Chapter 1

Introduction

1.1 Introduction to Buccal Drug Delivery System

In drug delivery advanced technologies turn the challenges into opportunities of drug deliveries. With the progressive explorations and developments in technologies, traditional drug delivery routes are being supplemented by versatile and sophisticated approaches of drug deliveries. Oral transmucosal route is most preferable route for systemic delivery. It is the most preferable and attractive route for the administration of drug as it provides some advantages for people over other injectable and enteral methods (Al-Hilal et al. 2013).

During the last decade it has been thoroughly investigated to overcome drawbacks of the orally delivered drug via GI tract. The oral transmucosal system bypasses the hepatic presystemic metabolism lead to higher bioavailability whereas oral mucosa having small surface area which may lead to drug loss because of uncontrolled salivary flow and uncontrolled swallowing (Shojaei et al. 2001).

The non-keratinized buccal mucosa of human is multistratified and drug diffusion across the buccal epithelium involves both the paracellular and transcellular routes. Several authors have been reviewed of the generalized structure of human buccal mucosa and its physiology, diffusion mechanism, various transport systems for drug and available different drug delivery system (Makwana 2012; Bruschi & de Freitas 2005; Harris & Robinson 2016; Pather et al. 2008; Rossi et al. 2005; Shojaei 1998; Sudhakar et al. 2006; Smart 2005). Various formulations have been explored and developed for the drug delivery by means of the mucosa of the oral cavity which include solids like tablets, patches, films, lozenges, wafers, microparticles; liquids dosage forms like solutions and suspensions; semisolid dosage forms like creams, gels, ointments, emulgels and chewing gums and sprays (Patel et al. 2011).
Development of new single chemical molecule requires huge amount of time and money so, there is a need to think about new delivery strategies to improve efficacy of already approved drugs. In spite of having enormous improvement in various delivery systems, the most preferable way for drug administration is oral route because of high patient compliance, low cost, ease of administration (self-administration) is possible. But along with these advantages it has several potential disadvantages like reduced efficacy some drugs due to unfriendly conditions for drugs in GIT especially polypeptides and proteins, GI intolerance, presystemic degradation of drug in the GIT, extensive hepatic presystemic metabolism and unpredictable and erratic absorption which results in lower availability of drug at the site of action, frequent administration requirement and compromised dosing accuracy has encouraged the consideration of another potential route for administration. Parenteral is the merely recognized route with the purpose of reducing all disadvantages affiliated with ineffective or less effective oral drugs but they are expensive and require repeated administration. Additionally they have least patient compliance and other detrimental effects with this route. Many pharmaceutical researchers from all over the world are attempting to investigate various routes like transmucosal and transdermal as they are more preferable route of administration than injectable routes over the last few decades. Different transmucosal sites are available but the buccal mucosa provides the most suitable and easily available site for both the systemic and local delivery. The atmosphere of the buccal mucosa can be manipulated and controlled for the optimization of rate of drug permeation and drug dissolution with the perfect design of dosage form. Presently various formulations including tablets, films, sprays, gels, mouthwashes, pastes are used for the oral transmucosal delivery. Spray formulations have added value in the field of pharmacy as novel and patient convenient dosage forms (Sudhakar et al. 2006; Patel et al. 2011).

1.1.1 Oral cavity

It is the first region of digestive system. For the local and systemic therapy oral mucosal cavity provides an attractive route for the administration of drug because it
has an outstanding penetrability and reasonable patient compliance. Oral cavity is outlined by the hard palate, soft palate, cheeks, lips and mouth floor (Figure 1.1).

It may be divided into two regions. The first one is the oral cavity involving mouth floor, hard palate, soft palate, and tonsil whereas the second one involve outer oral vestibule, surrounded by the cheeks and lips. The drug undergoes systemic absorption by means of capillaries and arteries. The blood is supplied to oral cavity by the major artery called external carotid artery. The veins backflow the blood by means of bunches of veins and capillaries lastly absorbed by the jugular vein (Rossi et al. 2005; Sudhakar et al. 2006; Patel et al. 2011; Madhav et al. 2009; Michael, Rathbone, Jonathan, Michael 2002).

Figure 1.1 Anatomy of oral cavity

1.1.1.1 Drug Delivery via Oral cavity (Madhav et al. 2009)

Drug delivery via oral cavity is classified as following:

**Buccal delivery:** The administration of drug occurs by means of the mucosa of buccal part composed of the area between the gums and upper and lower lips and the lining of the cheek to the systemic circulation.
**Sublingual delivery:** The drug distribution occurs by means of the mucosa of sublingual part consisting of mouth floor and membrane of the ventral surface of the tongue to the systemic circulation.

**Periodontal, gingival and odontal delivery:** This delivery is used to treat various conditions like fungal and bacterial infections, ulcers and periodontal diseases in oral cavity. Their anatomy, ability to permeate of drug and retention capability of the dosage form for the required time period varies from each other.

1.1.1.2 **Buccal Mucosa: Anatomy and Physiology** (Rossi et al. 2005; Michael, Rathbone, Jonathan, Michael 2002)

The super most layer of buccal mucosa is Stratified Squamous Epithelium (SSE) and the innermost layer is submucosa. Between the supermost and innermost layer a basement membrane and lamina propria are present (Figure 1.2).

![Figure 1.2 Schematic Diagram of Buccal Mucosa](image)

**Stratified squamous epithelium:** They are analogous to other epithelia found in rest of the body. They are thick and having about 40 to 50 cell layers. It consists of a basal cell layer; numerous layers are intermediary, and a superficial layer. Lining
epithelium of buccal mucosa is non-keratinized SSE that is approximately 500 to 600 μm thick and having 50 cm² surface areas with a cellular turnover time of 5-6 days. The topmost layer is made up of flattened compact differentiated cells they are 150 μm thick. Oral mucosa is leaky epithelia intermediate between the epidermis and intestinal mucosa. They have 4 to 4000 times more permeability than skin. To defend the underlying tissue from the destructive agents and fluid loss is the main function of the epithelia of oral cavity.

**Lamina propria:** It lies underneath the basement membrane and contain constant layer of extracellular substances and separates connective tissues and the basal layer of epithelia. It comprises collagen fibrils which support connective tissue, smooth muscle and blood vessels. Its structure is not much thick and it doesn’t hinder the permeation of drug. The capillaries and nerve fibers that supply the mucosa are present in this region. It is filled with lots of capillaries and blood vessels that open to the internal jugular vein.

**Submucosa:** This innermost layer exists below the superficial layer of epithelial layer and lamina propria. It is a dense connective tissue situated below the epithelial layer and contains some accessory salivary glands known as mucus acinus which are surrounded by myoepithelial cells which help in salivation.

### 1.1.1.3 Secretions of Oral cavity (Shakya et al. 2011)

The secretions include saliva, mucus and crevicular fluid.

**Saliva**

Saliva is consisting of both inorganic and organic substances. Sublingual, parotid and submandibular are the major salivary glands which create saliva same way minor salivary glands also make saliva. They are found in tissues lining of the oral cavity. Each major salivary gland is located outside the oral cavity. Mainly the sublingual gland produces viscous saliva with limited enzymatic activity and the submaxillary and parotid glands produce watery secretion. Approx. 0.5 and 2.0 L total
salivary volume is formed every day and in an adult, about 750 ml of over-all average salivary volume is made every day. Chemically they contain 95% water and 0.5% solutes like various ions namely chloride, sodium, magnesium, potassium, bicarbonate and phosphate. It also contain mucin, globulin, urea, serum albumin, uric acid, enzymes like lysozyme and amylase (ptyalin) and dissolved gases. The flow rates of saliva depend upon the person’s age and sex by his health status, day time, the category of stimulus used, time period for glands to be stimulated. Generally 0.3 ml/ min is the average resting flow rate for whole saliva and 1.7 ml/min average flow rate is for stimulated saliva. The pH of entire saliva differs between 6.5 and 7.5. The major buffering system is the bicarbonate system but protein and phosphate buffers exert a slight role.

There are various physiological functions of saliva. It cause variation in oral flora. It neutralize the acid in esophagus and oral cavity so regulate the pH. Teeth can be remineralized with calcium phosphate salts. It lubricates and cleans the mucosa of oral, esophagus and pharynges. It helps in development of bolus. Epithelial proliferation can be stimulated by saliva. The digestion of starch and fat can be initiated.

**Crevicular Fluid**

The gingival glands of oral cavity secrete the crevicular fluid.

**Mucus**

Mainly mucus is comprised of electrolytes, water and a combination of some glycoproteins, which included huge polysaccharides bound through minor quantities of protein. It is secreted more than many biological membranes of the body for example, throughout the gastrointestinal tract walls. Mucosa secretes mucus. Mucus admixtures with saliva secreted by salivary glands to form entire saliva.

The mucus is mainly composed of 95% Water; 0.5 to 1% Mineral salts; 0.5 to 5 % Glycoproteins and Lipids and 0.5 to 1% free proteins.
Mucus has following functions:

Its hydrophobicity characteristics provide protective mechanism. The second function is to block the drug absorption in tissue and impacts the bioavailability. Its key role is to retain mucosal membrane wet. It has strong cohesion properties and definitely fixes with the cell surface of epithelia as a continuous gel layer. Frequent mucus secretion secret from the global cell which is required to recompense for the elimination of mucus layer because of degradation by bacteria, digestion and solubilization of mucin fragments.

The important characteristics of mucus are:

The glycoproteins of mucus have amphoteric properties and so they have ability to buffer small quantities of either acids or alkalis. It is strongly resist the digestion by proteases enzyme. Mucus possess adherent potentials to get adhere firmly to the food or other particles and to spread as a thin film over the surfaces.

Mucin layer provide adhesion of formulation in oral mucosa with the help of mucoadhesive substances to retain the drug for longer period of time. But this also acts as barrier for drug penetration.

1.1.2 Buccal Absorption Mechanisms (Swarbrick 2007; Hillery, Lloyd 2001)

The permeation of drug through the epithelial obstruction is by means of two major routes (Figure 1.3):

- The paracellular route: It involves the transportation of the molecules through an epithelium by passing through the intercellular space between the cells.
- The transcellular route: In this route the transfer of the molecules across the epithelial cells. This transcellular pathway involves various mechanisms like carrier medicated transport, passive diffusion and through endocytic processes.
1.1.2.1 The Paracellular Route:

The compounds which are water soluble in nature and having low molecular weight may absorb from the mucosa through the paracellular route. In oral epithelia tight junctions are rare. Thus in most of the cases, absorption of small hydrophilic molecule occur via paracellular penetration as claimed for drug delivery via the epidermis of the skin. In the oral cavity, the intercellular space contains lipidic material, deposited from the MCGs. The lipidic molecules can permeate through this lipidic environment and thus being absorbed via this route.

1.1.2.2 The Transcellular route:

It includes the transfer of the active drug through the epithelial cell, either by passive or by active transport which as described as below:

**Transcellular passive diffusion**

The lipophilic drugs of having low molecular weight get absorbed transcellularly, through passive diffusion. Transfer of molecule occurs down a concentration gradient. The stratified epithelium are lipophilic in nature so it is required to permeate through a number of cell layers to achieve the underlying blood capillaries.

**Carrier-mediated processes**

It is for small substances like amino acids and mono-saccharides. Though it has not been completely categorized regarding specificity, location or ability to convey.

**Endocytic processes**

Presently they are poorly characterized in the mucosa of the oral cavity. Though the mucosa of buccal region develops more potential location for the systemic absorption of the drugs particularly having high molecular weight because they are able to cross the epithelial cells by endocytic process.
1.1.3 **Buccal Drug Delivery System** (Patel et al. 2011; Madhav et al. 2009; Sudhakar et al. 2006; Kumar, Aggarwal, 2011; Jagadeeshwar Reddy, Maimuna Anjum 2013; Ajeet 2012; Roychowdhury et al. 2011)

Buccal mucoadhesive formulations can be considered into 3 categories according to geometry.

1) **Type-I:** Single layer is involves in this device drug release is multidirectional. But due to swallowing, type-1 formulation suffers from considerable drug loss.

2) **Type-II:** Double layered device is involved by the bioadhesive layer in which drug is loaded. On the top of this layer, an impermeable backing layer is present which avoids the loss of drug from the upper surface of the formulation.
3) Type-III: In unidirectional device, there is negligible loss of drug because drug release through side of mucosa. Buccal formulations can be categorized as either matrix or reservoir type.

- Reservoir type: An extreme quantity of the drug is in the reservoir which is bounded by a membrane of polymer and thus it regulates rate of drug release.
- Matrix type: The active compound is evenly distributed in the matrix polymer. The release of drug is being regulated by diffusion via the network of polymer.

Different kind of buccal formulations are discussed below:

1. **Buccal Tablets**
   Generally dosage form like tablet has been discovered formulation for the drug delivery to the buccal region. They are flat, oval, small and approx. 5-8mm diameter in size. They become softer and adhere to the mucosa which helps to retain in place till the drug release is completed. They can be intended to various regions of the buccal cavity involving the mucosa lining the cheek, the palate and between the gum and lips. Sequential tablets can be placed to another sides of the mouth. As they are physically less flexible which leads to decrease the patient compliance for repeated use. Generally they are made by direct compression technique but wet granulation method can also be applied. Tablets inserted into the buccal pouch for buccal administration may rupture slowly. Multilayered tablets can be formulated by serially addition and compression of the ingredients to layer by layer.

2. **Buccal Chewing Gum**
   Particularly medicated chewing gum is given for the treatment of buccal cavity and in nicotine replacement therapy. The most important disadvantage of this formulation is to regulate the administered dose. Nicotine chewing gum is one of the finest examples of buccal chewing gum formulation. It may get the acceptance in termination of smoking habit. They create a steady state plasma concentration slowly, instead of creating the sharp rapid peak which happens for the time period of the
smoking. Main negative aspect associated with this formulation is the considerable nicotine loss because of chances of swallowing that leads to hepatic presystemic metabolism and gastric discomfort, hence it reduce the efficacy of this preparation. Due to flexibility and patient comfort patches are more preferred as best mucoadhesive buccal drug delivery systems.

3. Buccal Patches/Films

They are comprised of an impermeable backing layer which provide bioadhesive surface that helps to adhere for mucosal attachment and thus prevent drug loss, regulate drug release path and reduce disintegration and deformation of the dosage form and a reservoir layer which is filled with drug molecules and it release drug in a precise method. In terms of comfort and flexibility they can be chosen over adhesive. Oral gel is washed away easily by saliva so it provide short residence time however patches overcome this problem and defend the surface of wound, decrease pain and thus treat the disease condition more efficiently.

4. Buccal Gels and Ointments

They are the semisolid formulations. Ease of distribution throughout the buccal mucosa is the advantage of these formulations but exact drug dosing may not be possible as compared to other formulations like tablets, films or patches. By using bioadhesive formulations poor retention can be overcome in case of gel formulation. Poloxamer 407, carbopol, sodium carboxymethylcellulose, xanthan gum and hyaluronic acid are the examples of such bioadhesive polymers which go through a changes in phase from a liquid to a semisolid which increases the viscosity and thus releases drug in a controlled and sustained manner. Hydrogels are produced using polymers that can be hydrated in an aqueous atmosphere and entrapped the drug moieties for slow release by diffusion and thus it becomes a favorable formulation.
5. **Multiparticulates, Microparticles and Nanoparticles**

They show value-added performance as compared to the monolithic matrix tablets. As a result of relative small size, they exhibit higher residence time in gastrointestinal by the diffusion into the mucous gel layer. Recent study has revealed that, along with chemistry and size, shape is also a vital characteristic of particles involved in this kind of formulations and can control diffusion, particle velocity and adherence of the formulation in a complex manner.

6. **Sprays**

It is one of the most appropriate formulation which carry the drug moieties into the saliva or to the surface of mucosa and make it highly accessible for the absorption. The lag time is reduced because it carries the dose in fine particles.

7. **Wafer**

It is a novel periodontal delivery system, used to treat microbial infection. Bulk layer of this formulation composed of biodegradable polymers, matrix polymer and antimicrobial agents whereas surface layers having adhesive properties.

8. **Lozenges**

They include antibiotics, corticosteroids, local anesthetics, antimicrobials and antifungal. They can apply topically within mouth. Multiple dosing is required daily in lozenges due to drug release is primarily high and then decline quickly in case of buccal cavity.

9. **Gels**

In this formulation, cross linked polyacrylic acid is used as gel forming bioadhesive polymers. Such gel forming bioadhesive polymers have been used for the adherence in a controlled manner. Gel formulations are widely used in buccal cavity. Benefits of this gel formulation involve rapid release of drug at absorption site and the capability to make intimate contact with the membrane of mucosa whereas drawback
of this formulation involve the lack of capability to convey a measured drug dose to
the site. Generally bioadhesive gel, ointment and powder have less patient acceptance
than solid adhesive formulations. Mostly they are used for local treatment in the buccal
cavity. For example, it has been intended locally for administration of steroids to treat
ulcer in buccal mucosa.

10. Gel forming liquids and *in situ* gel

Primarily viscous solutions have been explored to cover the buccal mucosa by
acting as a protectant. It is used locally to treat fungal infection and motility dysfunction
by acting as a vehicle for drug delivery. Pharmaceutical researchers has revealed that
the use of suspension of sodium alginate as novel bio adhesive liquid which coat the
surface of esophagus provide protection against reflux and can transport drugs to the
injured mucosa. The ability of retention was estimated on the surface of esophagus
under conditions imitating salivation. Both xanthan gum and polycarbophil exhibited
brilliant bioadhesive potential whereas thermosensitive poloxamer and carmellose
sodium exhibited lower retention. Poloxamer linked covalently to carbopol and
polyacrylic acid. Upon oral administration this “esophageal bandage” showed
remarkable retention within the esophagus.

1.1.4 Advantages of Buccal Drug Delivery Systems (Rossi et al. 2005; Patel et al.
2011; Hillery, Lloyd 2001; Bhattacharjee et al. 2014)
The following advantages can be obtained with this buccal drug delivery system.

Accessibility

For the application and removal of the various formulations, the buccal cavity
provides a large amount of available surface for the delivery of drug. This availability
gets rid of the requirement of complex devices for the delivery of the drug to the
absorption site. Thus simply designed devices are used for buccal than those
administration to transport drugs to the alveolar area of the lung.
Easy to use

Due to ease of administration these buccal devices such as films, tablets and sprays gain more patient compliance as compare to formulations like suppositories and pessaries used for rectal and intravaginal routes respectively.

Rich blood supply

The extremely vascularized buccal surface as well as also maintains sink condition. Additionally buccal region exhibits benefit of fast onset of action and the capability of sustained drug delivery for numerous hours.

Low metabolic activity

The buccal cavity show less metabolic activity than gastrointestinal zone which makes it the most preferable option for enzymatically labile drugs such as proteins and peptides. It undergoes the presystemic metabolism in liver or in the intestine before reaching to the systemic circulation.

Low variability

Buccal route of administration has less variability as compared to oral route. The only factors that influence a degree of variability in buccal route are salivary flow and disease condition whereas intestinal motility, extremes of pH and presence of food can contribute to make oral route highly variable.

Robust

The mucosa of oral cavity is usually displayed a large amount of various foreign substances, comparatively strong and less susceptible to irritation than mucosa of nasal cavity.

Prolonged retention

If the proper drug delivery system is used, the drug retention period can be prolonged in buccal cavity which reduces the frequency of drug dose.
Intestinal alternative

The buccal cavity is a valuable substitute for the absorption of drug in condition where the GI route is not feasible. In case where patients are suffering from nausea, vomiting and swallowing difficulties should prefer the buccal delivery. Some drugs are susceptible to irritation in the gastric region, unstable in GI fluids and undergo considerable presystemic metabolism in the gastrointestinal wall or hepatic presystemic metabolism.

Zero order controlled release

They have the ability to attain zero-order controlled release which offers some advantages. It reduce the frequency of dose whereas it increase patient compliance. It avoid the risk of ineffectiveness and toxicity of conventional treatment.


They can be described as follows:

Limited to potent molecules

Drugs having large molecular size get poorly absorbed so buccal delivery system is restricted to potent drug moieties only; normally for the drugs having low effective plasma concentrations.

Adverse reactions

Sensitive and irritable drugs should be used carefully for the local treatment in buccal cavity. But as explained ahead the epithelial region of oral cavity is comparatively robust which is not as restrictive as in other extremely sensitive nasal mucosa.
Metabolic activity

Buccal route of administration has less metabolic activity for proteins and peptides as compared to GI tract. Mucosa and secretions of oral cavity are capable to degrade drugs and that measures might be essential to get control of this.

Mucus and salivary clearance

It reduces the drug retention period within the buccal cavity which gives chances for absorption.

Mucus barrier

The diffusion of drug can be restricted by the physical obstruction of the mucus layer and by the nonspecific or specific binding of drugs to the mucus layer.

Patient acceptance

Buccal film like novel formulation is put in an unusual site so complications may occur for getting acceptance of patients towards this route. The risk observed with this system that it would be swallowed. Eating and drinking may become restricted.

Commercial

The use of novel dosage forms like buccal adhesive film for the systemic drug delivery for high molecules which involve a vast time input, money, effort, and related with a large amount of risk which make considerable delay in the formulation and advertising of novel system and also comparatively produce them costly.
1.2 Introduction to Diabetes (Bluestone et al. 2010; Akerblom & Knip 1998; Kahn 2003; Deshpande et al. 2008; Grant et al. 2009)

Definition: Diabetes mellitus can be defined as a metabolic disorder of multiple etiologies described by long-lasting hyperglycemia with disruptions in the metabolism of fat, protein and carbohydrate which results from lack in secretion of insulin, its action or both.

The World Health Organization declares the range of blood glucose indicative of normal people or non-diabetic person is as follows:

- Fasting blood glucose level should be between 70-130 mg/dl
- Postprandial blood glucose level should be below 180 mg/dl

Symptoms of Type 1 Diabetes

- Increased urinary frequency
- Increased thirst
- Unexplained weight loss
- Increased appetite
- Muscle weakness
- Poor blood flow
- Blurred vision
- Feeling tired
- Muscle wasting
- Very dry skin
- Sores that are slow to heal
- More infections than usual
- Tingling or numbness in hands or feet

Along with some above symptoms, nausea, vomiting or stomach pains may be observed in sudden onset of IDDM, now called type 1 diabetes.

1.2.1 Roles of Insulin and Glucagon in regulating the normal blood glucose level

A healthy person’s body maintains normal blood glucose range through some complex mechanisms. The two hormones like Insulin and glucagon are produced from the pancreas which help in regulating the glucose level in bloodstream:
• β-cells of islets produce insulin that lowers elevated level of glucose in bloodstream.

• α-cells of islets produce glucagon that raises lower level of glucose in bloodstream.

After meal when the levels of glucose get higher in bloodstream the insulin is released from the pancreatic β-cells into the bloodstream.

• This produced Insulin helps the various body cells (i.e. fat, liver and muscle cells) to take up glucose molecules from the bloodstream hence lowering the glucose levels in the bloodstream.

• Insulin also excites the muscle and liver tissue to store extra amount of glucose that are known as glycogen.

• Insulin also lowers the levels of glucose in blood by reducing the formation of glucose in the liver.

When the levels of glucose in blood drop overnight or due to heavy exercise or by skipped meal, the glucagon is released from the pancreatic α-cells into the bloodstream.

• This produces glucagon sends signals to the tissues of liver and muscle to convert glycogen (the stored form of glucose) into glucose molecules and released the glucose molecules into the bloodstream hence it raises the level of glucose in bloodstream.

• If the body is required to have more glucose molecules for energy, glucagon leads to the formation of glucose from amino acid by stimulating the liver.
1.2.2 Types of Diabetes

Generally two types of diabetes are there: Type 1 and Type 2

1) Type 1 Diabetes

It is an autoimmune type disease because body’s immune system attacks and damages the pancreatic β-islet cells, which is responsible for the insulin production. β-cell destruction leads to the shortage of insulin, thus it causes Type 1 Diabetes Mellitus. In this type, destruction of β-cell may occur for more than several years but its symptoms generally develop over a short period of time. Its occurrence may be in children and young adults or before the age of thirty. Previously it is well-known as juvenile diabetes or insulin-dependent diabetes mellitus because it needs insulin dose every day. It may account for 5% to 10% of all diagnosed cases of diabetes mellitus. Genetic, autoimmune and environmental factors are the examples of the associated risk factors with type 1 DM. Thus insulin secretions from the β-cells decreases and ultimately ends completely.
2) Type 2 Diabetes

This is most common type and caused by insulin resistance (reduction in sensitivity towards insulin), a situation in which the body cells of fat, liver and muscle do not utilize insulin efficiently. Diabetes mellitus type 2 develops when the body does not create adequate amount of insulin to recompense the impaired capacity to use insulin. Its symptoms may appear slowly and can be slight and in some cases people remain undiagnosed for years.

Previously it is known as non-insulin-dependent diabetes mellitus or adult-onset diabetes. Generally diabetes mellitus type 2 occurs in either middle-aged people associated with obesity or overweight or those older than 40 years of age. Researchers assume that environmental factors and genetic susceptibility are the most associated risk factors which triggers of diabetes mellitus type 2. It may account for about 90% to 95% of all diagnosed cases of diabetes. Obesity, prior history of gestational diabetes, family history, physical inactivity, impaired glucose tolerance and nationality are the associated risk factors with Diabetes mellitus Type 2.

1.2.3 Consequences (Loghmani 2005)

Type 1 Diabetes

Prior to diagnosis of diabetes mellitus type 1, the level of glucose in blood is high and symptoms seen are increased thirst, appetite and urination. Additionally it involves weight loss or decrease in grow rate. After diagnosis, patient must follow a daily treatment which involve injections of insulin on a regular basis, examination of glucose level in blood, awareness regarding food intake particularly carbohydrates. The course of therapy may include stress to families so they must get wide-ranging education regarding diabetes, training for the self-management of diabetes, checking out frequently, and social support commonly because the trouble of having a chronic disease and living the life with such disease is a result that is often ignored.
Serious consequences develop with too much administration of insulin or insufficient administration of insulin. Insufficient food intake or too much insulin leads to hypoglycemia, unconsciousness and seizures. Insufficient administration of insulin reflects chronic hyperglycemia which results in various complicated diseases associated with eyes and kidney. It also involves nerve damage, and risk of cardiovascular complications are increased which become visible in 12-14 years after diagnosis.

Type 2 Diabetes

Prior to diagnosis of diabetes mellitus type 2, the high level of blood glucose is there with no signs. After diagnosis, patients must follow a daily treatment which involves awareness regarding food intake particularly carbohydrates, fats and total energy intake, examination of glucose level in blood and exercise on a regular basis. Medication like insulin or glucose lowering oral therapeutic agents may also be required. The course of therapy may include stress to families so they must get wide-ranging education regarding diabetes, training for the self-management of diabetes, follow-up frequently, and social support on a regular basis. Severe consequences will depend on the prescribed medication. People administered with insulin or sulfonylureas are at risk because of lowering the glucose level in blood stream. Consequences for long term are similar to the consequences with diabetes mellitus type 1 which include complicated disease related to various organs of the body like eyes and kidney. Additionally it involves damage in nerves and greater risk of cardiovascular complications.

1.2.4 Diabetes and its Complications

Diabetes and its complications prove to be major reason of mortality and morbidity around the world. It has an effect on various organs in the body which leads to very serious complications. The complications can be categorized as macrovascular and microvascular. The former one includes stroke, peripheral vascular disease and cardiovascular disease whereas latter one involves neuropathy (Damage to nervous system), nephropathy (renal system damage) and retinopathy (eye disease). Peripheral
vascular disease may lead to injuries or bruises that do not cure after a while cause gangrene and ultimately segregation. (Deshpande et al. 2008) Other complications with diabetes mellitus may involve loss of mobility with aging, enhanced exposure to other diseases, depression, and pregnancy problems.

1.2.5 Therapy to treat the Diabetes Mellitus type 1 (Loghmani 2005)

Insulin Therapy

Insulin is the only prescribed medicine which effectively reduces glucose level in bloodstream in the patients having type 1 diabetes mellitus. Various factors like food, stress, illness and physical activity should be managed on a regular basis because they significantly affect the insulin dose during the treatment with insulin injections. Rapid-acting insulin can be administrated before a meal, during a meal or instantly after a meal. Insulin taken after a meal helps in lowering the postprandial hyperglycemia related with food involving high fat.

- Rapid acting insulin analogs (e.g. Lyspro, Aspart): It is used in children and in conditions where other techniques become ineffective to regulate post-prandial hyperglycemia.
- Long acting insulin analogs (e.g. Glargine): It is applied to treat type 1 diabetes. Additionally it can be applied to treat diabetes type 2 who need long-acting insulin to regulate the blood glucose levels.

Insulin can be injected subcutaneously to the various parts of the body like thigh, upper arm, buttocks and the abdomen. Thigh and abdomen are the most suitable sites for self-administration of insulin injection. The quantity of insulin injections/day will differ; insulin can be administered using various devices like insulin syringes, insulin pens or insulin inhalers etc.

**Insulin Syringes**: To inject insulin subcutaneously using needle and syringe is the most common form of delivery. Syringes comes in a range of different sizes.
Insulin Pens: They appear similar to extra-large ink pen. The pen cap is removed and a pen cartridge is attached then the cartridge is inserted subcutaneously and delivers the insulin dose by pressing the knob. Several devices are available. In some devices replaceable needles of insulin are used whereas some pens are disposable. The major benefit is that insulin pen provide accurate dose of insulin. The pen needles are slightly thin and in some cases shorter than syringe needles which make it more comfortable. Due to this advantage over syringes they are easy to use. They deliver the insulin in a careful manner which allows the people to administer whenever they want.

Insulin Pumps: Currently it is available for continuous subcutaneous insulin infusion in the country. Its use may be restricted to diabetes specialists with relevant experience.

Insulin Jet Injectors: A high-pressurized air is used to transport a fine spray of insulin. They are novel but complex. Additionally this device is costly and involves frequent sterilization.

Insulin Inhalers: In this device, compressed air is used to carry dry insulin which can be inhaled by the mouth and reaches directly into the lungs where dose of dry insulin is absorbed and passes into the bloodstream.

Treatment for Type 2 Diabetes

Glucose Lowering Therapeutic Agents

They are intended for to treat the patients having non-insulin diabetes mellitus, failing to acquire favorable glycemic control with sufficient diet/exercise. The selection of these agents should be done based on patient’s condition with consideration specified to the pharmacological and safety profile of each glucose lowering agents. Primarily the dose of drug should be low and then dose should be increased as per requirement for acquiring the glycemic control.
1.2.6 Side Effects associated with Oral Hypoglycemic Agents (ICMR 2005)

Sulphonylureas:

Hypoglycemia is the commonest side effect and possible to be prolonged and profound with long acting sulphonylureas, like glibenclamide and chlorpropamamide and hence they should be used with extreme caution in the elderly. The associated side effects are as follows:

- Mild nausea or vomiting. It may be reversed with discontinuity.
- Uncommonly anemia, skin rashes, steven-Johnson’s syndrome, cholestatic jaundice, leucopenia, granulomatous hepatitis or thrombocytopenia may happen.
- People with diabetes gain weight by some sulphonylureas.
- Photosensitivity reactions to sulphonylureas (hyperpigmentation of exposed parts) may occur.
Non-Sulphonylureas:

**Meglitinide analogues:** They produce fewer and milder hypoglycemic episodes and other side effects compared to sulphonylureas.

**Biguanides:** GI side effects like diarrhea and abdominal discomfort may occur in some people with diabetes. Metformin administration after meals with slow titration of doses can use to minimize these GI side effects. Lactic acidosis is a rare side effect and is rare in the absence of other serious hypoxic medical disorders.

**Alpha-glucosidase inhibitors:** Abdominal discomfort, bloating, flatulence and diarrhea like gastrointestinal side effects are common.

**Thiazolodenediones:***

- Treatment with glitazone combined with sulphonylurea or insulin produce mild to moderate hypoglycemia in people with diabetes undergoing therapy with glitazone in combination with a sulphonylurea or insulin.
- Weight gain may be quite significant in many people with diabetes and its dose related.
- Edema and cardiac failure are reported in some people with diabetes, especially when combined with insulin.
- Other adverse events of glitazone include anemia and haemodilution.
- Liver dysfunction is relatively rare with rosiglitazone and pioglitazone compared to troglitazone.

Thus most of them have numerous severe adverse effects; so, the search for more efficient and safer hypoglycemic agents is one of the important areas of investigation.
1.3 Introduction to Medicinal Plants

In ancient Ayurvedic medical texts such as Charak Samhita and Sushruta Samhita, glycosuria was known as a symptom of diabetes and therefore treatment of diabetes was phenomenal (Nagarajan et al. 1982).

Apart from insulin and oral hypoglycemic agents, phytotherapy are the most alternative therapy which includes a variety of herbal medicinal plants whose raw materials showing hypoglycemic activities therefore they can be recommended for diabetic people. The traditional medicinal plants are evaluated and believed to be an outstanding candidates for diabetes and have been recommended as non-toxic, efficient and with less or no side effects by the World Health Organization (World Health Organization 1980).

However some herbal extracts have been verified for hypoglycemic effects in diabetic models using human and animals in diabetes mellitus type 2. A less toxic biguanide for example Metformin is potent oral glucose lowering agent which was actually developed from Galenga officinalis and is being used to treat diabetes. So, medicinal plants in various oral formulations have been suggested for the treatment of diabetes.

Many plants are recognized with hypoglycemic activities from across the world since times immemorial. In the last few decades various researchers performed on plants and cited them in ancient literature or traditionally used for diabetes, have presented antidiabetic activity. The plant species have confirmed their efficiency to lower the glucose level in blood and therefore one of alternative to treat diabetes mellitus. Recently herbal products have started gaining importance as an alternative and complementary medicine to treat DM. some plant reviews with known antidiabetic property or conventional use as antidiabetic therapy have been published (Bailey et al. 1986; Bailey & Day 1989; Ivorra et al. 1989).

Gupta et al.(2013) have reviewed some medicinal plants that have a varying degree of hypoglycemic activity along with hypolipidemic and antioxidant properties. The significant potent medicinal plants have negligible side effects than currently available allopathic oral hypoglycemic drugs. The antidiabetic property of various
herbal medicinal plants are recognized due to the presence of terpenoids, polyphenols, alkaloids, flavonoids, glycosides and some active constituents which shows glucose lowering properties. Several mechanisms of actions have been predicted for those plant extracts. Several herbal drugs have sound effects on the activity of pancreatic β-cell (release of insulin and regeneration of β-cell) or some drugs enhance the insulin sensitivity and some of the plant extracts exhibit insulin-like activity. Other mechanisms may involve improved glucose homeostasis and some others like because intestinal glucose absorption is inhibited, glycemic index of carbohydrates is reduced or effect of glutathione is decreased. These mechanisms of actions may be liable to reduce and/or to abolish diabetic complications.
1.4 Introduction to *Gymnema sylvestre*

Botanical Name: *Gymnema sylvestre* R.Br.

Family: Asclepiadaceae

![Figure 1.6 Gymnema sylvestre R.Br.](image)

Vernacular names

<table>
<thead>
<tr>
<th>English</th>
<th>Miracle fruit</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gujarati</td>
<td>Dhuleti, mardashingi, Gurmar</td>
</tr>
<tr>
<td>Hindi</td>
<td>Gurmar</td>
</tr>
<tr>
<td>Sanskrit</td>
<td>Meshashringi, madhunashini</td>
</tr>
<tr>
<td>Marathi</td>
<td>Kavali, vakundi, kalilardori</td>
</tr>
<tr>
<td>Telugu</td>
<td>Podapatri</td>
</tr>
<tr>
<td>Tamil</td>
<td>Adigam, cherukurinja</td>
</tr>
</tbody>
</table>

1.4.1 Description

1.4.1.1 History

In India *Gymnema sylvestre* (GS) has been used to treat the diabetes for more than 2,000 years. Primarily it was applied for non-insulin dependent diabetes mellitus, a state for which it is suggested continuously in India. Additionally, *Gymnema sylvestre* leaves were used to treat constipation, liver disease, water retention and stomach ailments.
1.4.1.2 Botanical Description

It is consists of the leaves of a perennial woody climber plant known as *Gymnema sylvestre* belonging to family Asclepiadaceae. It is found in India mostly in the tropical forests of Southern and Central India. It is also present in Konkan, Banda, Western Ghats and Deccan peninsula and in Northern and Western region of India. (Grover et al. 2002; Kritikar 1998; Yoganarasimhan 1990) Leaves are elliptic or ovate with acute or acuminate apex. The lower surface of leaf is more pubescent and base is rounded or cordate. Non-glandular trichomes are present on both the surfaces of leaf. It bears small yellow colored flowers in the form of cymes (Potawale et al. 2008; Kritikar 1998; Nadkarni KM 1976).

1.4.2 Traditional uses

Sushrut samhita defines *Gymnema sylvestre*, as a destroyer of madhu meha (glycosuria) and other urinary disorders. Mainly leaves of *Gymnema* have been used for the treatment of madhu meha or “honey urine” for more than 2,000 years in India. By virtue of its property of abrogating the essence of sugar it has been given the name of gur-mar due to its importance of sugar annihilation in the body in Diabetes mellitus (Khanna, Krishnan 2009). Thus traditionally, the term “destroyer of sugar” is used for GS because chewing of leaves will discriminate the sweetness.

Among various activities, the plant is attributed to other activities like biting, astringent, harsh, thermogenic, calming, anodyne, stomach related, liver tonic, emetic, diuretic, stomachic, stimulant, anthelmintic, purgative, cardiotonic, expectorant, antipyretic and uterine tonic. This medicinal plant is also valuable in dyspepsia, clogging, jaundice, hemorrhoids, renal and vesical calculi, cardiopathy, asthma, bronchitis, amenorrhoea, conjunctivitis and leucoderma (Saneja et al. 2010; Satdive et al. 2003). *Mahakalyanakaghrtam, Ayaskri, Varunadi kasaya and Varunadighrtam* like various Ayurvedic preparations involve this medicinal plant in their composition.
1.4.3 Pharmacological review

Anticancer activity

Various extracts like chloroform, ethyl acetate and alcoholic extract of *Gymnema sylvestre* were tested on epithelial cells of human lung cancer cell lines and human breast cancer cell lines *in vitro*. Among them, ethyl acetate and chloroform extracts showed comparatively better result than alcoholic extract on epithelial cells of human lung cancer cell lines (Malik et al. 2008).

Antimicrobial activity

The ethanolic concentrate of *Gymnema sylvestre* leaves revealed antimicrobial action against *B. subtilis*, *Bacillus pumilis*, *Staphylococcus aureus* and *Pseudomonas aeruginosa*.

Antihyperlipidemic activity

The effect of *Gymnema sylvestre* extract was significantly antihyperlipidemic that can be due to flavonoids, tannins, phenols, and triterpenoids which are present in phytochemical screening (Fushiki et al. 1992).

Anti-inflammatory activity

After administration of the *G. sylvestre* aqueous extract it reduced the paw oedema volume by 48.5% within 4 hrs which showed significant reduction in granuloma weight, when compared to control group (Shanmugasundaram et al. 1990).

Free radical scavenging activity

Significant results were observed when antioxidant potential of aqueous extract of *G. sylvestre* was evaluated *in vitro* against DPPH radicals and LDL oxidation.
**Antihyperglycemic activity**

Since times immemorial, *Gymnema sylvestre* (GS) has been applied to treat the diabetes mellitus as Ayurvedic medicine of India. Phytochemically, several glycosides (various gymnemic acids) exhibit selective anaesthetic effect. Experimental studies involving rats fed on high carbohydrate diet showed minor reduction in blood sugar in normal rats, whereas marked reduction in anterior pituitary treated hyperglycemic rats. The drug influenced the disturbed carbohydrate metabolism in hyperglycemic animals by limiting the carbohydrate turnover and thus inhibiting the vicious cycle of hyperglycemia (Liu et al. 1992). Water soluble fractions (GS3, GS4) of *Gymnema sylvestre* were evaluated against streptozotocin induced diabetes for their effect on blood glucose homeostasis and pancreatic endocrine tissue which displayed blood glucose homeostasis through increased serum insulin levels provided by repair/regeneration of the endocrine pancreas (Sahu et al. 1996).

1.4.4 **Phytochemistry**

Phytochemical investigations of *Gymnema sylvestre* leaves revealed presence of triterpene saponins belonging to oleanane and dammarene classes, the majority of which are a group of oleanane type triterpenoid saponins known as “gymnemic acids”. Besides these, some additional plant constituents are phytin, flavones, resins, anthraquinones, lupeol, d-quercitol, α and β- chlorophylls, hentriacontane, pentadecacontane, formic acid, tartaric acid, butyric acid, β-amyrin related glycosides and stigmasterol (Davis 2016; Holstein et al. 2001; Gregorio et al. 1996).
1.4.5 Mechanism of Action

The hypoglycemic effect of *Gymnema sylvestre* leaves was first documented in the late 1920s. According to recent reports, various formulations of gymnemic acid have been found very effective against obesity which can be attributed to the ability of gymnemic acids to delay the absorption of glucose in the blood (Yoshikawa et al. 1993). The gymnemic acid and glucose molecules possess similar atomic arrangement so the molecules of gymnemic acid fill the receptor on the taste buds. In this manner it prevents the activation by sugar molecules present in the food and thus curbing craving for sugar. Same way they occupy the receptor of the absorptive external layers of the intestine and thus preventing the sugar absorption by the intestine, which lowers the level of sugar in blood (Sahu et al. 1996). Some possible mechanisms by which the leaves of *Gymnema sylvestre* and especially Gymnemic acids exhibit its hypoglycemic effect, which may be increase in secretion of insulin and utilization of glucose. It also promotes regeneration of islet cells and inhibits absorption of glucose from intestine, the exact action being unknown. It could also be involved in one or more mechanisms (Nakamura et al. 1999).