1.1 Immune system:

The immune system is a complex defense network of the body that protects us from diseases. Normally immune responses are divided into innate or first line or nonspecific and specific adaptive immune response. The cellular components involve in innate immune response include neutrophils, monocytes, macrophages, natural killer cell and natural killer T cells. The adaptive immune response is mediated by the production of antibodies by lymphocytes\(^{(1)}\). There are basically two different types of lymphoid cells, T and B cells which mediate ‘cellular’ and ‘serologic’ or ‘humoral’ immunity, respectively\(^{(2)}\). The critical steps of consequences in an effective immune response are generated by the antigen presentation, activation of T- and/or B-lymphocytes and the resultant secretion of immune effectors molecules such as antibodies (Abs) and cytokines. Natural and synthetic substances can also modulate immune function by stimulating or suppressing beneficial or deleterious immune responses. Plant derived medicines could also mediate such types of effects through targeted modulation of key cellular and molecular interactions in antigen-presenting cells (e.g. Dendritic cells), T-lymphocytes and B-lymphocytes\(^{(3)}\).

1.1.1 Immunotherapy and immunostimulation:

Immunotherapy is a medical term defined as the treatment of disease by inducing, enhancing, or suppressing an immune response. Immunomodulation is the modulation of immunity either by amplify an immune responses are classified as immune stimulation. On the other hand immune therapies that reduce or suppress the immune system are classified as immunosupression. There are two main categories of immunostimulants such as specific immunostimulants which provide antigenic specificity in immune response like in case of
vaccines or any other antigen. Non-specific immunostimulants act irrespective of antigenic specificity to augment immune response or stimulate components of the immune system without antigenic specificity, such as immunologic adjuvant (4).

Other mechanisms of immunotherapy such as autologous immune enhancement therapy where patients own peripheral blood-derived NK cells, cytotoxic T Lymphocytes and other relevant immune cells are expanded in vitro and then re-infused to treat disease like cancer(5).

A special types of genetically engineered T cells are created by infecting patient's cells with a virus that contain a copy of T cell receptor (TCR) gene which is specialized to recognize tumor antigens. The virus is not able to reproduce within the cell, only integrates into the human genome. Patient’s own T cells are exposed to these viruses and then expanded non-specifically or stimulated using the genetically engineered TCR (6).

1.1.2 Drugs used to stimulate immune system: (7)

Immunostimulants or immunopotentiators are drugs leading predominantly to a non-specific stimulation of immunological defense mechanisms. Change in the immunity leads to incurable life threatening diseases like cancer and AIDS. So, stimulation of the host immune response presents an interesting supplementary approach for conventional immunodeficiency disorders. There are many synthetic, semisynthetic and natural derived products found to have immunostimulant property. Few examples are:

- **Levamisole**

  Levamisole is an anti helmintic drug that also restores functions of B lymphocytes, T lymphocytes, monocytes and macrophages.
Chapter 1

- **Thalidomide:**
  Different effects of this old drug have been utilized in conditions like erythema nodosum leprosum, multiple myeloma and rheumatoid arthritis.

- **BCG vaccine:** BCG vaccine is used in carcinoma bladder.

- **Recombinant cytokines**
  Interferons: In tumors and chronic hepatitis B and C.
  Interleukin 2 (aldeslukin): has been used in renal cell carcinoma and melanoma.

### 1.1.3 Immunostimulatory agents obtained from herbal source

The stimulation of immune response by herbal products, as a possible therapeutic measure has become a subject of scientific investigations. The basic concept has, however, existed in the ancient Vedic scripture, the Ayurveda, and has been practiced in Indian traditional medicine for many centuries. One of the therapeutic strategies in Ayurveda medicine is to increase body’s natural resistance to the disease causing agent rather than directly neutralizing the agent itself; in practice, this is achieved by using extracts of various plant materials called *rasayanas* (Charak Samhita, 1000 BC)\(^{(8)}\). This concept in modern scientific understanding would mean enhancement of immune responsiveness of an organism against a pathogen by non-specifically activating the immune system using immunostimulatory agents of plant origin.\(^{(9)}\).

Immunostimulatory plant-derived medicines may be a crucial alternative in developing regions of the world – where burden of disease is high and access to medical care is limited. Hence, plant derived immunostimulators will serve as a low cost alternative to achieve a better health status through enhanced protective immunity\(^{(10)}\).
Plant derived immunostimulants generally mediate their effects through targeting modulation of key cellular and molecular interactions of antigen-presenting cells (e.g. dendritic cells), T-lymphocytes and B-lymphocytes by following ways-

1. Modulation of dendritic cell activity.
3. Modulation of T-lymphocyte function by-
   a) Modulating CD4+ T-lymphocytes:
   b) Modulating CD8+ T-lymphocyte function.\textsuperscript{(11,12)}

1.2 Bioactive polysaccharides:

Polysaccharides are basically biopolymers consist of monosaccharides linked together through glycosidic bonds. The structures of polysaccharides can be linear or contain branched side chains. All polysaccharides have a general formula of C\textsubscript{x} (H\textsubscript{2}O)\textsubscript{y} where x is usually a large number between 200 and 2500. Considering that the repeating units in the polymer backbone are often six-carbon monosaccharides, the general formula can also be represented as (C\textsubscript{6}H\textsubscript{10}O\textsubscript{5})\textsubscript{n} where 40\textless n\textless 3000.\textsuperscript{(13)}

Different type polysaccharides widely distributed in the plants, microorganism (fungi and bacteria), algae, and animals. Together with proteins and polynucleotides, they are essential biomacromolecules in the life activities and play important roles in cell–cell communication, cell adhesion, and molecular recognition in the immune system fertilization, pathogenesis prevention, and blood clotting.\textsuperscript{(14)} In recent research evidences shows that polysaccharide have broad spectrum of biological effects; such as antibiotic, antioxidant, anti-mutant, anticoagulant, antitumour and immunostimulation activities.\textsuperscript{(15)}
Chapter- 1

During the past three decades, a number of bioactive polysaccharides have been isolated from mushrooms, fungi, yeast, algae, lichens, and plants, and these compounds have attracted significant attention because of their immunomodulatory and antitumor effects. For example, plant polysaccharides have been shown to increase macrophage cytotoxic activity against tumor cells and microorganisms, activate phagocytosis, increase reactive oxygen species (ROS) and nitric oxide (NO) production, and enhance secretion of a variety of cytokines and chemokines polysaccharides and polysaccharide–protein complexes\(^{(16)}\).

Most polysaccharides derived from higher plants are relatively nontoxic and do not cause significant side effects compared with immunomodulatory bacterial polysaccharides and synthetic compounds. Hence, they are the ideal candidates for immunomodulatory therapies\(^{(17)}\). In the recent past different water soluble polysaccharides with reported immunostimulating potential have been isolated from higher plants. They have produce the immunostimulating effect by enhancing the phagocytosis of granulocytes and macrophages, inducing the Interferone (INF), Interleukine(IL), Tumor necrosis factor(TNF) production and t'complement activation\(^{(18)}\).

1.2.1 Types of Bioactive polysaccharide:

Bioactive polysaccharides are structural carbohydrates and the non-structural carbohydrates also known as storage carbohydrates. These are the two broad classifications used for polysaccharides in plant kingdom. The structural carbohydrates consist of components like cellulose, glycan, pectin, etc. while the non-structural carbohydrates include sucrose, starch and fructan\(^{(19,20)}\).
1.2.1.1 Bioactive immunostimulatory polysaccharide:

Immunostimulatory polysaccharide of plants generally causing immunostimulation without altering the effect of the other organ which is a major problem associated with immunomodulatory bacterial polysaccharides and synthetic compounds.

Polysaccharides especially like fructans and mannans are derived from higher plants has been reported for having immunostimulatory property. There are a lots of other plant polysaccharide which have immunostimulating effect are as follows-

- **Fructans**: Fructans are a distinct group of non-structural carbohydrates with numerous physiological benefits. For example - Inulin type fructans ($2 \rightarrow 1$ linked) are the prototypes for most of the studies carried out on medicinal aspects so far. Recently it was observed that inulin and oligofructose modulate functions of the immune system which include concentration of IgA in the ileum and cecum, natural killer cell cytotoxicity as well as splenocyte enhanced natural killer cell cytotoxicity in gut associated lymphoid tissue (GALT). Numerous plants like *C. borivilianum, A. pyrethrum, Articum lappa, A. racemosus, Matricaria maritima, A. sativum, Vernonia kotschyana* rich in inulin type fructans have been reported to possess potent immunomodulatory activity.

- **Glucans**: The $\alpha (1 \rightarrow 4)$ glucan from Guduchi (*Tinospora cordifolia*) is the first glucans from ayurvedic herbs reported to possess potent immunostimulatory effect. The immunostimulatory effect produced by cell signalling mechanism by
activation of macrophages via TLR6 signaling, NF-κB translocation and cytokine production. From Tulsi (*Ocimum sanctum*) and *O. basilicum* the major water soluble polysaccharides that exhibiting biological effectiveness by modulating the immune system were found to be β 1→3 linked glucans.

- **Acetylated glucomannans**: First isolated acetylated glucomannan obtained from Salep, where both the monomers were linked β- (1 → 4), and an acetyl groups were located on different positions on the polymer. Ghritkumari (*Aloe vera*) containing 1→4 acetylated galactomanan possessing *in vitro* and *in vivo* complement fixing ability by activation of macrophages. For this activity they are used as adjuvant for treatment of viral and tumoral diseases.

- **Xylans**: Xylans and xylo-oligosaccharides (XOS) have become important nutritional products for human as well as veterinary usage. The hetero and acidic xylans isolated from Bhumi Amla (*P.niruri*) are reported for having immunostimulatory activity (20).

1.3 Immunomodulation & it’s relation with the structure of Polysaccharide:

The main important feature of the bioactivity of polysaccharides on immune system depends on its structural and functional relationship. The molecular weight, structure and composition of polysaccharides have been contributed to the bioactivity of polysaccharide. In general, polysaccharides in a configuration with β-1-3, 1-4, or 1-6 branch chains are necessary for activity and complex branch-chained polysaccharides with anionic structures and higher molecular weights have greater immunostimulating activities (21).
Arabinogalactans extracted from *Chlorella pyrenoidosa* cells have immunostimulatory activity which depend upon their molecular weights. The higher molecular weight arabinogalactans exhibited immunostimulatory activity compare to the lower molecular weight fractions (22).

The chemical structures and activities of some polysaccharides isolated from natural resources are shown in the Table: 1.1 (21)

**Table 1.1. Chemical structure of polysaccharide and their activity on immune system:**

<table>
<thead>
<tr>
<th>Polysaccharides</th>
<th>Chemical Structure</th>
<th>Activities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Lentinan</td>
<td>β-1,3-D glucan containing glycopyranosyl residues branched in position 6 of glucose unit.</td>
<td>Antitumor, antiviral immunomodulatory and anticoagulatory.</td>
</tr>
<tr>
<td><em>Ganoderma</em></td>
<td>(1-4)-α-D-mannoxylan or (1-3)-β-D-glucan backbone branched with mono-, di- and oligosaccharide side chains, a fucose-containing glycoprotein, or acidic protein bound polysaccharide.</td>
<td>Antitumor, immunomodulating activity,</td>
</tr>
<tr>
<td><em>Auricularia</em></td>
<td>Hetero polysaccharides consisted of a backbone chain of β-1,3-D-glucose residues with various branch residues, such as mannose, glucose, xylose and glucuronic acid.</td>
<td>Antitumor, immunomodulatory.</td>
</tr>
</tbody>
</table>
1.4 Synergistic combinations of bioactive polysaccharide with other drugs:

Bioactive polysaccharides have synergistic activity in combination with chemotherapeutic agents via immunopotentiation for the treatment of cancer. They exert their anti-cancer effect by enhancing the host’s immune system rather than via a direct cytotoxic effect.

This involves the stimulation of macrophages, natural killer (NK) cells, and cytotoxic T-lymphocytes (CTL) activities along with their secretory products like TNF, reactive nitrogen and oxygen intermediates, and interleukins (23).

In recent time various bioactive polysaccharide were reported to combine with other drugs showing promising synergistic effect in the treatment of various diseases enlisted in the table 1.2.
### Table 1.2. Synergistic effect of polysaccharide and other drugs:

<table>
<thead>
<tr>
<th>Polysaccharide Combination</th>
<th>Synergistic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>MD-fraction of <em>G. frondasa</em> IFN-α2b</td>
<td>Triggers DNA-PK activation and induces cancer cell arrest at the G1 cell cycle checkpoint (^{(24)}).</td>
</tr>
<tr>
<td>MD-fraction of <em>G. frondasa</em> cisplatin</td>
<td>Enhance the anti-tumor and anti-metastatic activity of cisplatin. At the same time decrease cisplatin-induced immunosuppression and nephrotoxicity in mice (^{(26)}).</td>
</tr>
<tr>
<td>PSK (Protein bound β-glucan) of <em>Trametes versicolor</em> trastuzumab 58</td>
<td>Increase the efficacy of trastuzumab 58 action via immunostimulation (^{(27)}).</td>
</tr>
<tr>
<td>C. Sinensis Polysaccharides Cisplatin</td>
<td>Enhance cytotoxicity (^{(28)}).</td>
</tr>
<tr>
<td>β-glucan of (Saccharomyces cerevisiae) bevacizumab</td>
<td>Antitumour effect (^{(29)}).</td>
</tr>
</tbody>
</table>
1.5 Pharmaceutical Excipients:

The International Pharmaceutical Excipients Council defines an excipient as any substance other than the active drug or pro drug that is included in the manufacturing process or is contained in a finished pharmaceutical dosage form \(^{(31, 32)}\).

The US Pharmacopeia–National Formulary (USP–NF) categorizes excipients as binders, disintegrants, diluents, lubricants, glidants, emulsifying–solubilizing agents, sweetening agents, coating agents and antimicrobial preservatives. In addition to this functional quality excipient should be chemically stable, nonreactive with the drug and other excipients, inert in the human body, have low equipment and process sensitivity, have pleasing organoleptic properties and are well characterized and well accepted by the industry and regulatory agencies. There are limited choices of excipients presently available in the market which can fulfill with all of these attributes. Hence, the selections of suitable excipients for designing a successful formulation are a challenging task.

Excipients are categorized as compendial or noncompendial materials. Compendial excipients have composition consistent with monographs published in compendia such as

<table>
<thead>
<tr>
<th>Polysaccharide Combination drug</th>
<th>Synergistic effect</th>
</tr>
</thead>
<tbody>
<tr>
<td>ginseng Herbal dietary</td>
<td>Inhibited the proliferation of wild-type HCT116 colon cancer effects(30)</td>
</tr>
<tr>
<td>polysaccharide supplement in human colon cancer cells via its pro-apoptotic effects</td>
<td></td>
</tr>
</tbody>
</table>

Ginseng polysaccharide In inhibited the proliferation of wild-type HCT116 colon cancer in human colon cancer cells via its pro-apoptotic effects.
as USP–NF. The compendial excipients are the better characterized excipients and most likely to possess the above mentioned desirable qualities. These materials are recognized as preferred excipients for pharmaceutical formulations. Noncompendial excipients might also be applied in pharmaceutical formulations. The use of these noncompendial materials is supported by Type IV drug master files (DMFs) in regulatory dossiers (i.e. new drug applications, abbreviated new drug applications, and investigational new drug applications.

1.6 The need of excipients in Drug Delivery:

Pharmaceutical excipients are substances other than the API which have been appropriately evaluated for safety and are intentionally included in a drug delivery system. Excipients can aid in the processing of the drug delivery system during its manufacture, support or enhance stability, bioavailability or patient acceptability, overall safety, effectiveness or delivery of the drug during storage or use. To meet emerging challenges include continually increasing drug development costs, slowing new product approval, blockbuster drug patent expirations, price pressure and global competition pharma industries have been continuously working on new formulations with new excipients. The focus on drug development costs is driving the industry to consider outsourcing, relocating production and sourcing ingredients from lower-cost locations. To overcome the challenges of price pressure due to "generitization" of branded drugs, as well as severe global competition, even in the generic sector, many pharmaceutical companies are seeking new proprietary drug delivery formulations. Innovative and new excipients offered by various excipient suppliers enable the development of new dosage forms, improve efficiency and may reduce the cost of drugs. Excipients can offer
opportunities to introduce new dosage forms and thus facilitate the extension of patent life.

1.7 Tablet Excipients:

The characterisation of pharmaceutical excipients using material science approach has helped in the design of drug formulations to obtain a desired set of performance properties (33).

The ingredients or excipients used to make compressed tablets are numerous and can be classified by their use or function as: fillers, binders, disintegrants, lubricants, glidants, wetting agents, antioxidants, preservatives, colouring agents and flavouring agents. Fillers, binders, glidants and lubricants help to impart satisfactory processing and compression characteristics to the formulation. Disintegrants, wetting agents, antioxidants, preservatives, colouring and flavouring agents help give additional desirable physical characteristics to the finished tablets. Preformulation studies demonstrate the influences of excipients on stability, bioavailability and the process by which the dosage forms are prepared. This calls for the need for acquiring more information and use standards for excipients (34).

1.8 Principal requirements of excipients

The importance of excipients in pharmaceutical formulations has generally been underestimated, as they were cheap ingredients viewed solely as inert supports for
medicaments. Today, this view is outdated and on the basis of what we have said above, we may say that excipients are rather more than the sugar in the pill \(^{(35)}\).

There are the three basic requirements of excipients for both in quality and safety as mentioned in figure 1.1. The requirement of therapeutic efficacy for drugs is replaced by that of functionality for excipients, defined as ‘the physical, physicochemical and biopharmaceutical properties’ of the same.

Less attention has been devoted to the safety of excipients, because their inertia and innocuity were taken for granted. The considerations made in the preceding paragraphs and the continual evolution of pharmaceutical technology, with the growing use of new ‘tailor-made’ materials, suggest that a new look at the so-called pharmacological and toxicological inactivity of these components of pharmaceuticals is in order \(^{(36, 37)}\).

To this end, we shall examine three issues that may compromise the safety of pharmaceuticals: (a) production, distribution and use; (b) pharmaceutical-excipient interactions; and (c) toxicity, which may be the cause of frequent and sometimes notable ‘adverse effects’.

![Figure 1.1. Prime objectives of pharmaceutical excipients](image)

*Figure 1.1. Prime objectives of pharmaceutical excipients*
1.9 Polysaccharides as a pharmaceutical excipient

Polysaccharides are the polymers of monosaccharides. In nature, polysaccharides have various resources from algal origin (e.g. alginate), plant origin (e.g. pectin, guar gum), microbial origin (e.g. dextran, xanthan gum), and animal origin (chitosan)\(^{(38)}\).

Polysaccharides possess a large number of reactive groups, a wide range of molecular weight (MW), varying chemical composition, which contribute to their diversity in structure and in property. The structures of important polysaccharides are mentioned in figure 1.2. From the viewpoint of polyelectrolyte, polysaccharides can be divided into polyelectrolytes and non-polyelectrolytes, the former can be further divided into positively charged polysaccharides (chitosan) and negatively charged polysaccharides (alginate, heparin, hyaluronic acid, pectin, etc.). The novel characteristics of natural polysaccharides are highly stable, safe, non-toxic, hydrophilic and biodegradable in nature. In addition, polysaccharides have abundant resources in nature and low cost in their processing. Particularly, most of natural polysaccharides have hydrophilic groups such as hydroxyl, carboxyl and amino groups, which could form non-covalent bonds with biological tissues (mainly epithelia and mucous membranes) forming bioadhesion\(^{(39)}\).

In recent years, a large number of studies have been conducted on polysaccharides and their derivatives for their potential application in drug delivery systems\(^{(40)}\).
Figure 1.2 Chemical structures of important polysaccharides.

1.9.1 Polysaccharide hydrocolloids or gums and mucilage

Mucilage, gums and glucans which are considered as polysaccharide hydrocolloids abundant in nature and commonly found in many higher plants. These polysaccharides constitute a structurally diverse class of biological macromolecules with a broad range of physicochemical properties which are widely used for various applications in pharmacy and medicine. Recent trend towards the use of vegetable and nontoxic products demands the replacement of synthetic additives with natural one. Many natural polymeric materials have been successfully used in sustained-release tablets. These materials include: guar gum, ispaghula husk, pectin, galactomannan from *Mimosa scabrella*, *Gleditsia triacanthos* Linn (honey locust gum), *Sesbania* gum, mucilage from the pods of *Hibiscus esculenta*, tamarind seed gum, gum copal and gum dammar, agar, konjac, chitosan etc. Some newly explored gums viz. sesbania gum, tara
gum are also reported for having sustained release properties. These have found application not only in sustaining the release of the drugs but are also proving useful for development of gastro retentive dosage form, bioadhesive system, microcapsules etc.

1.10 Polysaccharide-Based excipients and their advantages in Drug Delivery:

Polysaccharides are a diverse class of polymeric materials of natural origin formed via glycosidic linkages of monosaccharides. On the basis of monosaccharide units, polysaccharides can have a linear or branched architecture. In addition to structural diversity, polysaccharides have a number of reactive groups, including hydroxyl, amino, and carboxylic acid groups, indicating the possibility for chemical modification. Herein, we will describe characteristics, including biocompatibility, solubility, potential for modification and innate bioactivity, of several polysaccharides that lend credence to their potential for use in drug delivery systems.

1.11 Biodegradability and Biocompatibility of Polysaccharide:

Polysaccharides possess very low (if any) toxicity levels in comparison to many synthetic polymers. For example, dextrans are low toxicity and highly biocompatible biopolymers composed of glucose with α-1,6 linkages, with possible branching from α-1,2, α-1,3, and α-1,4 linkages. Consequently, dextrans have formed the basis of biocompatible hydrogels for prolonged therapeutic release. Likewise, dextran microspheres has exhibited biocompatibility when administered subcutaneously in rats with no inflammatory response. Also owing to their native presence within the body, most polysaccharides are subject to enzymatic degradation. Through enzyme catalysis, polysaccharides can be broken down to their monomer or
The functional groups along polysaccharide backbones, particularly hydroxyl and amine groups, typically yield high aqueous solubility but it can often be adjusted via monomer modification. For example, chitosan, composed of $\beta$-1,4 linked $N$-acetyl-D-glucosamine and D-glucosamine is prepared via deacetylation of chitin. By varying the degree of deacetylation of the parent compound, the solubility of chitosan in acidic conditions can be modified. In case of Higher degrees of deacetylation correspond to an increased number of available protonated free amino groups along the polysaccharide backbone consequently enhanced solubility (50). Likewise, $O$-
acetylation of glucomannan can be used to modulate the formation of intermolecular hydrogen bonds with water, thereby altering aqueous solubility\(^{(51)}\).

### 1.13 Bioadhesive and Mucoadhesive Drug Delivery Systems:

Adhesion is a process and is simply defined as the “fixing” of two surfaces to one another. In case of bioadhesion in which two materials among them at least one is biological in nature are held together for an extended period of time by interfacial forces \(^{(52)}\). So, Bioadhesion can be defined as the binding of a natural or synthetic polymer to a biological substrate. When this substrate is a mucous layer, the term mucoadhesion is often used \(^{(53)}\).

Bioadhesive drug delivery systems are used as a delivery device within the human to enhance drug absorption in a site-specific manner. Bio adhesive polymers are used to deliver the drug which can adhere to the epithelial surface of the stomach and have gastro retentive property. Thus, they improve the prolongation of gastric retention.

In mucoadhesion, the attachment of the drug occurs along with a suitable carrier to the mucous membrane. Mucoadhesion is a complex phenomenon which involves wetting, adsorption and interpenetration of polymer chains. The process of mucoadhesion has the following mechanism\(^{(54)}\).

- Initiate contact between a bioadhesive and a membrane (wetting or swelling phenomenon).
- Penetration of the bioadhesive into the tissue or into the surface of the mucous membrane (interpenetration)

In biological systems, bioadhesion can be classified into 3 types:
Type 1:- adhesion between two biological phases, for example, platelet aggregation and wound healing.

Type 2:- adhesion of a biological phase to an artificial substrate, for example, cell adhesion to culture dishes and bio film formation on prosthetic devices and inserts.

Type 3:- adhesion of an artificial material to a biological substrate, for example, adhesion of synthetic hydro gels to soft tissues and adhesion of sealants to dental enamel.$^{(55)}$

1.13.1 Advantages of bio and mucoadhesive drug delivery systems:

There are several advantages in using bio/mucoadhesive drug delivery systems:

1. Formulation stays longer time due to the adhesion and intimate contact, thus at the delivery site improving bioavailability of active pharmaceutical ingredients (API). Hence, using lower API concentrations for disease treatment we get optimum results.

2. Application of specific bioadhesive molecules which allows us to target a particular sites or tissues, for example the gastro retentive tablets use in gastrointestinal (GI) tract.

3. Increase in residence time combined with controlled API release may lead to sustained release pattern of drug release.

4. The drug degradation due to the first-pass metabolism can be avoided.

5. Additionally significant cost reductions may be achieved and dose-related side effects can be reduced due to API localisation at the disease site.$^{(56-59)}$
1.13.2 Theories of Mucoadhesion:

The basic concept of mucoadhesion is the dosage form can stick to the mucosal surface for long time and can be described by following theories:\textsuperscript{(58-63)}:

1. The wetting theory, which is based on the ability of bioadhesive polymers to spread and develop intimate contact with the mucous layers.

2. The diffusion theory, which proposes physical entanglement of mucin strands the flexible polymer chains, or an interpenetration of mucin strands into the porous structure of the polymer substrate.

3. The absorption theory, suggests that bioadhesion is due to secondary forces such as Vander Waal forces and hydrogen bonding.

4. The electron theory, which proposes attractive electrostatic forces between the glycoprotein mucin net work and the bio adhesive material.

1.13.2 Factors affecting mucoadhesion:

Different polymeric properties such as functional groupings, conformational structure of polymer, molecular weight and pH, amount of mucus and hydration etc. have immense effect on the degree of polymer-mucus interaction as well as on mucoadhesion.

1.13.2.1 Functional group contribution:

The attachment and bonding of bioadhesive polymers occurs mainly through interpenetration followed by secondary non-covalent bonding between substrates and the adhesion polymers occur to biological substrates. These secondary bonding are due to the
Chapter- 1

formation the hydrogen bond. Mucoadhesive polymers possessing hydrophilic functional
groups such as, carboxyl (COOH), hydroxyl (OH), amide (NH₂) and sulphate groups
(SO₄H) may be more favourable in formulating targeted drug delivery platforms typically
by physical entanglements and secondary interactions (hydrogen bonds) contribute to the
formation of a strengthened network. Therefore, polymers that exhibit a high density of
available hydrogen bonding groups would be able to interact more strongly with mucin
glycoproteins⁶⁴.

1.13.2.2 Degree of hydration:

Mucoadhesive strength of polymeric components depends on the degree of
hydration. Many polymers will exhibit adhesive properties under conditions where the
amount of water is limited and adhesion is thought to be a result of a combination of
capillary attraction and osmotic forces between the dry polymer and the wet mucosal
surface which causes dehydration and strengthening of the mucus layer⁶⁵. Although this
kind of “sticking” has been referred to as mucoadhesion, it is important to clearly
distinguish such processes from “wet-on-wet” adhesion in which swollen mucoadhesive
polymers attach to mucosal surfaces⁶⁶. Whilst hydration is essential for the relaxation and
interpenetration of polymer chains, excess hydration could lead to decreased
mucoadhesion and/or retention due to the formation of a slippery mucilage⁶⁷.
1.13.2.3 Molecular weight Polymer, length of the chain, conformation, and degree of cross-linking:

The fact was established that structural polymeric components significantly influence the extent of diffusion, entanglement and hence causes mucoadhesion. Polymeric molecular weight, chain length, conformation, degree of cross-linking were the main factors for this reaction.

For example, Dextran, with molecular weights of 19,500,000 and 200,000 possess similar bioadhesive strength which may be explained in terms of the helical conformation resulting in shielding of potential bioadhesive sites inside coiled conformers at higher molecular weights. On the other hand, poly(acrylic) acid has an optimal MW of about 750,000, whereas polyethylene oxide has an optimum MW closer to 4,000,000 (68) (69). Whilst a critical length is necessary to produce bioadhesive interactions, additionally the size and shape of the interpenetrating polymeric chains must be considered (69), (70). The degree of cross-linking within a polymer system significantly influences chain mobility and resistance to dissolution. Cross-linked hydrophilic polymers swell in the presence of water allowing them to retain their structure, whereas similar high molecular weight linear hydrophilic polymers are swellable and readily dispersible (71).

1.13.2.4 pH and charge:

The charge density of macromolecules is an important factor for bioadhesion with polyanions preferred to polycations when considering bioadhesion. Macromolecular charge is affected by the pH of the physiological environment due to the dissociation of...
functional groups, forming polymer mucus hydrogen bonding with undissociated anionic pendant functional groups \(^{(72)}\). In carboxylated polymers containing carboxylic groups and pH values below the respective pKa value would then be more favourable. \(^{(73),(74)}\). It was recognised that mucoadhesion processes are optimised in low pH environments but mucoadhesion may not be completely lost at higher pH values. At higher pH levels, repulsion of “like” COO\(^-\) functional groups changes the spatial conformation from a coiled state into a “rod-like” structure making them more readily available for inter-diffusion and interpenetration \(^{(74),(75)}\).

1.13.2.5 Polymer concentration:

Polymer concentration has also been shown to influence the strength of mucoadhesion. Optimal polymer concentration is dependent on physical state of the delivery system, with observational differences between semisolid and solid-state platforms. In the semisolid state, an optimum concentration exists for each polymer beyond which reduced adhesion occurs because a lower number of polymer chains are available for interpenetration with mucus. On the other hand, solid dosage forms such as buccal tablets exhibit increased adhesive strength as the mucoadhesive polymer concentration increases \(^{(76)}\).

1.13.2.6 Environmental and physiological factors:

There are numerous environmental and physiological factors that will have a marked effect on the mucoadhesive strength.
1.13.2.6.1 The variable mucus turnover at the applied surface/site throughout the body.

The maximum duration in which a mucoadhesive system can adhere to the mucosal tissue will be limited by the turnover time of the mucus gel layer. In case of some mucosal tissues where mucus turnover is relatively low (e.g. mouth or vagina), this may be of less critical importance. However, in the areas such as in the intestine where markedly high mucus turnover is occurred the adherence time is probably not longer than a couple of hours \(^{(77)}\). Mucus gel layer viscosity can vary throughout the body, with variability increasing in certain disease states. Low mucus viscosity results in a weak, easily detachable polymer/mucus bond, making targeted drug delivery extremely difficult. In contrast an extremely viscous mucus layer, such as those thickened due to white blood cell DNA, dead cells and inflammatory mediators limits the degree of interpenetration and also increases the diffusion pathway through which the active agents pass \(^{(78),(79)}\).

1.14 Mucoadhesive polymeric excipients.

The polymer attributes to high levels of retention at applied and targeted sites via mucoadhesive bonds include hydrophilicity, negative charge potential and the presence of hydrogen bond forming groups. Additionally, the surface free energy of the polymer should be adequate so that ‘wetting’ with the mucosal surface can be achieved. The polymer should also possess sufficient flexibility to penetrate the mucus network, be biocompatible, non-toxic and economically favourable \(^{(80)}\). The polymers used for oral mucoadhesive drug delivery system are classified as Hydrophillic polymers and Hydrogels.
Hydrophillic polymers: It contains carboxylic group and possess excellent mucoadhesive properties. These are PVP (poly vinyl pyrrolidine) MC (methylcellulose) SCMC (sodium carboxy methyl cellulose) HPC (hydroxyl propyl cellulose).

Hydrogels: These swell when in contact with water and adhere to the mucus membrane.

These are further classified according to their charge.

i) Anionic polymers- Polycarbophil (Noveon®) and carbomer(Carbopol®) polyacrylates.

ii) Cationic polymers- Chitosan.

iii) Neural/ non ionic polymers- Eudragit analogues.

They can also be classified as,

i) Synthetic polymers: Cellulose derivatives, carbops.

ii) Natural polymers: Tragacanth, pectin, gelatin sodiumalginate, acacia

1.14.1 Disadvantages of synthetic polymers in pharmaceutical excipients:

The synthetic polymers possessing certain disadvantages such as high cost, toxicity, environmental pollution during synthesis and variable side effects.

Acute and chronic adverse effects (skin and eye irritation) have been observed in workers handling the related substances methyl methacrylate and poly- (methyl methacrylate) (PMMA). Reports of adverse reactions to povidone primarily concern the formation of subcutaneous granulomas at the injection site produced by povidone.
Chapter- 1

Acute oral toxicity studies in animals have indicated that carbomer-934P has a low oral toxicity but the dust is irritating to the eyes, mucous membranes and respiratory tract.

Studies in rats have shown that 5% polyvinyl alcohol aqueous solution injected subcutaneously can cause anaemia. Polymer like polyglycolides, polylactides and their copolymers have an acceptable biocompatibility but exhibit systemic or local reactions due to their acidic degradation products. An initial mild inflammatory response has been observed when using poly-(propylene fumarate) in rat implant studies.

1.14.2 Natural mucoadhesive polymer.

For bioadhesion commonly used materials are poly acrylic acid, chitosan, cholestyramine, sodium alginate, hydroxypropyl methylcellulose (HPMC), tragacanth, dextrin, polyethylene glycol (PEG) and polylactic acids\(^{81,82}\).

Because of the rapid turnover of mucus in the gastrointestinal tract (GIT), it is very difficult to maintain bioadhesion effectively, even though some of these polymers are effective at producing bioadhesiveness.

In recent time, plant based natural mucoadhesive polymers take more interest because these natural materials have advantages over synthetic ones as they are chemically inert, nontoxic, less expensive, biodegradable and widely available\(^{81}\). The specific application of plant-derived polymers in pharmaceutical formulations include their use in the manufacture of solid monolithic matrix systems, implants, films, beads, nanoparticles, microparticles, as well as viscous liquid formulations. Within these dosage forms, polymeric materials have fulfilled different roles such as binders, matrix formers or drug release modifiers, film coating formers, thickeners or viscosity enhancers, stabilisers,
disintegrants, solubilisers, emulsifiers, suspending agents, gelling agents and mucoadhesives.

Polymers are often utilised in the design of novel drug delivery systems such as those that target delivery of the drug to a specific region in the gastrointestinal tract or in response to external stimuli to release the drug. This can be done via different mechanisms including coating of tablets with polymers having pH dependent solubilities or incorporating non-digestible polymers that are degraded by bacterial enzymes in the colon. Non-starch, linear polysaccharides are resistant to the digestive action of the gastrointestinal enzymes and retain their integrity in the upper gastrointestinal tract. Matrices manufactured from these polysaccharides therefore remain intact in the stomach and the small intestine, but once they reach the colon they are degraded by the bacterial polysaccharidases. This property makes these polysaccharides exceptionally suitable for the formulation of colon-targeted drug delivery systems (84).

1.14.2.1: Various plants derived natural mucoadhesive polymeric excipients:

Natural biomaterials such as polysaccharide are highly stable, safe, non-toxic, low cost, hydrophilic and biodegradable abundant resources in nature in addition. Particularly, most of natural polysaccharides have hydrophilic groups such as hydroxyl, carboxyl and amino groups, which could form non-covalent bonds with biological tissues (mainly epithelia and mucous membranes), forming bioadhesion. In recent years, a large number of studies have been conducted on polysaccharides and their derivatives for their potential application in drug delivery systems (83, 85). Some of the applications of plant derived polysaccharide are depicted in table 1.2.
Table 1.3. Plant derived polysaccharide as pharmaceutical excipients (86):

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Biological Source</th>
<th>Pharmaceutical Application</th>
<th>Formulation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cactus</td>
<td><em>Opuntia ficus indica</em></td>
<td>Gastroretentive agent</td>
<td>Flowting tablet</td>
</tr>
<tr>
<td>Gellan Gum</td>
<td><em>Pseudomonas elodea</em></td>
<td>Used in <em>insitu</em> gelling</td>
<td>Flowting matrix tablet</td>
</tr>
<tr>
<td>Hekea</td>
<td><em>Hakea gibbosa</em></td>
<td>Mucoadhesive in buccal delivery</td>
<td>Buccal tablet</td>
</tr>
<tr>
<td>Karaya Gum</td>
<td><em>Sterculiaurens,</em></td>
<td>Mucoadhesive</td>
<td>Flowting matrix tablet</td>
</tr>
<tr>
<td></td>
<td>Family Sterculiaceae</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mucuna Gum</td>
<td><em>Mucuna flagillepes</em></td>
<td>Mucoadhesive</td>
<td>Microsphere</td>
</tr>
<tr>
<td></td>
<td>(fam. Papilionaceae)</td>
<td></td>
<td>Microsphere</td>
</tr>
<tr>
<td>Orka</td>
<td><em>Hibiscus esculentus</em></td>
<td>Hydrophillic matrix</td>
<td>Mucoadhesive</td>
</tr>
<tr>
<td></td>
<td>fam. Malvaceae</td>
<td>control drug delivery</td>
<td>tablet</td>
</tr>
<tr>
<td>Carageenan</td>
<td><em>Boswellia Serrata</em></td>
<td>Mucoadhesive drug delivery</td>
<td>Gastroretentive</td>
</tr>
<tr>
<td></td>
<td>fam. Bruseraceae)</td>
<td></td>
<td>mucoadhesive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ophylane tablet.</td>
</tr>
<tr>
<td>Chitosan</td>
<td><em>Opuntia ficus indica</em></td>
<td>Mucoadhesive drug delivery</td>
<td>Gastroretentive</td>
</tr>
<tr>
<td></td>
<td>(fam. Cactaceae)</td>
<td></td>
<td>mucoadhesive</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>ophylane tablet.</td>
</tr>
</tbody>
</table>
1.15 Common sites of application for mucoadhesive dosage form:

1.15.1 Buccal drug delivery:

The main advantages of buccal site are high accessibility and low enzymatic activity. Additionally, buccal drug delivery can be promptly terminated in cases of toxicity through the removal of dosage form thereby offering a safe and easy method of drug utilization\(^{(88)}\).

Orabase\(^{\circ}\) a first-generation mucoadhesive paste has long been used as barrier system for mouth ulcers.

1.15.2 Ophthalmic applications:

The delivery of therapeutic agents to the eye may be achieved using various types of dosage forms including liquid drops, gels, ointments and solid ocular inserts (both degradable and no degradable)\(^{(89–91)}\). Another interesting delivery platform is in situ gelling polymer that undergoes a phase transition after application. Pre-application these systems are in the liquid state and are easily administered, whereas post-application they are transformed in highly viscous rheological structured networks\(^{(92)}\).

1.15.3 Vaginal drug delivery systems:

Vaginal drug delivery offers many advantages; the avoidance of hepatic first-pass metabolism, a reduction in the incidence and severity of gastrointestinal side effects, a decrease in hepatic side effects and avoidance of pain, tissue damage, and infection commonly observed for parenteral drug delivery routes of administration\(^{(93)}\). Whilst the vagina provides a promising site for systemic drug delivery because of its large surface
area, rich blood supply and high permeability, poor retention due to the self-cleansing action of the vaginal tract is often problematic \(^{(94)}\).

More recently ACIDFORM\(^{®}\) has been shown to be present intra-vaginally 12 h after insertion \(^{(95)}\).

1.15.4 Nasal Applications:

From a histological point of view, the nasal mucosa provides an attractive route for systemic drug delivery. The total area of the human nasal mucosa is about 150 cm\(^{2}\), which is surrounded by a dense vascular network, thus providing an excellent absorptive interface \(^{(96)}\). The nasal epithelium exhibits a relatively high permeability, with only two cell layers separating the nasal lumen from the dense vasculature within the lamina propria. Intranasal delivery of the peptide desmopressin that exerts its action on the kidneys, mimicking the action of antidiuretic hormone, used mainly in Diabetes insipidus. Other such formulations include Imigran\(^{®}\) (sumatriptan) and Miacalcic\(^{®}\) (Calcitonin) nasal sprays that are used in the treatment of acute migrane and post-menopausal osteoporosis, respectively \(^{(97)}\).

1.15.5 Mucoadhesive formulations for Gastro Intestinal (GI) Tract:

Principally mucoadhesive polymers may increase contact time with the lining of the GI tract and hence bioavailability. Furthermore, “absorption windows” within the GI tract such as those making up the gastro-associated lymphatic tissue (GALT) may be targeted allowing for the absorption of larger poorly soluble therapeutic agents. Despite a few notable exceptions, mucoadhesive drug delivery systems have not reached their full potential within oral drug delivery, because of insufficient adhesion within the GI tract to
provide a prolonged residence time. In this context targeted drug delivery systems have focused on developing mucoadhesive patches and microparticles by using first-generation polymers. The major problem with mucoadhesive tablets is the poor adherence to mucosal surfaces, mass combined with the vigorous movement of the gastrointestinal tract\(^{(98)}\).

Recently a thiolated chitosan tablet has been developed for the oral delivery of insulin which significantly decreased glucose levels in non-diabetic rats compared to unmodified polymer insulin tablets\(^{(99)}\).

Furthermore, the potential of microcrystalline chitosan granules have experimented for the delivery of furosemide\(^{(100)}\).

1.16 Currently available Mucoadhesive formulations\(^{(101)}\)

Currently mucoadhesive dosage form like Lozenges, troches and tablets for systemic delivery across the oral mucosa are commercially available consisting of drugs like nitroglycerin and fentanyl. Some of the commercially available mucoadhesive formulation is presented in table 1.3.

- **Tablets:** Solid formulations such as tablets and lozenges dissolve into the saliva and get absorbed by utilizing the whole surface area of the oral cavity. Nitrogard\(^{®}\) is a mucoadhesive tablet which delivers nitroglycerin for longer term angina relief and prevention. Miconazole buccal mucoadhesive tablets (Lauriad\(^{®}\)) were found to be non-inferior to 500 mg miconazole oral gel for the treatment of oral candidiasis in head and neck cancer patients.
Sprays: The Generex Biotechnology Corporation has developed a RapidMist™ spray which is used to deliver large molecules, such as insulin across the oral mucosa.

Pastes: The use of pastes as a drug delivery vehicle is a relatively under investigated method with most current literature focussing on the intra-canal delivery of antimicrobial pastes in endodontics. One currently commercially available mucoadhesive paste is Orabase® an oral adhesive paste that is available as a carrier alone or containing 0.1% triamcinoloneacetonide (Kenalog in Orabase®) for treating immunologically mediated oral mucosal conditions.

Table 1.4 Some commercially available oral mucoadhesive formulations:

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage form</th>
<th>Type of release</th>
<th>Product Name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fentanyl citrate</td>
<td>Lozenge</td>
<td>Quick</td>
<td>ACTIQ</td>
<td>Cephalon</td>
</tr>
<tr>
<td></td>
<td>Tablet</td>
<td>Quick</td>
<td>FENTORA</td>
<td>Cephalon</td>
</tr>
<tr>
<td></td>
<td>Film</td>
<td>Quick</td>
<td>ONSOLIS</td>
<td>Meda Pharmaceutical Inc</td>
</tr>
<tr>
<td>Buprenorphine HCl</td>
<td>Tablet</td>
<td>Quick</td>
<td>SUBUTEX</td>
<td>Reckitt Benckiser</td>
</tr>
<tr>
<td>Buprenorphine HCl and naloxone</td>
<td>Tablet</td>
<td>Quick</td>
<td>SUBUTEX</td>
<td>Reckitt Benckiser</td>
</tr>
<tr>
<td>Prochlorperazine</td>
<td>Tablet</td>
<td>Controlled</td>
<td>BUCCASTEM</td>
<td>Reckitt Benckiser</td>
</tr>
</tbody>
</table>
## Drug Dosage Form

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dosage form</th>
<th>Type of release</th>
<th>Product Name</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Testosterone</td>
<td>Tablet</td>
<td>Controlled</td>
<td>STRIANT SR</td>
<td>Columbia pharmaceuticals</td>
</tr>
<tr>
<td>Nitroglycerine</td>
<td>Tablet</td>
<td>Quick</td>
<td>NITROSTATE</td>
<td>Wlambert-p Davis</td>
</tr>
<tr>
<td></td>
<td>Spray</td>
<td>Quick</td>
<td>NITROSTATE</td>
<td>pfizer pharmaceuticals</td>
</tr>
<tr>
<td>Glyceryltriminitrate</td>
<td>Spray</td>
<td>Quick</td>
<td>NITROMIST</td>
<td>NovaDel</td>
</tr>
<tr>
<td>Zolpidem</td>
<td>Spray</td>
<td>Quick</td>
<td>ZOLPIMIST</td>
<td>Nova Del</td>
</tr>
<tr>
<td>Tablet</td>
<td>Quick</td>
<td>SUSCARD</td>
<td>Forest laborerries</td>
<td></td>
</tr>
<tr>
<td>Nicotine</td>
<td>Chewing gum</td>
<td>Quick</td>
<td>NICORETTE</td>
<td>GSK Consumer Health</td>
</tr>
<tr>
<td>Lozenge</td>
<td>Quick</td>
<td>NICOTINELLE</td>
<td>Novartis Consumer</td>
<td></td>
</tr>
</tbody>
</table>
1.17 Plant profile: *Aegle marmelos*.

1.17.1. General information

*Aegle marmelos* Correa, commonly known as bael fruit in english, belongs to the family Rutaceae, genus *Aegle* and species *marmelos*. Its stem, bark, root, leaves and fruits have medicinal value, and is traditionally used in herbal medicine. The medicinal properties of this plant have been described in the Ayurveda. In fact, as per Charaka (1500 B.C.), no drug has been longer or better known or appreciated by the inhabitants of India than the bael\(^{102}\).

![Flowers and fruit of Aegle marmelos](image)

Figure 1. 4. Flower (a) and fruit (b) of *Aegle marmelos* Correa. (Beal)

1.17.2 Distribution of the plant\(^{103},(104)\)

Bael tree is native to India and is found growing wild in Sub-Himalayan tracts from Jhelum east wards to West Bengal, in central and south India.
1.17.3 Biological Source:

Scientific classification:

**Kingdom:** Plantae

**Order:** Sapindales

**Family:** Rutaceae

**Subfamily:** Aurantioidae

**Tribe:** Clauseneae

**Genus:** Aegle

**Species:** A. marmelos

**Binomial name:** Aegle marmelos

1.17.4 Vernacular Names:

- English (bael fruit, holy fruit, golden apple, elephant apple, stone apple);
- Bengali: Bel
- Assamese: Bel
- Marathi- Bael
- Hindi (baelputri, bela, sirphal, siri-phal, kooralam);
- Oria- Belo,
- French (oranger du Malabar, cognassier du Bengale, bel indien);
- German (Belbaum, Schleimapfelbaum, Baelbaum);
1.17.5 Botanical Description:

*Aegle marmelos* is a slow-growing, medium sized tree, up to 12-15 m tall with short trunk, thick, soft, flaking bark, and spreading, sometimes spiny branches, the lower ones drooping. Young suckers bear many stiff, straight spines. A clear, gummy sap, resembling gum arabic, exudes from wounded branches and hangs down in long strands, becoming gradually solid. It is sweet at first taste and then irritating to the throat.

The deciduous, alternate leaves, borne singly or in 2’s or 3’s, are composed of 3 to 5 oval, pointed, shallowly toothed leaflets, 4-10 cm long, 2-5 cm wide, the terminal one with a long petiole. New foliage is glossy and pinkish-maroon.

Fragrant flowers [Figure1.4(a)] in clusters of 4 to 7 along the young branch lets, have 4 re-curved, fleshy petals, green outside, yellowish inside, and 50 or more Greenish-yellow stamens. The fruits are round, pyriform, oval, or oblong, 5-20 cm in diameter, may have a thin, hard, woody shell or a more or less soft rind, gray-green until the fruit [Figure 1.4(b)] is fully ripe, when it turns yellowish. It is dotted with aromatic, minute oil glands. Inside, there is a hard central core and 8 to 20 faintly defined triangular segments, with thin, dark-orange walls, filled with aromatic, pale orange, pasty, sweet, resinous, more or less astringent, pulp embedded in the pulp are 10 to 15 seeds, flattened oblongs, about 1 cm long, bearing woolly hairs and each enclosed in a sac of adhesive, transparent mucilage that solidifies after drying.
1.17.6 Chemical constituents of fruit:

The fruit pulp of *Aegle marmelos* contains carbohydrates, proteins, vitamin C, vitamin A, angelenine, marmeline, dictamine, O-methyl fordinol and isopentyl halfordinol. The natural oligosaccharides were characterized as 3-O-beta-D-galactopyranosyl-L-arabinose, 5-O-(beta-D-galactopyranosyl-L-arabinose and 3-O-beta-D-galactopyranosyl-D-galactose and acidic oligosaccharides as 3-O-(beta-D-galactopyranosyluronic acid)-D-galactose and 3-O-(beta-D-galactopyranosyluronic acid)-3-O-beta-D-galactopyranosyl-D galactose (105).

1.17.7 Traditional Uses of the various parts of the plant

All parts of *Aegle marmelos* are medicinally useful like leaves, fruit pulp, flower, stem bark, root bark etc.

- **Leaves:** Leaves are use as mild laxative, or the inflammation of mucus membrane having a free discharge and for asthma. The decoction of the leaves promote the removal of mucus secretion from the bronchial tubes. The leaf juice is given in dropsy or abnormal accumulation of liquid in the cellular tissue accompanied with constipation and jaundice. A hot poultice of the leaves is applied in ophthalmia or severe inflammation of conjunctiva with acute bronchitis and inflammation of the other body part (106).

- **Root:** The decoction of the root and sometimes the stem bark is useful in intermittent fever, also hypocondriasis and palpitation of heart. The decoction of the root is given with sugar and fired rice for checking diarrhoea and gastric
irritability of children \(^{107}\). Root is an ingredient of Dasamoola a standard ayurvedic remedy for loss of appetite and puerperal disease e.g. inflammation of uterus \(^{108}\).

**Flower:** Distillation of flower yielded a drug used as tonic for stomach and intestine, anti-dysenteric, ant diabetic, ant diaphoretic and as local anesthetic. It also used in epilepsy and expectorant \(^{109}\).

**Fruit:** Fruit is eaten during convalescence after diarrhea. It is valid for its mild astringency and as remedy for dysentery. The traditional healers of southern Chhattisgarh use dry powder of fruit with mustard oil for the treatment of burn cases. Fruits are also used in gastric troubles, constipation, laxative, tonic, digestive, stomachic, brain and heart tonic, ulcer, antiviral, gonorrhea, epilepsy and intestinal parasites \(^{111}\).

**Ripe fruit:** The ripe fruit promotes digestion and helpful in treating inflammation of the rectum. The ripe fruit extract shows antiviral activity against ranikhet disease virus. Fruit pulp also used as prevention during cholera epidemics, prevent the growth of piles, useful in the patients suffering from chronic dysentery condition alternating diarrhoea and constipation. Fresh juice is bitter and pungent helpful in lowering blood sugar \(^{110}\).

**Unripe fruit:** Fine powder of unripe fruit showed significant effect on the intestinal parasites and also effective against *Entamoeba histolitica* and *Ascaris lumbricoides*. Decogtion of unripe fruit is given as astringent in dirrhoea and dysentery \(^{110,111}\).
1.18 Drug Profile:

1.18.1 Levamisole Hydrochloride: 

Levamisole, marketed as the hydrochloride salt under the trade name Ergamisol (R12564), is an anti-helminthic and immuno-modulator belonging to a class of synthetic imidazo-thiazole derivatives. In the year of 1966 in Belgium the drug was discovered by Janssen Pharmaceutica. Initially it was prepared in the form of a racemate consist of two stereoisomers. Among them one called tetramisole and the other levorotatory isomer was given the name levamisole. Levamisole has been used in humans to treat parasitic worm infections. It has been used in combination with other chemotherapeutic agent as an adjuvant with chemotherapy for the treatment of colon cancer, melanoma and head and neck cancer. In some of the leukemic cell line studies, both levamisole and tetramisole showed similar effect (112-114).

1.18.1.1 General Informations:

- **Levamisole:** The levorotatory isomer of tetramisole
- **Synonym:** Levamisol, L-Tetramisole; dl-Tetramisol, Phenyl imidothiazole, Levamisolum, Levamisole hydrochloride.
- **Drug Category:** Adjuvants, Immunologic, Antinematodal agents, Anti-rheumatic Agents
- **Dose:** A single dose of 2.5 mg/kg has been recommended for antihelminthic therapy in humans and a dose of 50 mg, three times a day.

1.18.1.2 Chemistry:

- **Chemical Name:** (-)-2, 3, 5, 6-Tetrahydro-6-phenylimidazo-[2, 1-b]thiazole monohydrochloride
- **Molecular formulae:** C₁₁H₁₃ClN₂S
- **Chemical Structure:**

![Figure1.5. Structure of Levamisole Hydrochloride](image)

- **Molecular weight:** 240.7

1.18.1.3 Physicochemical properties:

- **Description:** A white crystalline stable powder.
- **Taste:** Metallic
- **Odour:** Characteristic odour.
- **Melting Point:** 227°C to 229°C.
- **Solubility:** Soluble in water, methanol, slightly soluble in ethanol, very slightly soluble in chloroform, insoluble in acetone
Optical Rotation: $[\alpha]_D^{20} = -124^\circ \pm 2^\circ (c=0.9 \text{ in water})$

PH: Levamisole solution has a pH of 3–4.5

UV maxima: 213 nm

Heavy metals: maximum 20 ppm. 12 ml of solution S complies with limit test A. Prepare the standard using lead standard solution (1 ppm Pb) R.

Loss on drying: maximum 0.5 per cent, determined on 1.000 g by drying in an oven at 100-105 °C for 4 h.

Sulphated ash: maximum 0.1 per cent, determined on 1.0 g.

Dissociation Constant: pKa 8

Partition Coefficient: Log P (octanol/water) = 0.64

Storage: Levamisole to be stored in well-closed, light resistant container

1.18.1.4 Pharmacology of Levamisole:

Levamisole acts as an anti parasitic, immunomodulators and adjuvant in colorectal cancer. It appears to restore depressed immune function through stimulating antibody formation and enhance T-cell response by stimulating T-cell activation and proliferation. Anti parasitic action appears to be tied to its agonistic activity towards nicotinic receptors in nematode muscles resulting in spastic paralysis. The net effect is a paralyzing of the worm, which is then expelled live\textsuperscript{(112-116)}. 
1.18.1.5. Mechanism of action:

The mechanism of action of levamisole as an antiparasitic agent appears to be tied to its agonistic activity towards the L-subtype nicotinic acetylcholine receptors in nematode muscles. This agonistic action reduces the capacity of the males to control their reproductive muscles and limits their ability to copulate. The mechanism of action of levamisole as an anticancer drug in combination with fluorouracil is unknown. The effects of levamisole on the immune system are complex. The drug appears to restore depressed immune function rather than to stimulate response to above-normal levels. Levamisole can stimulate formation of antibodies to various antigens, enhance T-cell responses by stimulating T-cell activation and proliferation, potentiate monocyte and macrophage functions including phagocytosis and chemotaxis, and increase neutrophil mobility, adherence, and chemotaxis.\(^{114-116}\).

1.18.1.6 Indications of the drug:

Levamisole is an antihelminthic drug approved for use in human medicine as well as veterinary medicine.

Other pharmacological useses of levamisole as-

1. It used as an immunomodulator in rheumatoid arthritis and colorectal cancer therapy.

2. A single dose of 2.5 mg/kg has been recommended for antihelminthic therapy in humans and a dose of 50 mg, three times a day, for three days every other week for colon cancer therapy.
3. Levamisole is used for adjuvant treatment in combination with fluorouracil after surgical rejection in patients with Dukes' stage C colon cancer.

4. Levamisole is also used to treat malignant melanoma and head/neck cancer.

1.18.1.7 Adverse drug reactions

Significant adverse reactions of levamisole are agranulocytosis, skin rash, and febrile illness, rheumatoid arthritis, blood dyscrasia. Nausea, vomiting, diarrhea, mouth sores, loss of appetite, stomach pain, change in taste and smell, muscle aches, fatigue, dizziness & headache.

1.18.1.8 Drug interactions

1. Anticoagulant effect increases when levamisole combines with Anisindione, Dicumarol, Acenocoumarol, Warfarin.

2. Levamisole hydrochloride has been reported to produce "ANTABUSE"-like side effects when given concomitantly with alcohol.

3. Concomitant administration of phenytoin and Levamisole fluorouracil has lead to increased plasma levels of phenytoin. The physician is advised to monitor plasma levels of phenytoin and to decrease the dose if necessary.

4. By taking levamisole and warfarin sodium, prolongation of the prothrombin time beyond the therapeutic range found in patients, it is suggested that the prothrombin time should be monitored carefully, and the dose of warfarin sodium or other coumarin-like drugs should be adjusted accordingly, in patients taking both drugs.
1.18.1.9 Pharmacokinetics of Levamisole:

Levamisole is rapidly absorbed from the GI tract and extensively metabolized in the liver and excreted mainly by the kidneys (70% over 3 days). Pharmacokinetic parameter is given in table 1.4 shows its plasma elimination half-life is 4.4-5.6 hours. Due to short elimination half-life, blood levels of levamisole fall more rapidly and go unnoticed in toxicological examination. Data from few clinical studies indicate that the consumption of 50-200 mg/day of levamisole causes agranulocytosis in 0.08 – 5% of the studied population (WHO, FAS 33) (117-119).

Table 1.5. Pharmacokinetics parameters of Levamisole

<table>
<thead>
<tr>
<th>Sl. No.</th>
<th>Pharmacokinetic Parameters</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Absorption</td>
<td>Rapidly absorbed from gastrointestinal tract</td>
</tr>
<tr>
<td>2.</td>
<td>Fraction Bioavailable (%)</td>
<td>47– 74.2%</td>
</tr>
<tr>
<td>3.</td>
<td>Elimination half-life (h)</td>
<td>4.4 – 5.6</td>
</tr>
<tr>
<td>4.</td>
<td>Volume of distribution (lit/Kg)</td>
<td>2-6</td>
</tr>
<tr>
<td>5.</td>
<td>Protein Binding (%)</td>
<td>20-25%</td>
</tr>
<tr>
<td>6.</td>
<td>Clearance (lit/min)</td>
<td>0.19 – 0.26</td>
</tr>
<tr>
<td>7.</td>
<td>C&lt;sub&gt;max&lt;/sub&gt; (ng/ml)</td>
<td>82 – 100</td>
</tr>
<tr>
<td>8.</td>
<td>T&lt;sub&gt;max&lt;/sub&gt; (hr)</td>
<td>3</td>
</tr>
<tr>
<td>9.</td>
<td>Steady state plasma concentration (mg/lit)</td>
<td>1</td>
</tr>
</tbody>
</table>
1.18.1.10 Metabolism & Excretion:

A single oral labeled levamisole dose in humans was eliminated in urine (60%) and feces (4%) within 24 hours. Urinary products included unchanged drug (3.2%) and free (1.6%) and conjugated (11%) p-OH-levamisole. Aminorex has been identified as a minor urinary metabolite in the horse; reported urine concentrations of levamisole and aminorex following oral or subcutaneous levamisole administration ranged from 210–2754 or 20–330 µg/L, respectively (118-122).
Chapter- 1

REFERENCE:


Chapter- 1


Chapter- 1


Chapter- 1


Chapter- 1


44. Ratner, B.D. and Bryant, S.J. (2004). Biomaterials: Where we have been and where we are going. *Annu Rev Biomed Eng*, 6, 41–75.


Chapter- 1


Chapter- 1


Chapter- 1


Chapter- 1


Chapter- 1


