lower end of the esophagus where stomach juice frequently refluxes. A peptic ulcer called a ‘marginal ulcer’ also frequently occurs whenever surgical opening, such as a gastrojejunostomy, is made between the stomach and some portion of the small intestine.

Types of Peptic Ulcer Disease:

Peptic ulcers arise as either acute or chronic ulcers.

Acute Peptic Ulcer and Erosions:

Acute erosions and ulcers usually occur in the stomach, though Cushing’s and Curling’s ulcers also occur in the duodenum.

Acute peptic ulcers develop:

- As part of an acute gastritis
- As a complication of a severe stress response
- As a result of extreme hyperacidity
Aetiology:

Deeper extension of the erosions in acute gastritis resulting from NSAIDs or acute alcohol over dosage can produce frank ulcers. About half of the cases follow ingestion of nonsteroidal antiinflammatory drugs (NSAIDs) like aspirin or butazolidine.

These agents can damage the gastric mucosa directly and by acting systemically, inhibiting enzyme cyclooxygenase that result in decreased prostaglandin synthesis and impaired mucosal defense (prostaglandins have a protective effect on gastric mucosa). They tend to cause erosions rather than acute ulcers.

The urea splitting organism H. pylori has been shown to exist on the epithelial surface deep to the mucous layer in a number of patients with gastroduodenitis and is thought to interfere with cytoprotection by virtue of the formation of ammonia from urea.

Acute ulcers occur also in a heterogeneous group of conditions where stress seems to be the common denominator. Stress ulcers may result due to prolonged hypotension following major trauma or cerebrovascular accidents, surgery, sepsis, severe burns (Curling's ulcer), myocardial infarction and respiratory failure. Extreme hyperacidity, can lead to multiple acute ulcers in the antrum, the duodenum and even the jejunum.

The erosions are mostly multiple and may occur in any part of the stomach, but in the duodenum they are mostly confined to the first part. They do not penetrate the muscularis mucosa. Acute ulcers have the same distribution, but penetrate the muscularis mucosa for a variable distance. When healing occurs they do not leave any scar.
**Chronic Peptic Ulcer:**

The term peptic ulcer covers both duodenal and gastric ulcers. Gastric and duodenal ulcers differ in their epidemiology, incidence and pathogenesis.

A peptic ulcer may occur either if the acidity is very high or if there is lowered resistance of the mucosa. It is believed that the first factor is responsible for the duodenal ulcer and the second for gastric ulcer. Chronic peptic ulcers seem to occur most frequently at mucosal junctions.

**Gastric Ulcer:**

It may be simpler to differentiate two types of gastric ulcers. The common variety, where gastric acid secretion is normal or low and the ulcer is situated on the lesser curve near the incisura angularis.

The less common variety, where it occurs in association with duodenal ulcer, is located close to the pylorus and the gastric acid secretion is similar to that of duodenal ulcer.

**Pathology:**

The primary event in the pathogenesis of gastric ulcer is considered to be the altered mucosal resistance with consequent damage by acid, pepsin and other destructive agents.

Gastric ulcer is larger in size than a duodenal ulcer. A chronic gastric ulcer may become malignant, but the incidence does not exceed 0.5% that occurs later in life. Males are slightly more affected than females and peak incidence occurs between the ages of around 55 and 65 years of age. Interestingly there is a slightly greater increase in incidence in people who are of the blood group A.
An array of pathophysiological abnormalities found in gastric ulcer patients include:

- Gastric motility and emptying defects.
- Duodeno-gastric reflux, bile reflux due to pylorus defects.
- Gastritis.
- Mucosal ischaemia.
- Altered Bicarbonate production.

Besides these, other imbalances may occur in aggressive factors and defensive factors.

**Duodenal ulcer:**

Duodenal ulcers account for the majority of PUD ulcers, occurring 5 times more frequently than gastric ulcers. In the duodenum, the ulcer is nearly always situated in the first part and is most common on the anterior wall. The ulcer may at times be on the posterior wall.

The situation, in which there is both a posterior and an anterior duodenal ulcer, is referred to as "kissing ulcers". Occasionally the ulceration may be so extensive that the entire duodenal cap is ulcerated and gets devoid of mucosa. A chronic duodenal ulcer penetrates the mucosa and into the muscle coat leading to fibrosis. The fibrosis causes deformities such as pyloric stenosis. When an ulcer heals a scar can be observed in the mucosa. Sometimes there may be more than one duodenal ulcer.

In the 19th century, gastric ulcers were common than duodenal ulcers. But in the 20th century a change has taken place, and duodenal ulcers have overtaken gastric ulcers. Duodenal ulcers are much more common and higher rate of occurrence is observed in males between 20 and 30 years of age.
Patients of duodenal ulcers have one or more physiologic defects which either alone or in combination increase the concentration of aggressive factors present in the duodenum.

These defects include:
- Increased number of parietal cells.
- Increased parietal cell sensitivity to gastrin.
- Increased basal and stimulated secretory rates of acids and pepsin.
- Increased meal stimulated gastrin release.
- Increased serum pepsinogen concentration.
- Increased rate of gastric emptying.
- Decreased acid induced inhibition of gastrin release of acid secretion.
- Impaired bicarbonate secretion in pancreatic juice and hence failure to buffer gastric acid secretion.
- Impaired duodenal mucosal defense.

2.6b Pathophysiology of Peptic Ulceration:

Pathophysiology of gastric ulcer has still not been fully elucidated. It is generally accepted that pathophysiology of peptic ulcer disease lies mainly in imbalance of acid secretory mechanisms (aggressive factors) and local mucosal defensive factors (defensive factors). Dole et al. (1994) gave the classification of factors that contribute to peptic ulcer disease.

(A) Aggressive Factors:
1. Endogenous factors
   - Gastric acid secretion
Review of Literature

- Pepsin secretion

2. Exogenous factors
- NSAIDs
- Corticosteroids
- Smoking
- H. Pylori infection
- Alcohol
- Caffeine
- Oxygen free radicals

(B) Defensive Factors:

(1) Gastric Mucosal Barrier:

(a) Pre-epithelial Factors
- Mucous
- Bicarbonate

(b) Epithelial Factors
- Hydrophobic cell membrane
- Rapid cell turnover
- Chemical agents
- Restitution

(c) Sub-epithelial Factors
- Blood flow
- Angiogenesis

In addition to above other factor such as pancreatic enzyme like secretin inhibits gastric acid secretion and pepsin secretion. Endothelial growth factor also promotes peptic ulcer healing by inhibiting gastric acid secretion. Besides these local factors of GI tract, general factors like vagal effects, hormonal effects (Histamine, Nor adrenaline), insufficient circulation, shock, general ischaemia etc. can also become a part of pathogenesis of peptic ulcer...
disease. Constitutional and environmental factors like sex, age, temperament, family history; social class, geographical difference, occupation, food habit etc. can also be a cause of peptic ulcer disease.

(A) Aggressive Factors:
Aggressive factors that damage the mucosa of the gastrointestinal tract may originate endogenously or exogenously.

1. Endogenous Factors:
(a) Gastric acid secretion:
Gastric acid is one of the most important causative factors of peptic ulceration. The acid gradient is created in the parietal cells using adenosine triphosphate as an energy source. H⁺-K⁺ ATPase is a primary active transporter that maintains the hydrogen gradient at a cell lumen ratio of 1:2,000,000. This activity has been shown to be the final step in acid production. Several endogenous substances are known to stimulate H⁺-K⁺ ATPase by interaction with parietal cell receptors. Histamine acts at a H₂ receptor on the parietal cell. Acetylcholine acts at a muscarinic receptor on the parietal cell.

(b) Pepsin:
Pepsin is a proteolytic enzyme. It is secreted as a zymogen, pepsinogen that is activated in presence of HCl. The secretion of pepsinogen is stimulated by gastrointestinal hormones (CCK, gastrin), neuropeptides (VIP), histamine and isoproterenol. These stimuli activate two different intracellular effectors systems in chief cells. CCK and gastrin appear to stimulate the hydrolysis of phosphatidylinositol, resulting in an increase in cytosolic calcium and activation of protein kinase C. Histamine, secretin, VIP and isoproterenol stimulate pepsinogen secretion by activating adenyl cyclase, leading to an increase in intracellular levels of cyclic AMP and activation of cyclic AMP dependent protein kinase. The optimum pH required for pepsin activity is
1.8 to 3.5 and beyond pH 5, it is almost inactive. In gastric and duodenal ulcer patients, there is a greater proportion of pepsin I than in normal subjects. This result could be significant because pepsin I appear to digest mucus more rapidly than the major form of the enzyme, pepsin III\textsuperscript{131}.

2. Exogenous Factors:

(a) NSAIDs:
NSAIDs produce a spectrum of injury to the gastro-duodenal mucosa, from hemorrhages and petechies to erosions and ulcers. A number of epidemiological studies indicate that NSAIDs are more likely to induce GU rather than DU, though they induce bleeding and perforation in both types of ulcers. The likely causes of NSAIDs-induced gastroduodenal mucosal injury are:

*Inhibition of prostaglandin synthesis:*
Reduction of endogenous gastroduodenal PG concentrations by NSAIDs is a consequence of inhibition of the enzyme cylooxygenase (COX-1), in the PG cascade. It forms the basis of their anti-inflammation action as well.

*Back-diffusion of acid:*
Intracellular accumulation of protons dissociated from high concentration of the acidic drug, which rapidly accumulate in mucus (superficial) and parietal cells. This causes localized acid accumulation (back-diffusion of acid)\textsuperscript{132}.

*Prolongation of bleeding time:*
Aspirin, in doses below 75 mg selectively inhibits thromboxane synthesis without significantly affecting the synthesis of PGs, including PG\textsubscript{I\alpha}, and induce prolongation of bleeding time and can increase spontaneous bleeding rate from human gastric mucosa\textsuperscript{133}.
Relative increase in leukotrienes levels:
Selective inhibition of the cylooxygenase pathway, in eicosanoid synthesis, by NSAIDs can induce a relative increase in products derived from the alternate lipoxygenase pathway. Thus, indomethacin is known to induce a relative increase in leukotriene C₄ at the cost of reduced PGE₂ levels, which may induce mucosal vasoconstriction and enhance NSAID-induced injury. Sloughing of the protective mucus layer, discharge of mucus from epithelial cells and cell desquamation due to denaturation of mucus glycoproteins and mucus cell proteins.

(b) Alcohol:
It has been well known that intra-gastric installation of absolute alcohol causes focal area's gastric mucosal hyperemia, necrosis, edema and hemorrhage.

Ethanol rapidly penetrates the gastric mucosa, and apparently causes cell and plasma membrane damage, that results in increased membrane permeability leading to intracellular accumulation of sodium and water.

When the increased membrane permeability fails to maintain the normal electrolyte distribution between intracellular and extra cellular compartments, the massive intracellular accumulation of calcium represents a major step in the pathogenesis of gastric mucosal injury.

In the gastric mucosa, these damages result in cell death and exfoliation in the superficial epithelium, i.e. erosion. Ethanol produces damage to gastric mucosa by various contributing factors, which includes mucosal blood flow, platelet thromboxane, damage to capillary endothelium, release of LTC₄/D₄ and platelet activating factor. Further, gastric lesions caused by ethanol
have been attributed to free radical damage, which results in lipid peroxidation products\textsuperscript{139}.

(c) Cigarette Smoking:
Study after study has repeatedly shown that smoking affects ulcer disease in three ways. It’s estimated that smokers are twice more likely to develop an ulcer in the first place than non-smokers\textsuperscript{140}.

It slows down the rate of healing\textsuperscript{141,142}. Cigarette smoking increases your risk of an ulcer recurring after it has been successfully treated. Smoking stimulates and increases the acid output in the stomach. It decreases blood flow to the area. Smoking reduces the level of protective prostaglandins in the area, impairs therapeutic effects of H\textsubscript{2} antagonists, may stimulate pepsin secretion, increases the risk of harmful effects of H. pylori and increases the production of free radicals, vasopressin, secretion of endothelin by the gastric mucosa and production of platelet activating factor.

(d) Diet:
There is some evidence that a high fiber intake is protective against the risk of developing duodenal ulcers. This could be due to the high levels of carbohydrates in fiber rich foods, which seem to have a protective effect. Eating a high fiber diet also seems to reduce the rate of duodenal ulcer recurrence (remission).

The existence of a dietary protective factor has been proposed, the nature of which little is known but is likely to be lipid or peptide present in cereal fiber, pulses and green vegetables\textsuperscript{143}. 
(e) Ulcer Bug (H. pylori):
H. pylori is a gram negative, spiral shaped with a smoother outer coat with 4-6 bulbous tipped sheathed flagella at one end, which help it to penetrate the mucosa and colonize on the surface of gastric antrum.

It naturally infects humans and monkeys. It is noninvasive, living in the mucus and some adherent to mucosa. Its strong urease property causes the release of ammonia, which provides the environment of increased pH in its surroundings, enabling the organism to survive in highly acidic gastric atmosphere\textsuperscript{144}.

H. pylori infection is present in 90-95 % of the patients with duodenal ulcer and 70% of those with gastric ulcer. Remaining 30% of the ulcers are attributed to the use of NSAIDs.

\textbf{Its Role in Ulcer Disease}

\textit{Altering the Acid / Alkaline Balance:}

H. pylori display potent urease activity, a property that may have important implication in the pathogenesis of ulcer\textsuperscript{145}. Urease, in turn, converts the urea present in stomach juices into bicarbonate and ammonia. These substances work together to neutralize stomach acids, and cause the stomach to become an alkaline environment, extremely suitable for the survival of the bacteria.

\textit{Damaging the Stomach Lining:}

Because of their spiral shape and the way they move, the H. pylori bacteria have been shown actually to penetrate the stomach's protective mucous lining. Here they can safely produce substances that weaken the stomach's protective mucus and make the stomach cells more susceptible to the damaging effects of acid and pepsin.
Sticking to Stomach Cells:
Excess stomach acid and other irritating factors cause inflammation of the upper end of the duodenum. In some people, over long periods of time, this inflammations result in the production of stomach-like cells called duodenal gastric metaplasia. Because H. pylori is very attracted to stomach cells, it attacks these areas of gastric metaplasia, causing further tissue damage and inflammation, which may result in an ulcer.

Stimulating acid flow:
Studies now suggest that H. pylori also stimulate the stomach to produce more acid. It does this by increasing the circulation and release of gastrin. They decrease the amount of somatostatin that is released onto the neighboring G cells, which triggers the G cells to secrete more gastrin and acid.

Inflammatory mediators release:
H. pylori produce vaculoating cytotoxin (VacA), ammonia and ammonium products (mono-N-chloramine), phospholipase (Phospholipase C and phospholipase A₂), chemotoxins and platelet-activating factor (PAF), which act on epithelial cell surface and damage the defense system. Urease also stimulates dose dependently the production of IL-1β, IL-6, IL-8, TGFα peptides and mRNA. This local production of cytokines by urease stimulates mononuclear phagocytosis and this may play a key role in H. pylori induced mucosal inflammation. There is increasing evidence that these organisms have a cytopathogenic role, with the mucus secreting cells as the likely target. Weakening of the mucosal barrier renders the mucosa exposed to acid-pepsin assault. The bacteria also appear to play a significant role in non-ulcer dyspepsia.
(f) Free Radicals:
Gastric damage which results from inadequate vascular perfusion could subsequently result in release of tissue damaging mediators, among which oxygen derived free radicals are likely candidates. These highly labile and reactive moieties have the capacity to disrupt cell membranes by lipid peroxidation of membrane constituents and destroying the interstitial matrix following degradation of collagen and hyaluronic acid. The cellular injury can induce the further release of endogenous cytotoxic agents, including lysosomal enzymes. Such damage is reduced by agents that interfere with free radical generation, or by radical scavengers. The ever-growing significance of antioxidant nutrients such as alpha tocopherol, beta-carotene and ascorbic acid has come to light.  

(g) Corticosterone:
Corticosteroids decrease the mucosal barrier and increases acidity. Steroids reduce the rate of shedding of gastric mucosal cells by decreasing the rate of cell renewal. Steroids potentiate the development of ulcers due to other factors (e.g. aspirin ingestion).  

(B) Defensive Factors:
1. Gastric Mucosal Barrier:

Taken in isolation, mucus and bicarbonate secretion appear to have limited effect against luminal acid. However, when considered as a single co-joint system, they provide an effective mean of mucosal protection against acid-pepsin assault. It has been divided into three mechanisms:
(a) Pre-epithelial Mechanisms:
It includes mucus gel layer and trapped bicarbonate.

Mucus:

Gastroduodenal Mucus Gel:
Mucus is a viscous gel that adheres to and blankets the entire gastrointestinal tract, keeping it lubricated and protected from infective, chemical and physical insults. Mucus is secreted into the gastroduodenal lumen by surface epithelial cells and mucus neck cells (goblet cells) and submucosal Brunner's glands. The gel is 95% water and 5% glycoprotein. The secretion has two components, a water insoluble gel adherent to the mucosal surface and soluble mucus in the lumen. Mucus has several functions, which contribute significantly to cytoprotection:

Lubrication and mechanical protection:
The mucus gel covers the entire surface of the gastroduodenal mucosa with a variable thickness of less than 500 μm, lubricating the mucosa and forming the first line defense against noxious gastric contents. It exists in a dynamic balance between production on one hand and degradation by pepsin and shear forces on the other.

Mixing barrier:
Mucus is readily permeable to H+ ions and provides a mixing barrier at the mucosal surface, preventing the relatively small amounts of bicarbonate ions from mixing with the bulk of H+ ions in the lumen, thus confining the neutralization at the mucosal surface, ensuring a pH gradient across the mucus layer. The glycoprotein molecules appear to retard the diffusion of H+ ions, the rate of diffusion being four times slower than that in unstirred water. However, this retardation of H+ ion diffusion across the mucus layer is unlikely to be the sole factor protecting the mucosa from acid since this
would require the renewal of a mucus layer of at least 0.4 mm thickness, ten times each second, in order to maintain epithelial surface neutrality.

*Unidirectional flux of H+ ions:*
The passage of acid secreted by the parietal cells appears to occur through mucus channels, which are highly sulphated. Periodically, instead of an increasing pH gradient across mucus, there is a sudden drop in pH. A concentration gradient of Na+ ions appear to be generated across the mucus layer by the continuous activity of Na+/K+-ATPase at basolateral membrane of mucus cells. Sodium diffusing across this gradient will generate a diffusion potential positive at the cell-facing surface of the mucus gel. It is postulated that this potentially moves H+ ions into the lumen and retards back diffusion.

*Prevention of back diffusion of pepsin and pepsinogen activation:*
Since mucus exploits the phenomenon of phase separation, it attenuates back diffusion of macromolecules like pepsin from gastric juice. Mucus also has a function in transporting pepsinogen and preventing its activation into pepsin.

*Repair of superficial mucosal damage:*
Following damage of surface cells, there is rapid release of large amounts of mucus and plasma proteins, which together with the cellular debris form a coating over the destroyed area, providing a favorable microenvironment for repair and restitution.

*Antibacterial activity:*
Identification of bacteria in human stomach in gastritis indicates that normal gastric mucus may have antibacterial activity.
Bicarbonate Secretion:
It has been long realized that the stomach secretes alkali apart from acid. Secretion of alkali by surface epithelial cells into the unstirred mucus layer, with consequent formation of a pH gradient across the gel, would afford far more protection than mucus alone. There is now evidence that the proximal duodenal mucosa also secretes bicarbonate. 

The quantum of alkali secretion by the stomach and proximal duodenum is 5-10% of maximal acid output. The gastric fundus appears to transport these ions by a metabolically dependent trans-cellular route while the antrum transports about one third of its bicarbonates by inter-cellular channels by passive diffusion. The situation is more complex in the duodenum. Pancreatobiliary secretion and possibly the Brunner’s glands, obviously contribute to net duodenal alkali. The endogenous bicarbonate secretion by the surface duodenal cells contributes minimally to overall alkalinization and the extra-cellular bicarbonate remains the major source of transported alkali.

The vascular arrangement of the mucosa helps in facilitating the transport of bicarbonate released by the parietal cells towards the bicarbonate secreting cells of the surface epithelium.

The mucus gel provides an ideal zone for the restriction of acid-bicarbonate interaction close to the cell surface. Mucus and bicarbonate secretion complement each other and little protection can be afforded by either functioning in isolation. Bicarbonate flux alone cannot lower the H+ ion concentration in the mucus gel to an extent, which can provide the apical membrane of the surface epithelium with a near neutral pH.
(b) Epithelial Mechanisms:
This mechanism includes chemical groups (sulfhydryl compounds), which fight against oxidative stress as well as hydrophobic cell membrane, lipid cell turnover and processes of restitution are also important in maintaining the epithelial cell layer\textsuperscript{138}.

Sulfhydryl Compounds:
Non-protein sulfhydryl compounds (SC) are present in high concentrations in gastric epithelium. The major component of SC is reduced glutathione, which is capable of binding reactive free radicals that accumulate during tissue ischaemia and injury induced by noxious agents like ethanol. Reduced glutathione induces gastric mucosal protection by increasing SC levels. They appear to be involved in PG synthesis and PG receptor activation, and may also directly influence membrane permeability, cell adhesion, toxicity of free radicals and prevent the release and effects of mediators likely to be involved in inducing mucosal damage\textsuperscript{152}.

Hydrophobic Cell Membrane:
One of the important properties of the stomach wall is its hydrophobicity in spite of its hydrophilic mucoid layer. This is attributed to the surface active phospholipids (SAPs) present largely as the inter-granular matrix material of unsecreted mucus, which in any case provides the major resistance to hydrogen ions diffusing from the lumen of the stomach to the vital organelles of surface mucus cells. While each of the barrier breakers displays greater affinity for SAP, Bile salts chemically complex with SAP while NSAIDs inhibit the production of PG controlling SAP synthesis\textsuperscript{131}.

Mucosal Restitution:
The process of restitution involves rapid re-epithelization characterized by the migration of remaining viable surface mucus cells and mucus neck cells from
the crypts to cover the damaged surface. The morphological healing is accompanied by physiological return of function characterized by concomitant flux of bicarbonate ions across the epithelium. This restitution process is augmented by high bicarbonate concentrations and is inhibited by luminal pH below 4. PGs do not appear to be involved in restitution.

**Gastric Mucosal Renewal:**
The rapid proliferation of the gastric mucosa plays an important role in mucosal protection during normal state and following mucosal damage. In the latter situation, the undifferentiated neck cells proliferate, migrate towards the lumen and differentiate into surface epithelial cells. Other cells migrate downwards towards the depth of the gland and differentiate into parietal and chief cells. An increase in gastric mucosal cell turnover contributes significantly to mucosal defense since damaged cells are rapidly replenished.

(c) **Sub-epithelial Factors:**

**Blood Flow in the Gastric Mucosa:**
An adequate blood flow is essential for gastric mucosal tissue to withstand the challenge of both endogenous and exogenous aggressors. Blood flow enhances the mucosal defense mechanisms by ensuring the delivery optimum quantum of oxygen, nutrient and bicarbonate to the surface epithelial cells and removing H+ ions, which have penetrated the mucus-bicarbonate and epithelial barrier.

**Angiogenesis:**
Angiogenesis, the formation and growth of new capillary blood vessels, is an important process in many physiological conditions such as embryonic development and wound healing. It prevents the mucosal damage by ulcers and maintains blood flow.
Neuronal and Endothelin Derived Mediators in the Gastric Mucosa:  
Cholinergic and Non-cholinergic Processes:
The direct effects of cholinergic stimulation on the gastric microcirculation may be obscured by concurrent induction of acid secretion and hence an accompanying vasodilation. However, an increase in mucosal blood flow following vagal stimulation has been observed to precede the secretion of acid, indicating a direct vasodilator action\textsuperscript{155}.

Sensory Neurons:
The release of vasodilator neuropeptide calcitonin gene related peptide (CGRP) from afferent sensory neurons has been suggested to be a protective mechanism in the gastric mucosa. A pungent principle of red pepper can deplete primary afferent sensory neurons of their neuropeptide content and cause their functional ablation\textsuperscript{156}.

Endothelium – derived Factors:  
Endothelin (ET - 1):
Vascular endothelial cells synthesize a 21 – residue peptide known as endothelin-1 (ET-1) that can exert vasoconstrictor actions both in vitro and in vivo\textsuperscript{157}. Local intra-arterial infusion of pico mole quantities of ET-1 induces substantial gastric mucosal vaso-congestion and hemorrhagic injury in the rat\textsuperscript{158}. ET-1 and ET-3 are equipotent in inducing rat gastric hemorrhage following intravenous infusion\textsuperscript{159}.

Interactions of Endothelin in the Gastric Mucosa:
The injurious action of ET-1 on the rat gastric mucosa following its intra-arterial infusion was not inhibited by pretreatment with atropine nor α - and β- adrenoreceptor antagonists, indicating no involvement of adrenergic or cholinergic mechanisms. In addition, pretreatment with a 5-lipoxygenase
inhibitor had no effect on the mucosal injury, showing that local release of vasoconstrictor leukotrienes\textsuperscript{158}.

**Prostacyclin:**
Prostacyclin can inhibit gastric acid secretion in a number of experimental preparations\textsuperscript{160, 161} and can stimulate the secretion of bicarbonate, a luminal protective factor\textsuperscript{162}. Prostacyclin and its more stable analogues, like other prostanoids particularly of the E series\textsuperscript{143}, exert potent protective actions against gastric mucosal damage in a number of experimental models\textsuperscript{163}.

**Nitric Oxide:**
NO plays an important role in the modulation of mucosal blood flow under resting and stimulated conditions, and has a key interactive role in the regulation of mucosal integrity; however, in excess, unregulated, liberation of NO has also ulcerogenic potential.

Both NO and CGRP appear to be involved in the processes underlying the mucosal vasodilatation following sensory nerve activation through acid back-diffusion into the mucosal tissue\textsuperscript{164}. Endogenous NO, sensory neuropeptides and prostanoids, which have distinct biochemical origins, may not only exert local vasodilator actions on the microcirculation essential for adequate blood flow under physiological conditions, but may act to enhance or preserve endothelial cells function and continuity, especially under conditions of challenge.

NO and enzymes that form prostacyclin are highly susceptible to attack by free radicals\textsuperscript{165} and interference with the actions of these mediators may be involved in the microvascular injury seen following the local release of free radicals. A reduction in NO formation or its inactivation by free radicals in
both endothelial cells and neutrophils\textsuperscript{166} may serve to initiate or to amplify endothelial injury.

2. \textit{Endogenous Prostaglandins}:
Endogenous Prostaglandins enhance mucosal resistance and protect the gastroduodenal mucosa against macroscopic damage induced by a variety of agents. The term "cytoprotection" was initially coined to describe the ability of PGs to augment gastroduodenal mucosal resistance to injury, independent of acid secretion inhibition. However, the term has fallen into disrepute since it implies that PGs directly enhance mucosal resistance, whereas the potential mechanisms involved in PG action are multiple, including stimulation of mucus and bicarbonate output\textsuperscript{167} gastric mucosal blood flow \textsuperscript{168}, strengthening of gastric mucosal barrier, decreasing gastric motility, increasing the release of endogenous mediators of gastric cytoprotection like sulfhydryls and EGF\textsuperscript{169}, scavenging free radicals\textsuperscript{152}, decreasing release of endogenous mediators of gastric injury-vasoactive amines and leukotrienes and stimulation of cellular growth and repair\textsuperscript{133}.

3. \textit{Others}:

\textbf{Gamma Amino Butyric acid (GABA)}

CNS mechanisms are important in the regulation of acid secretion. GABA and GABA mimetic have an ulcer attenuating effect not associated with central sedation or decreased acid secretion but could be by augmenting gastric mucosal defenses\textsuperscript{170}.

\textbf{Epidermal Growth Factor}

Epidermal growth factor (EGF) plays an important role in ulcer healing. EGF has been shown to be identical to urogastrone and is now designated URO / EGF. It is a potent inhibitor of gastric acid secretion.
The inhibition of acid secretion, and stimulation of cell proliferation and regeneration, induced by URO/EGF, can make significant contribution to ulcer healing\textsuperscript{171}.

2.6c Treatment of Peptic Ulcer Disease:

The lifetime prevalence of peptic ulcer disease is approximately 10\% and some physician estimate that 50\% of healthy individuals experience heartburn on a daily basis. The goals of therapy for ulcers are relief from pain, promotion of healing, prevention of recurrence and avoidance of ulcer perforation, gastric obstruction etc.

So, the therapeutic strategies are aimed at balancing aggressive factors against defensive or cytoprotective factors.

Classification of antiulcer agents:

1. Drugs with acid neutralizing activity:

Antacids:

Magnesium Oxide and Hydroxide, Magnesium Carbonate, Magnesium trisilicate, Aluminum Hydroxide, Sodium Carbonate.

The antacids diminish the quantity of free hydrochloric acid in the stomach by three mechanisms. (1) Direct neutralization of preformed acid (2) Adsorption of hydrogen ion plus adsorption and inactivation of pepsin as brought about by aluminum antacids and anion exchange resin. (3) Some new formulation of antacids in colloidal suspension, which form a protective coating over the gastric mucosa and the ulcer crater.

2. Drugs with antisecretory activity:

(A) Proton pump inhibitors (PPI)

(a) Reversible proton pump inhibitors (RPPI)
SK & F 96067 inhibits H+-K+ ATPase competitively with respect to the secondary transport ion, K+. It has shorter duration of action than Omeprazole.

(b) Irreversible proton pump inhibitors
Substituted Benzimidazoles like Omeprazole, Lansoprazole, Pantoprazole, Rebaprazole, Laminoprazole.

The H+-K+ ATPase located in the tubular vesicular and secretory membranes of the parietal cells in the stomach is the final common step in the secretion of hydrogen ions into the gastric lumen. Omeprazole is concentrated in the parietal cells (with a very low pH), where it gets protonated, trapped and converted to the active form, Sulphenamide, which forms stable disulfide linkage with sulfhydryl groups on H+-K+ ATPase. It cause 100% inhibition of acid secretion, both resting as well as stimulated but has no effect on bicarbonate secretion.

(B) H2 receptor antagonists
Cimetidine, Ranitidine, Famotidine, Nizatidine, Ebritidine, Roxatidine.

The H2 receptor antagonists inhibit gastric acid secretion elicited by histamine, gastrin and to a lesser extent by muscarinic agonists. The maximal acid inhibition achieved is approximately 80% and the volume and H+ ion concentration decrease. Amount of pepsin secreted also falls, as does the secretion of intrinsic factor. Cimetidine (200mg 3 times /day and 400mg each evening) for 4 weeks heals duodenal ulcers in about 60% of patients. Cimetidine is also effective in promoting healing gastric ulcer and treatment of oesophagitis, hemorrhagic gastritis or stress ulceration.
(C) Antimuscarinic agents
Pirenzepine, Telenzepine
Pirenzepine is a selective M1 receptor blocker with little affinity for M2, M3, M4 receptors. They reduce basal acid secretion and stimulated acid secretion to a lesser extent. They do not reduce food-stimulated secretion. In peptic ulcer patients where gastric hypermotility and muscle spasm are marked, anticholinergics may be useful.

(D) Histidine decarboxylase inhibitors
Epicatechin, Toxifen, Amentoflavon
These agents reduce the histamine content of fundus and antrum by inhibiting H+-K+ ATPase. The intensity of inhibition parallel with the number of phenoxy-OH groups in the molecules.

(E) Cholecystokinin Antagonist
Proglumide, Loxyglumide
It competitively blocks the gastrin receptors. It has been shown to be equally effective to Cimetidine in treating duodenal and gastric ulcer patients. No side effects have been reported. But high cost of this treatment than equally effective/ superior drugs.

3. Mucoprotective agents
(A) Prostaglandin analogues
Misoprostol, Rioprostil, Arboprostil, Trimoprostil, Enprostil
Nodoprost, Nileprost, Eniprost and Rosaprost are under clinical trial.
These are mainly PGE1 and PGE2 analogs.

(B) Bismuth compounds
Colloidal Bismuth subcitrate, Tri-potassium di-citrate bismuthate (TDB), Bismuth subsalicylate, Bismuth subgallate, Bismuth subnitrate.
Colloidal Bismuth subcitrate has negligible acid neutralizing capacity but can inhibit pepsin activity, increase mucus secretion and interact with proteins in the necrotic ulcer crater to form a barrier against acid diffusion. In addition it has been shown to cause detachment and lysis of Helicobacter pylori, which is thought to be an important etiologic factor in Type B, gastritis and peptic ulcer disease.

(C) Sucralfate
It is a complex of sucrose of octasulfate and polyammonium hydroxide and possesses negligible antacid activity. At pH less than 4, it undergoes extensive polymerization to form a sticky, viscous yellow white gel which adheres to the ulcer base and protects it from further acid assault.

(D) Carbenoxolone
It is a liquorice extract with a steroid like structure and possesses significant mineralocorticoid activity. It is thought to promote ulcer healing by increasing mucus secretion and altering the composition of mucus by augmenting glycoprotein synthesis. It also inhibits peptic activity by suppressing the activation of pepsinogen. It also inhibits enzymes that inactivate prostaglandins and increased half-lives of these prostaglandins, also enhance it beneficial effect. It may act partly by increasing the life span of the gastric mucosal cells and also by increasing pyloric tone to prevent bile reflux.

(E) Gefarnate
Gefarnate (Geranyl farnesylacetate) is a synthetic terpene that contains a number of isoprene units, the basic fragments from which pentacyclic ring structures such as steroids can be synthesized and with which the triterpenoid carbenoxolone bears some structural resemblances. It was originally extracted from the white headed cabbage. Gefarnate (200-400 mg
every 8 h) may possess the beneficial effects of carbenoxolone on gastric ulcer
disease but without side effects of the liquorice preparation.


Dual Therapy:

- TDB + metronidazole 80%
- Amoxicillin + Omeprazole 72-84%
- Ranitidine bismuth citrate + Clarithromycin 82-94%

Triple Therapy:

Triple regimens have the advantage of being shorter.

- Omeprazole + Clarithromycin + Amoxicillin 96.6%
- Omeprazole + Amoxicillin + plaunotol 100%

Quadrupole therapy:

It has advantage of shorter duration of treatment and thus the reduced cost
and a lower potential for development of antibiotic resistance.

Various regimens are

- Omeprazole + CBS + Tetracyclin + Metronidazole 98%
- Omeprazole + Amoxicillin + TDS + Metronidazole 95%
2.6d Plant Drugs as Antiulcer Agents:

Literature survey of plant drugs revealed that compounds with variety of chemical nature possess antiulcer activity. Many phytoconstituents and their derivatives established as antiulcer agents are listed here (Table: 2).

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<thead>
<tr>
<th>Name</th>
<th>Part</th>
<th>Chemical constituents</th>
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<tr>
<td>Anacardium occidentale Linn</td>
<td>Plant</td>
<td>Catechins&lt;sup&gt;172,173&lt;/sup&gt;</td>
</tr>
<tr>
<td>Asparagus racemosus Willd.</td>
<td>Root</td>
<td>Saponins&lt;sup&gt;174&lt;/sup&gt;</td>
</tr>
<tr>
<td>Azadirachta indica A. Juss.</td>
<td>Leaf, Fruit, Seed</td>
<td>Nimbidin&lt;sup&gt;16&lt;/sup&gt;</td>
</tr>
<tr>
<td>Brassica oleracea Linn</td>
<td>Leaf</td>
<td>Gefarnate&lt;sup&gt;13&lt;/sup&gt;</td>
</tr>
<tr>
<td>Bupleurum falcatum Linn.</td>
<td>Root</td>
<td>Polysaccharides&lt;sup&gt;175&lt;/sup&gt;</td>
</tr>
<tr>
<td>Centella asiatica Urban.</td>
<td>Leaf</td>
<td>Asiaticoside, madecassoside&lt;sup&gt;176&lt;/sup&gt;</td>
</tr>
<tr>
<td>Cinnamomum cassia Blume.</td>
<td>Bark</td>
<td>Volatile oil, Tannins</td>
</tr>
<tr>
<td>Croton cajucara Benth</td>
<td>Bark</td>
<td>Nor-clerodane diterpene trans-Dehydrocrotonin&lt;sup&gt;178&lt;/sup&gt;</td>
</tr>
<tr>
<td>Croton sublyratus Kurz.</td>
<td>Aerial parts</td>
<td>Plaunotol, registered with WHO under the code CS-684 and the drug known as Kelnac®&lt;sup&gt;179&lt;/sup&gt;.</td>
</tr>
<tr>
<td>Dalbergia monetaria L</td>
<td>Bark</td>
<td>Proanthocyanidins&lt;sup&gt;180&lt;/sup&gt;</td>
</tr>
<tr>
<td>Camellia sinensis Kuntze</td>
<td>Leaf</td>
<td>Catechins&lt;sup&gt;181&lt;/sup&gt;</td>
</tr>
<tr>
<td>Dittrichia viscosa (L.) W. Greuter</td>
<td>Flowering top</td>
<td>Flavonoids&lt;sup&gt;182&lt;/sup&gt;</td>
</tr>
<tr>
<td>Emblica officinalis Gaertn.</td>
<td>Fruit</td>
<td>Vitamin-c, corilagin, ellagic acid, Trigalloyl glucose&lt;sup&gt;183&lt;/sup&gt;</td>
</tr>
<tr>
<td>Glycyrrhiza glabra Linn.</td>
<td>Rhizome</td>
<td>Glycyrrhizinic acid, flavonoids&lt;sup&gt;13,14&lt;/sup&gt;</td>
</tr>
<tr>
<td>Gynostemma pentaphyllum</td>
<td>Plant</td>
<td>Quillaja saponins, gypenoside&lt;sup&gt;184&lt;/sup&gt;</td>
</tr>
<tr>
<td>Linderae umbellatae Ramus</td>
<td>Stem</td>
<td>Procyanidins&lt;sup&gt;185&lt;/sup&gt;</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Part</td>
<td>Constituents/Compounds</td>
</tr>
<tr>
<td>----------------------------------</td>
<td>----------</td>
<td>-----------------------------------------------------------------</td>
</tr>
<tr>
<td>Mikania cordata Burm.</td>
<td>Leaf</td>
<td>Mikanolide, flavone-mikanin, fumaric acid, epifriedelinol</td>
</tr>
<tr>
<td>Musa paradisiaca Linn.</td>
<td>Fruit</td>
<td>Carbohydrates, mineral vitamins</td>
</tr>
<tr>
<td>Ocimum basilicum Linn.</td>
<td>Aerial parts</td>
<td>Volatile oil</td>
</tr>
<tr>
<td>Panax ginseng Mey. C. A.</td>
<td>Leaf</td>
<td>Panaxosides, ginsenoside, polysaccharides</td>
</tr>
<tr>
<td>Salvia miltiorrhiza Bge</td>
<td>Root</td>
<td>Salvianolic acid</td>
</tr>
<tr>
<td>Sideritis mugronensis L.</td>
<td>Plant</td>
<td>Hypolaetin 8-glucoside</td>
</tr>
<tr>
<td>Sophora subprostata</td>
<td>Root</td>
<td>Sofalcone (derivative of sophoradin)</td>
</tr>
<tr>
<td>Silybum marianum</td>
<td>Plant</td>
<td>Silymarin</td>
</tr>
<tr>
<td>Spilanthes Ocymifolia</td>
<td>Plant</td>
<td>Lupeol acetate</td>
</tr>
<tr>
<td>Taraxacum officinale Weber</td>
<td>Plant</td>
<td>Taraxerol</td>
</tr>
<tr>
<td>Tectona grandis L.</td>
<td>Root</td>
<td>Lapachol</td>
</tr>
<tr>
<td>Terminalia chebula Rect. C. Clarke.</td>
<td>Fruit</td>
<td>Terchebin, chebulic acid</td>
</tr>
<tr>
<td>Trigonella foenum graecum Linn.</td>
<td>Seed</td>
<td>Steroidal saponins</td>
</tr>
<tr>
<td>Zingiber officinale Rosc.</td>
<td>Rhizome</td>
<td>Essential oil, 6-Gingesulphonie acid</td>
</tr>
</tbody>
</table>