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1.1 DIABETES - PERSPECTIVE OF AYURVEDA

Ayurveda (In Sanskrit “knowledge of life” or “knowledge of longevity”) is one of the most ancient traditions of India and it has now spread beyond India to other countries like, Sri Lanka, Malaysia, Mauritius, South Africa, Japan, Russia, Europe, and North America (Elder, 2004; Hankey, 2005; Patwardhan, 2010 and Vaidya, 2001). Herbs are commonly used for treatment in Ayurveda. Indian healthcare consists of various systems of medicines and ayurveda still remains dominant compared to modern medicine, particularly for treatment of a variety of chronic disease conditions. Considerable research on pharmacognosy, chemistry, pharmacology and clinical therapeutics has been carried out on ayurvedic medicinal plants (Patwardhan, 2004). The Ayurvedic Pharmacopoeia of India is especially rich in herbal treatments for diabetes (Ayurvedic Pharmacopoeia of India, 2008). Ethnobotanical studies of traditional herbal remedies used for diabetes around the world, have identified more than 1,200 species of plants with hypoglycemic activity although only a few of them have been scientifically studied (Ajgaonkar, 1979; Yoshiharu, 1994; Alarcon, 2000; WHO, 2005 and Vaidya, 1979). Medicinal plants used to treat hypoglycemic or hyperglycemic conditions are of considerable interest for ethno-botanical community as they are recognized to contain valuable medicinal properties in different parts of the plant and a number of plants have shown a varying degree of hypoglycemic and antihyperglycemic activity.

India is endowed with traditional wealth of medicines as is evident from the fact that the ‘Shushruta-Samhita’, the ancient repository, differentiated between genetically and the acquired forms of diabetes and recommended different treatments for the two types of diabetes (Grover, 2002). In India, plants have long been used for the empirical treatment of diabetes (Pulok, 2006 and Vaidya, 2008). The hypoglycemic activity of large number of these plants have been evaluated and confirmed in different animal models (Preston, 1985; Portha, 2007 a; Frode, 2008). Diabetes mellitus was well known to the ancient founders of Ayurveda, as judged from the detailed descriptions of the disease in the classic texts like Charaka –Samhita, Sushruta-Samhita and Bhrigu-Samhita etc. (Satyavati, 1989 and Dhanukar, 2000).
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It means, 'Madhumeha' is a disease in which a patient passes sweet urine and exhibits sweetness all over the body i.e. in sweat, mucus, breath, blood etc. (Ashtang Hridayam, 2000 and Subbalakshmi, 2001). Diabetes mellitus (DM) is described in Ayurveda as madhumeha kshaudrameha which literally means “excessive urine with sweet taste like honey,” or dhatupak janya vikriti which means a disease caused by a defective metabolism leading to derangement in body tissue (seven dhatus) transformation process (Subbalakshmi, 2001; and Dwivedi 2007).

Historically, Ayurvedic texts have described 20 types of urinary disorders (pramehas) based on the predominant doshas (10 kaphaja, 6 pittaja, and 4 vataja urinary disorders) and physical characteristics of the urine (e.g., volume, color, odor, taste, sediments, solid particles, presence of seminal fluid, and mucus). The urine is discharged in excessive quantities and is generally turbid. DM is one of these pramehas that may occur in any of the three (vata, kapha, or pitta) body constitutions. The Ayurvedic approach to DM management includes life-style dietary interventions, exercise, and a variety of hypoglycemic herbs and herbal formulas depending upon the predominant dosha. Cleansing procedures are unique to the Ayurvedic approach to DM. However, the Ayurvedic clinical description of DM, etiology, diagnosis, prognosis, and recommended lifestyle changes are basically similar to those described in Western medicine (Vaidya, 1971).

1.1.1 Clinical Description (Rog Viakhya) and Etiology (Vyadhi Haitu)

The major signs and symptoms of DM described in classic Ayurvedic texts consist of honey like sweetness of urine, thirst, polyphagia, tiredness, obesity, constipation, burning sensation in the skin, seizures, insomnia, and numbness of the body. Boils, wounds, and abscesses are often difficult to heal in a diabetic patient and are recognized in Ayurveda. All these symptoms are very similar to
those currently described in Western medicine. Ayurvedic physicians also use modern diagnostic chemical analysis of urine and blood for confirmation.

The etiology of DM in Ayurveda is multifactorial. DM may be a familial trait, and overweight (fat - meda) patients with this diagnosis may be engaged in a lethargic lifestyle and unhealthy diet (e.g., idle sitting, excessive sleep, overeating sweet and fatty food items, and lack of physical exercise) (Crawford, 1999; Vaidya, 1989, Vaidya, 2004 and Mishra, 2004).

Ayurveda divides DM into two categories:

- Genetic (sahaja), occurring in young age from the very beginning of life that has some similarities with the juvenile diabetes or insulin-dependent diabetes; and
- Acquired (apathyaja) due to an unhealthy lifestyle that occurs in old age and obese people and has similarities with type 2 DM.

In addition, Charak Samhita (100 to 400A.D.) describes two types of DM: one that occurs in very underweight people (krsa prameha) and one that occurs in obese people (sthula). The former DM requires restorative (santarpan) treatment along the line of insulin treatment and the latter requires fat-reducing (apatarparna) treatment (Sharma, 1998 and Bhaishajya Ratnawali, 1985).

Type 2 DM has even greater involvement of genetic factors than type 1 DM, but no specific gene has been linked to DM to account for the role. There is no evidence available to show the role of autoimmunity in type 2 DM. The two major problems in type 2 DM are the reduced secretion of insulin from beta cells and development of resistance to insulin in peripheral tissues (Vaidya, 2002). Obesity is another major etiological factor, as 80% of type 2 DM patients are obese. Sahaja or Beeja-dosha type of diabetes was said to be quiet recalcitrant (Asadhya). The obese patients were managed by langhana-fasting (calorie restriction) and the lean by additional nutrition (calorie supplementation and nutrients).

Apathya nimittaja or aberrant life-style as well as obesity are managed by correcting the life-style (diet, exercise, rest), samshodhana (Panchakarma procedures) and samshamana (medications etc.) (Singh, 1998)
1.1.2 Pathogenesis (Samprapti)

According to classic Ayurvedic texts, DM and all pramehas (urinary disorders) start with the derangement of kapha that spreads throughout the body and mixes with fat (Meda) that is similar in physical properties to kapha (mucus). Kapha mixed with fat passes into the urinary system, thereby interfering with normal urine excretion. Vitiated pitta, vata, and other body fluids (malas) may also be involved in this blockade. This blockade is believed to be the cause of frequent urination observed in DM. Pramehas left untreated may lead to deranged development of the bone marrow, body tissues, nutritional materials (fat, proteins, and carbohydrates), and hormones (ojas). The incurable stage of pramehas is madhumeha, which is insulin-dependent DM. Madhumeha may not be described precisely in Ayurveda, but it points in the direction of the current knowledge we have about the disease with respect to neurological damage and insulin (ojas) malfunctioning at the production (degeneration of islets of Langerhans in the pancreas) or at the utilization levels. The involvement of tissues (dushyas) leading to blood vessels, kidney, eye, and nerve damage is also described in Ayurveda as major complications. DM is described not only as a condition of madhumeha (sugar loss in urine), but also as a condition of ojameha (immunity and hormone loss) in Ayurveda for the purpose of treatment.

1.1.3 Clinical Course and Prognosis (Sadhyata)

According to classical Ayurveda, all pramehas have the potential to become incurable (madhumeha) if left untreated. The kaphaja urinary disorders (pramehas) are curable because the causative dosha and the affected tissues (dushya) have the same properties, thus requiring the same type of therapy. Although the pittaja urinary disorders are controllable (palliative), the resulting disorder may persist for life because the causative dosha is pitta, but the tissues and waste products (dushya) are different, requiring a different type of therapy. Vataja urinary disorders are considered incurable because tissues (dhatus) and hormones (ojas) undergo deterioration. Recent studies have observed a relationship between the body constitution and relative amounts of hyperglycemia and insulinemia consistent with the Ayurvedic prognosis (Bharti, 1995; Chandola, 1994; Kar, 1997).
Kapha constitution patients showed the highest level of insulinemia and the lowest levels of fasting blood sugar (FBS) and postprandial blood sugar (PPBS). Vata patients showed the lowest level of insulinemia and the highest levels of FBS and PPBS. Pitta patients were in the middle. Further studies are necessary to confirm these findings.

In Ayurveda the major complications of kaphaja urinary disorders are believed to be poor digestion, anorexia, vomiting, drowsiness, and coughing. Pittaja urinary disorder patients tend to exhibit a pricking pain in the urinary bladder, penis, and scrotum, as well as fever, burning sensations, thirst, sourness of the throat, fainting, and loose bowel movements. Vata urinary disorders (diabetes) patients often experience tremors, pain in the cardiac region, abdominal tenderness, insomnia, and dryness of the mouth. The major complications of vata DM most commonly include ulcers (eruptions) over joints, muscles, skin, blood vessels, as well as damage to the kidney and the retina.

1.1.4 Clinical Examination and Diagnosis (Rog Pariksha and Nidan)

Historically, Ayurveda diagnosis of DM was primarily based on the sweetness of urine that was identified by a swarm of flies and ants over the urine. Ayurvedic physicians currently use urine, blood sugar, and glycohemoglobin (HbA1c) levels to confirm the diagnosis. Ancient Ayurvedic texts give the following signs and symptoms of kaphaja, pittaja, and vataja pramehas for diagnosis. Recently, however, Ayurvedic doctors’ diagnostic tools evolved to more modern clinical and laboratory methods consistent with those of Western medicine:

➢ Kaphaja pramehas
   a. Udaka meha — The urine is clear; is in large amounts; is white, cold, and odorless; resembles water, sometimes with slight turbidity, and slimy.
   b. Iksu meha — The urine is like sugarcane juice and is very sweet.
   c. Sandra meha — The urine becomes thick when kept overnight.
   d. Sura meha — The urine resembles beer (sura) with a clear top and a cloudy bottom portion.
   e. Pista meha — The urine is white and thick, similar to a solution of corn flour.
   f. Sukra meha — The urine is like semen or mixed with semen.
   g. Sita meha — The urine is sweet and very cold.
h. **Sikata meha** — The urine contains sandlike particles.

i. **Sanair meha** — The urine is passed very slowly.

j. **Laala meha** — The urine is slimy and contains threads like that of saliva.

> **Pitta pramehas**

a. **Ksara meha** — The urine is like a solution of alkali in smell, color, and taste.

b. **Kala meha** — The urine is black.

c. **Nila meha** — The urine is bluish.

d. **Haridra meha** — The urine is yellowish, similar to tumeric.

e. **Manjistha meha** — The urine is foul smelling resembling *manjistha* (*Rubia cordifolia*), a slightly red solution.

f. **Rakta meha** — The urine is foul smelling, slightly salty, and blood red.

> **Vata pramehas**

a. **Majja meha** — The urine looks like marrow or marrow mixed.

b. **Ojas meha** — The urine looks like honey.

c. **Vasa meha** — The urine looks like liquid muscle fat and may be passed frequently.

d. **Hasti meha** — The urine is like that of an elephant in rut, being discharged continuously without force, mixed with lymph and without obstruction.

These urine characteristics may be found in a wide range of pathologies covering all kinds of urinary infections, obstructive uropathies, renal failures, and other health conditions. The *kaphaja iksu meha* and *vataja ojas meha* are the correlate of the modern understanding of DM. The results of diagnosis are correlated with body constitutions in order to design an individualized therapy.

### 1.1.5 Therapy (*Chikitsa*)

Traditional daily management of DM is carried out with appropriate palliative herbal therapies. These herbs are selected based on their properties, such as *rasa* (taste), *guna* (physicochemical properties), *veerya* (potency), *vipaka* (post digestive effect), and *prabhava* (unique action), that are necessary to bring about balance in *doshas*. On the basis of this approach, Charak Samhita has prescribed the following palliative treatments specific for *dosha* constitutions: 10 water
decoctions for kapha, 10 decoctions for pitta, and 1 ghee for vata in which 17 herbs are collectively cooked (Ashtang, 1998 and Ayurvediya Dravyaguna Vidnyan, 2000).

1.1.6 Combination Formulas

Individual herbs are generally not used in Ayurvedic therapies. Because therapies are based on a predominant dosha and body constitution, they always include formulas containing many herbs and sometimes various minerals. In Ayurveda, every disease has one or two predominant doshas that need to be balanced according to the constitution of the patient; therefore, one therapy may not be applicable to all patients even though the patients share the same disease. Ayurveda recommends that the patient’s lifestyle, age, type of work, psychosocial needs, and will power are considered in a management plan. The latter includes the necessary panchkarmas, herbal formulas, a healthy meal plan, and blood-sugar monitoring. A physician needs to evaluate the plan at each visit and make necessary modifications (Mary, 2001).

Following are some combination formulas used in Ayurveda:

- **Ayush-82**
  Ayush-82 is a mixture of four herbs: the seeds of *Mangifera indica*, *Syzygium cuminii*, and *Momordica charantia*, and the leaves of *Gymnema sylvestre*.

- **MA-471**
  MA-471 is a mixture of the following herbs: *Enicostemaa littorale*, *Phyllanthus niruri*, *Eugenia jambolana*, *Melia azadirachta* (indica), *Terminalia arjuna*, *Aegle marmelos*, and shilajit.

- **Abraga Chendooram**
  Abraga chendooram (AC) is a mixture of abragum (purified black mica, 80 g), vengaram (dehydrated borax, 0.5 g), and Saranaiver charu (juice of root of *Trainthema decandra* Linn.); Adathodaielai charu (juice from the leaves of *Adhatoda zeylanica* Linn.); and Alam Vizhuthu Kudineer (root of *Ficus benghalensis* Linn).

- **D-400**
  D-400 is a mixture of *Eugenia jambolana*, *Pterocarpus marsupium*, *Ficus*
glomerulata, Gymnema sylvestre, Momordica charantia, Ocimum sanctum, and shilajit.

- **Sandan Podia**
  Sandana is a mixture of six parts of *Tinospora cordifolia* sugar and one part of each of the following herbs: *Santalum album* (sandalwood) sawdust, *Andropogon citratus* (lemongrass) root, *Vitiveria zizanioides* (vetiver) root, *Syzygium aromaticum* (clove) flower bud, *Anacyclus pyrethrum* (pyrethrum) root, and purified Shilajit. The sugar is extracted from *tinospora* by suspending the crushed plant in water overnight, decanting and drying the extract in the sun, and resuspending the material in water overnight.

- **Kadal Azhinjil Choornam and Triphala Tablets**
  Kadal, a preparation containing roots and bark of *Salacia chinensis*, and triphala.

- **M-93**
  M-93 is a mixture of four herbs: *Aegle marmelos* (bilva), *Azadirachta indica* (neem, nimba), *Ocimum sanctum* (tulsai), and *Piper longum* (kalimircha).

1.1.7 Reasons for the Current worldwide Interest in Ayurveda

The great therapeutic success of synthetic antibiotics, hormones, and vaccines has created an expectation that conventional medicine will be able to discover a cure for every ailment. This expectation has been only minimally met for many diseases (e.g., cancer, arthritis, autoimmune diseases, and AIDS) even after spending hundreds of billions of dollars in research worldwide over the past 30 years. In addition, the synthetic antibiotics and steroids sometimes result in serious adverse effects, such as immunosuppression, gastrointestinal bleeding, and ulcers, after prolonged administration. Ayurvedic therapies generally provide relief without such adverse effects even after prolonged administration. Ayurvedic herbs and formulas often have a wide spectrum of therapeutic activity. Ayurvedic therapies are known to be relatively economic. Other alternative nondrug complementary therapies may be even more expensive. The relative safety of Ayurvedic medicine is another reason for its popularity. Ayurvedic formulas are time tested for safety.
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Globalization of Ayurveda has gained momentum. Many active groups have been formed in many parts of the world, including developed countries, to spread the concept and practice of Ayurveda.

This is due primarily to the following three reasons:

• The holistic approach advocated by Ayurveda in therapeutic practice,
• It has one of the most extensive and profound conceptual bases among the Traditional System of Medicines (TMSs) of the world, and
• Its survival for more than 2 millennia as a vibrant medical system.

In spite of the presence of number of marketed oral synthetic antidiabetic drugs, researchers have now diverted their attention to different herbs and medicinal plants in order to find out new active principles with better antidiabetic activity (Beigh, 2002; Ji, 2009; Thomas, 2011). Traditional antidiabetic plants might provide new oral hypoglycaemic compounds, which can counter the high cost and poor availability of the current medicines / present day drugs for many rural populations in developing countries (Noor, 2008).

Therefore, it is prudent to look for options in herbal medicine for diabetes as well.

1.2 REVIEW OF LITERATURE FOR DIABETES

1.2.1 Historical background

Diabetes mellitus (DM) is a chronic disorder characterized by impaired metabolism of glucose and other energy-yielding fuels, as well as the late development of vascular and neuropathic complications. Diabetes mellitus consists of group of disorders involving distinct pathogenic mechanisms in which hyperglycemia is the denominator. Regardless of the cause, the disease is associated with a common hormonal defect, namely, insulin deficiency, which may be total, partial, or relative when viewed in the context of coexisting insulin resistance. Lack of insulin plays a primary role in the metabolic derangements linked to diabetes, and hyperglycemia in turn plays a key role in the complications of the diabetes. It has been centuries, since this syndrome was first recognized. Credit for the initial observation that diabetes is not a single disorder rests with two Indian physicians—Charaka and Susruta (600 B.C.) who differentiated two forms of the disease, although most of the descriptions in the classic literature
probably relate to currently used term Type- I (insulin dependent) DM. Diabetes mellitus was recognized as early as 1500 B.C by Egyptian physicians, who described a disease associated with “the passage of much urine”. The term diabetes (the Greek word for siphon) was coined by Greek physician Artaeus - the Cappadocian. Artaeus noticed that patients with diabetes had a disease that caused the siphoning of the structural components of the body into the urine. Although it was known for centuries that the urine of patients with diabetes was sweet, it was not until 1674 that physician named Willis coined the term Diabetes Mellitus (from the Greek word for honey) (Setter, 2000). During the 18th and 19th centuries, a less clinically symptomatic variety of the disorder, identified by heavy glycosuria, often detected in later life and commonly associated with overweight rather than wasting, was noted, which is today recognized as Type-2 DM. When screening programs for DM commenced in the 20th century, it became apparent that there were many people who could be classified as having DM but who were in general “asymptomatic”. It has become apparent subsequently that the term diabetes mellitus covers a wide spectrum of disease, from those with acute and sometimes explosive onset to asymptomatic people whose disease is discovered by screening. In the mid 1930s, Himsworth proposed that there were at least two clinical types of DM, insulin deficiency and insulin dependent. Condition of his clinical observations came with Bornstein and Lawrence’s development of a bioassay for insulin, and when Radioimmunoassay for insulin became available a decade later Bornstein and Lawrence’s observations were confirmed (Harris, 2004).

1.2.2 Disease Profile

- **Definition**
  The term diabetes mellitus describes a metabolic disorder of multiple etiologies characterized by hyperglycemia with disturbance of carbohydrate, fat and protein metabolism resulting from defects in insulin secretion, insulin action or both. The effects of diabetes mellitus include long-term damage, dysfunction and failure of various organs. Diabetes mellitus may present with characteristic symptoms such as polyphagia, polydypsia, polyuria, blurring of vision, and weight loss. In its
severe forms, ketoacidosis or a non-ketonic hyperosmolar state may develop and lead to stupor, coma and in the absence of effective treatment to death.

Several pathogenetic stages are involved in the development of diabetes. These include the processes which destroy the beta cells of pancreas with consequent insulin deficiency, and others that result in resistance to insulin action. The abnormalities of carbohydrate, fat and protein metabolism are due to deficient action of insulin on target tissues resulting from insensitivity or lack of insulin (WHO, 1999 and Hongmei, 2005).

- **Global burden of Diabetes:**
  Despite using different methodologies, and at times showing large differences in country-specific estimates, reports have arrived at remarkably similar global figures of diabetes. It is estimated that approximately 285 million people worldwide, or 6.6%, in the age group 20-79, will have diabetes in 2010, some 70% of whom live in low-and middle-income countries. This number is expected to increase by more than 50% in the next 20 years if preventive programmes are not put in place. By 2030, some 438 million people, or 7.8% of the adult population, are projected to have diabetes (WHO, 1994). The largest increases will take place in the regions dominated by developing economies (Figure 1.1).

![Figure 1.1: Prevalence (%) estimates of diabetes (20-79 years), 2030.](image)

• **Epidemiology**
  
  Type-1 diabetes mellitus accounts for up to 10% of all cases of diabetes mellitus and results from an autoimmune destruction of the pancreatic β-cells. The prevalence of β-cell autoimmunity appears proportional to the incidence of Type-1 diabetes mellitus in various populations. For instance, countries of Sweden, Sardinia and Finland have the highest prevalence of islet cell antibody (3% - 4.5%) and are associated with the high incidence of Type-1 diabetes mellitus, 22-35 per 100,000. Type-2 diabetes mellitus is a heterogeneous disorder of glucose metabolism. Type-2 diabetes mellitus accounts for as much as 90% of all cases of diabetes mellitus and usually results from defects in insulin sensitivity and a relative defect in insulin secretion (Oki, 2002).

  The World Health Organization (WHO) has predicted that the global prevalence of Type-2 diabetes will be more than from 135 million in 1995 to 300 million in 2025 and that this increase will affect both industrialized and developing countries expecting the greatest increase in India, from 19.4 to 57.2 million. (Munuchoodappa, 2002).


  **1.2.3 Classification**

  • **Earlier Classification:**
    
    The first widely accepted classification for diabetes mellitus was published by WHO in 1980 and in modified form in 1985. Subsequent classifications of Diabetes were given by International Nomenclature of Disease (IND) in 1991 and tenth revision of the International Classification of Diseases (ICD-10) in 1992. The 1985 classification was widely accepted and is used worldwide. It represented a compromise between the clinical and etiological classification and allowed classification of individual subjects and patients in a clinically useful manner even when the specific cause or etiology was unknown. The recommended classification includes both staging of diabetes mellitus based on clinical descriptive criteria and a complimentary etiological classification.

  • **Revised Classification**
    
    The revised classification encompasses both clinical stages and etiological types of diabetes mellitus and other categories of hyperglycemia. The clinical staging
reflects that diabetes progresses through several clinical stages during its natural history. Moreover, individual subjects may move from stage to stage in either direction. Persons who have, or who are developing, diabetes mellitus can be categorized by stages according to the clinical characteristics, even in the absence of information concerning the underlying etiology. The classification by etiological type results from improved understanding of the causes of diabetes mellitus.

1.2.4 Etiological Types Of Diabetes (WHO, 1999)

- **Type-1 Diabetes mellitus**
  Type 1 indicates the process of beta – cell destruction that may ultimately lead to absolute insulin deficiency.
  - **Auto immune diabetes mellitus:**
    This form of disease encompassed by the terms insulin-dependent diabetes, type 1 diabetes or juvenile onset diabetes, results from auto immune destruction of the beta cells of the pancreas.
  - **Idiopathic:**
    There are some forms of Type 1 diabetes which have no known aetiology. Some of these patients have permanent insulinopenia and are prone to ketoacidosis, but have no evidence of autoimmunity.

- **Type-2 diabetes mellitus**
  By definition, in this type of diabetes the autoimmune destruction of pancreas does not occur and patients do not have other known specific causes of diabetes. Type 2 diabetes mellitus is a heterogeneous disorder characterized by some degree of insulin resistance with variable insulin secretion. Insulin secretion is set to be relatively deficient because many patients may have normal to elevated levels of insulin. However their blood sugars remain elevated because of tissue resistance to the action of the insulin that is not usually life threatening (Setter, 2000).
  Diabetes is becoming an epidemic disease in Asian countries like India (King, 1998 and Ramachandran, 2001). The healthy BMI for an urban Indian is <23 kg/m², and cutoff values for waist circumference as per reports are 85 cm for
men and 80 cm for women, and for waist-to-hip ratio they are 0.89 for men and 0.81 for women.

- **Other specific types**
  - **Genetic defects in β-cell function:**
    Genetic factors account for about one-third of the susceptibility to Type-2 diabetes. Over 20 different regions of the human genome show some linkage with Type-1 diabetes, but more interest has been focused on the Human Leucocyte Antigen (HLA) region within the major histocompatibility complex on the short arm of chromosome 6.
  - **Other genetic defects in insulin action:**
    There are some unusual causes of diabetes which result from genetically determined abnormalities of insulin action. The metabolic abnormalities associated with mutations of the insulin receptor may range from hyperinsulinaemia and modest hyperglycemia to symptomatic diabetes.
  - **Disease of the exocrine pancreas:**
    Any process that diffusely injures the pancreas may cause diabetes. Acquired processes include pancreatitis, trauma, infection, pancreatic carcinoma and pancreatectomy. With the exception for cancer, damage to pancreas must be extensive for diabetes to occur.
  - **Endocrinopathies:**
    Several hormones (e.g. growth hormone, cortisol, glucagon, epinephrine) antagonize insulin action. Diseases associated with excess secretion of these hormones can cause diabetes (e.g. Acromegaly, Cushing’s syndrome, Glucagonoma and Phaeochromocytoma). These forms of hyperglycemia typically resolve when the hormone excess is removed.
  - **Drug - or chemical - induced diabetes:**
    Many drugs can impair insulin secretion. These drugs may not, by themselves, cause diabetes but they may precipitate diabetes in persons with insulin resistance. In such cases, classification is ambiguous, as the primacy of beta cell dysfunction or insulin resistance is unknown. Certain toxins such as Vacor (a rat poison) and pentamidine can permanently destroy pancreatic beta cells. Many drugs and hormones (nicotinic acid and glucocorticoids) can also impair insulin action.
• **Infections:**
  Certain viruses have been associated with beta-cell destruction. Diabetes occurs in some patients with congenital rubella. In addition, Coxsackie B, cytomegalo virus and some other viruses (adenovirus and mumps) have been implicated in inducing diabetes.

• **Uncommon but specific forms of immune mediated diabetes mellitus:**
  Diabetes may be associated with several immunological diseases with a pathogenesis or aetiology different from that which leads to type 1 diabetes process. Postprandial hyperglycemia of a severity sufficient to fulfill the criteria for diabetes has been reported in rare individuals who spontaneously develop insulin auto antibodies. However these individuals generally present with symptoms of hypoglycemia rather than hyperglycemia.

• **Other genetic syndromes sometimes associated with diabetes:**
  Many genetic syndromes are associated with an increased incidence of diabetes mellitus. These include the chromosomal abnormalities of Down’s syndrome, Klinefelter’s syndrome and Turner’s syndrome. Additional manifestations include diabetes insipidus, hypogonadism, optic atrophy and neural deafness.

• **Personality Traits:**
  Several researches have reported that the following are the typical characteristics of Type A Personality: urgency, impatience, aggressiveness which show up as impatience, rudeness, being easily upset over small things and excessively strong achievement-orientation. They also seem to show characteristics as facial tension, tongue clicking, teeth grinding, dark circles under eyes, facial sweating. Patients with coronary heart disease are likely to have negative effects such as hypertension, job stress; social isolation (Mudgil, 1992;) and these behaviors are also found to be common among Diabetics as well. Research reports revealed that Type-A behaviour measure showed significant relationship to occupational stress and work motivation in relation to age, job level and overall well-being among nursing professionals (Virk, 2001).

1.2.5 **Diagnosis**
  Diabetes mellitus is diagnosed on the basis of WHO recommendations from 1999, incorporating both fasting and 2-h after glucose load (75 g) criteria into a practicable diagnostic classification that should be used (Table 1.1).
Table 1.1: Diagnostic criteria Of Diabetes Mellitus and other categories of hyperglycemia

<table>
<thead>
<tr>
<th>Glucose concentration in venous plasma (mmol/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diabetes mellitus</td>
</tr>
<tr>
<td>Impaired glucose tolerance</td>
</tr>
<tr>
<td>Impaired fasting glucose</td>
</tr>
<tr>
<td>Glucose load = 75 g glucose load orally</td>
</tr>
</tbody>
</table>

1.2.6 Pathogenesis of Type 2 Diabetes Mellitus

With the advent of 1990s it was known that type 2 diabetes was characterized by the classic triad of $\beta$-cell dysfunction, excess glucose production from the liver, and insulin resistance defined as impaired insulin-mediated glucose clearance into skeletal muscle (DeFronzo, 2004). However, knowledge at that time provided no physiological connection between these organs. Another conundrum was how excess adiposity, i.e., being fat, caused insulin resistance, which again is a defect in skeletal muscle physiology. Considerable progress has occurred over the last decade in the understanding of type 2 diabetes although all of the answers are not in yet.

Figure 1.2: Sequence of the key pathological features of type 2 diabetes
Adapted from: Jack, L., Pathogenesis of Type 2 Diabetes Mellitus. *Archives of Medical Research.* 36, 197–209, 2005.
• **Genetic Predisposition**
  The genetic basis for many monogenic forms of diabetes has been discovered, such as mitochondrial genome defects and the association with diabetes and deafness, Wolfram’s syndrome, several rare syndromes of extreme insulin resistance and obesity, and many of the MODY syndromes (maturity onset diabetes of youth). Still, these account for only a small proportion of diabetes. Many chromosomal hot spots have been identified in various populations and are under intense study to determine the genes involved, which is now easier because of the genetic map from the human genome project. Also, many research groups are interested in various gene polymorphisms, common variations in the sequence of genes, sometimes in noncoding regions that may affect transcriptional regulation, and may be linked to physiological differences (Figure 1.2).

• **Environment**
  An important concept is that the diabetes genotype typically causes only a predisposition for glucose intolerance. Whether one develops the diabetes phenotype depends on environmental factors to a considerable extent. The predisposing environmental factors share an ability to negatively impact the glucose homeostasis system through worsening of insulin resistance or to impair β-cell function. Superimposing these factors onto a genetically compromised glucose homeostasis system raises the risk of progressing to hyperglycemia. It is the rapid emergence of these disadvantageous environmental factors that is causing the worldwide diabetes epidemic (Leahy, 2005).

• **Acquired Organ Dysfunction**
  Both beta-cell dysfunction and insulin resistance occur very early in the course of type 2 diabetes long before blood glucose values reach a level that is defined as pre-diabetes. The primary events are believed to be an initial deficit in insulin secretion and, in many patients, relative insulin deficiency in association with peripheral insulin resistance.
• **The β-cell Dysfunction**

β -cell dysfunction is initially characterized by impairment in the first phase of insulin secretion during glucose stimulation and may antedate the onset of glucose intolerance in type 2 diabetes. Later in the course of the disease, during the second phase release of newly synthesized insulin is impaired, an effect that can be reversed, in part by restoring strict control of glycemia. This secondary phenomenon, termed desensitization or β - cell glucotoxicity, is the result of a paradoxical inhibitory effect of glucose upon insulin release and may be attributable to the accumulation of glycogen within the β -cell as a result of sustained hyperglycemia. Other candidates that have been proposed are sorbitol accumulation in the β -cell or the non-enzymatic glycation of β -cell proteins.

Other defects in β -cell function in type II diabetes mellitus include defective glucose potentiation in response to non-glucose insulin secretagogues, asynchronous insulin release, and a decreased conversion of proinsulin to insulin. Autoimmune destruction of pancreatic β -cells may be a factor in a small subset of type 2 diabetic patients and has been termed the syndrome of latent autoimmune diabetes in adults.

• **Insulin Resistance**

The presence of hyperinsulinism in type 2 diabetes, due to insulin resistance has been considered to play an integral role in the pathogenesis of the disease (Figure 1.3). As chronic hyperinsulinemia inhibits both insulin secretion and action, and hyperglycemia can impair both the insulin secretory response to glucose as well as cellular insulin sensitivity, the precise relation between glucose and insulin level as a surrogate measure of insulin resistance has been questioned.

• **The Liver**

Hepatic insulin resistance is characterized by a marked decrease in glucokinase activity and a catalytic increased conversion of substrates to glucose despite the presence of insulin. Thus, the liver in type 2 diabetes is programmed to both over produce and under-use glucose. The elevated free fatty acid levels found in type 2 diabetes may also play a role in increased hepatic glucose production.
1.2.7 Diabetes Complications

Chronic hyperglycemia is associated with long-term damage and dysfunction of small and large blood vessels resulting in failure of various organs. Common complications resulting from uncontrolled diabetes include heart disease, stroke, blindness, periodontal disease, nervous system damage, and kidney dysfunction. At the time of diagnosis, most patients with type 2 diabetes will have some symptoms of elevated glucose (ie, polyuria, polydipsia, polyphagia), microvascular symptoms (ie, blurred vision, numbness or tingling in hands or feet), and macrovascular complications (ie, cardiovascular disease) (Edelman, 2001). These patients have a high mortality rate because of macrovascular complications. In comparison, an increased incidence of microvascular complications is usually not observed until 10 years after the initial diagnosis in type 1 diabetes.
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➤ Pathogenesis

Hyperglycemia is considered a major factor in the development of diabetic complications and the adverse effects are recognizable through multiple pathways. The aldose reductase (polyol) pathway, advanced glycation end-product pathway, hexosamine pathway, and protein kinase C pathway provide evidence that elevated blood glucose promotes cellular dysfunction and damage. The polyol pathway converts excess intracellular glucose into sugar alcohols via activity of the enzyme aldose reductase. This enzyme catalyzes the conversion of glucose to sorbitol, and in turn, sorbitol triggers a variety of different intracellular changes in the tissues involved (Setter, 2003). Advanced glycation end products (AGEs) form at a constant rate in the normal body; however, in diabetes, this process is drastically increased.

Three main consequences have been found in association with AGEs inside cells:

a) Functional alterations of intracellular proteins,
b) Altered interaction with AGE receptors, and
c) Altered interactions with matrix and other cells.

The hexosamine pathway becomes activated when glucose levels are high in cells. It processes an upstream glycolytic intermediate, causing a permanent modification of proteins and transcription factors by the product of the pathway, N-acetyl-glucosamine. A high level of intracellular glucose activates the enzyme protein kinase C (PKC). When activated, this PKC enzyme alters cell function (Hammes, 2004).

➤ Clinical Manifestations: Microvascular

Over 200,000 people die each year because of diabetes related complications. Underlying diabetic complications such as nephropathy, neuropathy, retinopathy, cardiovascular disease, and peripheral vascular disease can be present for many years before an actual diagnosis is made (Spijkerman, 2003).

a) Nephropathy: Diabetic nephropathy is a clinical syndrome characterized by excessive urinary albumin excretion, hypertension, and renal insufficiency. In the United States, diabetic nephropathy accounts for about 40% of new cases of end-
stage renal disease (ESRD). Nephropathy is a frequent complication of type 1 and type 2 diabetes mellitus. Patients who have type 2 diabetes are commonly found to have albuminurias and overt nephropathy soon after or at the time of diabetes diagnosis. The natural history of diabetic nephropathy has 5 stages, which includes hyper filtration with normal renal function; histological changes without clinically evident disease; incipient diabetic nephropathy or microalbuminurias; overt diabetic nephropathy (macroalbuminuria, reduced renal function); and renal failure requiring dialysis.

b) **Neuropathy:** Diabetic peripheral neuropathy (DPN) is one of the most prevalent and complicated conditions to manage among diabetic patients. About 60% to 70% of people with diabetes have mild to severe forms of nervous system damage; resulting in impaired sensation or pain in the feet or hands, slowed digestion of food in the stomach, carpal tunnel syndrome, precursor for foot ulcers, and other nerve problems. Diabetes is the major contributing reason for non-traumatic lower extremity amputations (more than 60% of cases). The most common form of DPN involves the somatic nervous system; the autonomic nervous system may be affected in some patients (Boulton, 2005).

c) **Retinopathy:** Diabetic retinopathy is the most frequent cause of new cases of blindness among adults aged 20-74 years. Diabetic retinopathy can progress from mild non proliferative abnormalities, to moderate and severe non proliferative diabetic retinopathy, and finally, to proliferative diabetic retinopathy (Fong, 2004). Nonproliferative retinopathy produces blood vessel changes within the retina: bleeding (hemorrhages), weakened blood vessel walls (microaneurysms), leakage of fluid (edema or exudate), and loss of circulation.

➤ **Clinical Manifestations: Macrovascular**

Diabetes exerts a heavy toll on the vascular system. The hallmark of diabetic macrovascular disease is accelerated by atherosclerosis involving the aorta and large and medium-sized arteries. Macrovascular disease causes accelerated atherosclerosis among diabetics, resulting in increased risk of myocardial infarction, stroke, and lower-extremity gangrene (Maitra, 2005). Macrovascular
complications associated with diabetes include cardiovascular, cerebrovascular, and peripheral arterial diseases.

a) **Cardiovascular:** People with diabetes are 2 to 4 times more likely to develop cardiovascular disease (CVD) than those without diabetes. There are several risk factors that may contribute to the development of coronary heart disease (CHD), including lifestyle (eg, cigarette smoking and diet), hyperglycemia, hypertension, and high cholesterol. Additional mechanisms that contribute to the increased risk of CHD and worse outcomes in persons with diabetes include endothelial dysfunction, hyper coagulability, impaired fibrinolysis, platelet hyper aggregability, oxidative stress, sympathovagal imbalance, and glucose toxicity.

b) **Cerebrovascular:** Cerebrovascular disease is a term encompassing many disorders that affect the blood vessels of the central nervous system. These disorders result from either inadequate blood flow to the brain (ie, cerebral ischemia) or from hemorrhages into the parenchyma or subarachnoid space of the central nervous system (CNS). The risk factors that may predispose a patient to a stroke include smoking, obesity, hypertension, dyslipidemia, and transient ischemic attacks.

c) **Peripheral Arterial Disease:** Peripheral arterial disease (PAD) is an atherosclerotic occlusive disease. It is the major risk factor for lower extremity amputations. The abnormal metabolic state accompanying diabetes results in changes in the state of arterial structure and function predisposing people to PAD (Anversa, 2005). The risk of development of PAD increases threefold to fourfold in patients with diabetes mellitus (Murabito, 1997). Risk factors for the development of PAD include diabetes, hypertension, hyperlipidemia, cigarette smoking, and age. In people with diabetes, the risk of PAD is increased by age, duration of diabetes, and presence of peripheral neuropathy. Elevated levels of C-reactive protein (CRP), fibrinogen, homocysteine, apolipoprotein B, lipoprotein (a), and plasma viscosity are potential risk factors for PAD.
1.2.8 Treatment Targets for type 2 Diabetes Mellitus

The cellular lesions responsible for insulin resistance and β-cell dysfunction in type 2 Diabetes Mellitus are still imprecisely defined. Most presentations of the disease probably involve multiple signaling defects within insulin target tissues and multiple defects of stimulus secretion coupling in the β-cells. Thus, it is not possible to isolate a single drug target to reverse all aspects of the disease. Although there is ‘evidence-based’ justification for reducing hyperglycemia by any safe means, an ideal treatment will achieve this by correcting one of the underlying endocrine or metabolic disturbances (Figure 1.4) (UKPDS Group, 1995 a; UKPDS Group, 1995 b; UKPDS Group, 1998 c). Additional benefits against associated disorders of the metabolic syndrome are also sought because reductions in obesity, hypertension, hyperlipidaemia and hyperinsulinemia have been shown to assist glycaemic control and/or reduce vascular complications in T2DM. The pathogenic role of insulin resistance in T2DM has focused attention on new agents to improve sensitivity to insulin or partially mimic the action of insulin. The benzamido derivative repaglinide has recently been introduced as a rapidly absorbed and rapidly eliminated insulin releaser. Another approach to synchronize insulin secretion with meal consumption involves the intestinal hormone glucagonlike peptide-1 (7–36 amide) (GLP1). Other potential mechanisms to increase insulin secretion include inhibitors of phosphodiesterases, antagonists of α2-adrenoceptors, and metabolic stimulants such as succinate esters, which also stimulate insulin biosynthesis. Because reduced adiposity improves glycaemic control in obese T2DM patients, there is current debate over the use of anti-obesity agents in these patients. A recently introduced intestinal lipase inhibitor orlistat can decrease fat digestion and absorption by up to 30%. T2DM, especially in the elderly, is often associated with deficiencies in some of the minerals required for glucose metabolism. Agents that act directly to stimulate glucose metabolism could, in theory, overcome the under-utilization of glucose in muscle. However, none of the agents known to act in this manner has proved suitable for clinical development. Insulin remains the only effective alternative when oral antidiabetic drugs can no longer achieve adequate glycaemic control in T2DM (Standl, 1999; and Wild, 1999).
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Figure 1.4: Schematic representation of potential new drug target areas (right panel) to address the defects of insulin secretion by pancreatic B-cells and defects of insulin action (insulin resistance)(left panel) in liver, muscle and fat in type 2 diabetes mellitus.

**Abbreviations:** ↑, increase; ↓, decrease; GLUT4, insulin-stimulated glucose transporter isoform 4; PEPCK, phosphoenol pyruvate carboxykinase; PI 3-kinase, phosphatidylinositol 3-kinase; PKB, protein kinase B.

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**Therapy For Type 2 Diabetes Mellitus**

**Diet**

Diet therapy, although important for the prevention as well as the treatment of all stages of type 2 diabetes, continues to remain poorly understood and highly controversial (Franz, 1994; Henry, 1998). When obesity coexists with hyperglycemia, as seen in the majority of individuals with type 2 diabetes, weight reduction is the major goal of dietary therapy. Traditional recommendations emphasize reduction of both the total and saturated fat content and replacement with complex carbohydrates to 50–55% of the dietary calories. In type 2 diabetic patients, such diets may cause marked postprandial hyperglycemia. As there is considerable patient variability in the rate of glucose absorption, arduous attention to postprandial glucose monitoring and the addition of high fiber contents to the diet become critically important.

**Exercise**

Exercise has been shown to be beneficial in the prevention of the onset of type 2 diabetes mellitus as well as in the improvement of glucose control as a result of enhanced insulin sensitivity (Helmrich, 1994; Schneider, 1998). Decreased intraabdominal fat, an increase in insulin-sensitive glucose transporters (GLUT-4) in muscle, enhanced blood flow to insulin-sensitive tissues, and reduced free fatty acid levels appear to be the mechanisms by which exercise restores insulin sensitivity (Erisonn, 1997). In addition, exercise provides the added benefits of lowering blood pressure, improving myocardial performance, and lowering serum triglycerides while raising high density lipoprotein cholesterol levels.

**Pharmacotherapy for type 2 diabetes mellitus**

Current therapeutic agents available for type 2 diabetes mellitus include sulfonylureas and related compounds, biguanides, thiazolidinediones, α-glucosidase inhibitors and insulin (Table 1.2). A rational approach would be to begin with the agents particularly suited to the stage and nature of the disease, progressing, if necessary, to combination therapy. Pharmacological agents acting through different mechanisms of action should be chosen to improve glucose values while minimizing adverse effects.
Table 1.2: Oral agents used in the management of type 2 diabetes mellitus

<table>
<thead>
<tr>
<th>Class</th>
<th>Mechanism of action</th>
<th>Indication(s)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sulfonylureas and repaglinide</td>
<td>Increase insulin secretion</td>
<td>Insulinopenia</td>
</tr>
<tr>
<td>Biguanides</td>
<td>Decrease hepatic gluoneogenesis</td>
<td>Obesity + insulin resistance</td>
</tr>
<tr>
<td></td>
<td>Decrease peripheral insulin resistance</td>
<td></td>
</tr>
<tr>
<td>Thiazolidinediones</td>
<td>Decrease peripheral insulin resistance</td>
<td>Insulin resistance</td>
</tr>
<tr>
<td></td>
<td>Reduce fatty acids</td>
<td></td>
</tr>
<tr>
<td>a-glucosidase inhibitors</td>
<td>Slow absorption of carbohydrates</td>
<td>Postprandial hyperglycemia</td>
</tr>
</tbody>
</table>

**Sulfonylureas and Related Agents:**

Sulfonylureas have been used to treat type 2 diabetes since 1942 and require functional pancreatic β-cells for their hypoglycemic effect (Loubatieres, 1957; Groop, 1991). All currently available sulfonylureas bind to specific receptors on β-cells, resulting in closure of potassium ATP channels. As a result, calcium channels open, leading to an increase in cytoplasmic calcium that stimulates insulin release (Pilipson, 1995). To a lesser degree than insulin administration, sulfonylureas, through endogenous hyperinsulinemia, cause a propensity for hypoglycemia and weight gain (Harrower, 1994). Still controversial is the influence of sulfonylureas on cardiovascular mortality, an observation first described by the University Group Diabetes Program (Meinert, 1970). However, newer data has shown that sulfonylureas, with the exception of glimiperide, block the vasodilator response to ischemia in animals, thereby potentially increasing cardiovascular risk. At present, the question regarding sulfonylurea use in cardiac mortality in humans remains unanswered (Smits, 1995; Leibowitz, 1996).

**Biguanides:**

After withdrawal of the biguanide, phenformin, from the U.S. market in 1975, a second generation biguanide, metformin, was introduced and widely distributed throughout Western Europe, Canada, and Mexico. With a frequency of lactic acidosis 1/10th that of the parent compound and a strong record of safety and efficacy, the drug was carefully introduced into the American market in 1995.
Glucose lowering by the drug occurs primarily by decreasing hepatic glucose production and, to lesser extent, by decreasing peripheral insulin resistance. The drug acts by causing the translocation of glucose transporters from the microsomal fraction to the plasma membrane of hepatic and muscle cells. It does not stimulate insulin release and does not, when given alone, cause hypoglycemia (Mahler, 1995). Moreover, it does not cause weight gain, and it improves the lipid profile by causing a decline in total and very low density lipoprotein triglyceride, total cholesterol, and very low density cholesterol levels and an increase in high density lipoprotein cholesterol levels (Davidson, 1995; Wu 1990). It is ideally suited for obese patients with type 2 diabetes who are unresponsive to diet, one and are presumed to be insulin resistant. The major risk continues to be that of lactic acidosis, which occurs with a frequency of 1/20,000 patient/yr. As the major route of excretion of the drug is through the kidneys, it should not be given to those with renal disease.

**Thiazolidenediones:**

This new class of antidiabetic agents has been under investigation since 1983 (Fujita, 1983). Thiazolidenediones appears to act by binding to the peroxisome proliferator activator receptor-g (Lehman, 1995). This nuclear receptor influences the differentiation of fibroblasts into adipocytes and lowers free fatty acid levels (Spiegelman, 1998). Clinically, its major effect is to decrease peripheral insulin resistance, although at higher doses it may also decrease hepatic glucose production (Nolan, 1994). Measurement of transaminases and bilirubin monthly for the first 8 months of therapy and every 2 months thereafter for the first year of therapy is essential as early detection can help reversal of hepatotoxicity which is a side effect of this class of drugs (Watkins, 1983).

**α-Glucosidase inhibitors:**

Members of this class act by slowing the absorption of carbohydrates from the intestines and thereby minimize the postprandial rise in blood glucose (Coniff, 1995). Gastrointestinal side-effects require gradual dosage increments over weeks to months after therapy is initiated. Serious adverse reactions are rare, and weight gain may be minimized with this therapy. Acarbose, the agent of this class in clinical use, may be added to most other available therapies (Lebovitz, 1997).
Insulin:
Insulin therapy is indicated in the treatment of type 2 diabetes for initial therapy of severe hyperglycemia, after failure of oral agents, or during perioperative or other acute hyperglycemic states. Insulin has been used in multiple combinations in type 2 diabetes, and new insulin analogs are in clinical trials (Burge, 1997). The first available insulin analog is lispro insulin, representing a two-amino acid modification of regular human insulin. Insulin therapy can cause further weight gain in obese type 2 diabetics and increase the risk of hypoglycemia (Dagogo, 1997). In addition, the peripheral hyperinsulinemia achieved by exogenous insulin therapy may be a risk factor for cardiovascular disease (Wingard, 1995).

Combination therapies
Most available agents have been used in combination to treat type 2 diabetes. Although many combinations are not yet approved for use, a rational choice for combination therapy would include an agent that increases insulin levels and one that enhances sensitivity to insulin and lowers glucose production. This combination of agents would appear to correct most of the pathophysiological defects found in type diabetic individuals.

Investigational therapies based on incretin action:
It is well established that an oral glucose load evokes a greater insulin response than glucose given by the intravenous route. One of the gut polypeptides responsible for this observation is glucagon-like peptide (GLP-1). Given parenterally or through the buccal mucosa, GLP-1 lowers glucose levels, decreases glucagon levels, and delays gastric emptying. GLP-1 mimetics bind to GLP-1 receptors on pancreatic beta cells, but have a longer half-life because dipeptidyl peptidase-4 (DPP-4) enzymes cannot degrade the homologue or analogue peptides as rapidly as natural GLP-1. The first developed GLP-1-agonist is exendin-4 (exenatide). It is administered subcutaneously twice daily, with slow-release forms of exenatide with a once weekly administration being developed. Lowering of HbA1c levels by 0.5-1% may be expected, mainly by lowering postprandial blood glucose levels. The higher the baseline level, the greater the magnitude of HbA1c reduction. Hypoglycemia occurs rarely and only in patients receiving Sulphonyl urea in combination with exenatide. Another important
advantage is the progressive weight loss (up to 5 kg over 6 months), some of which may be a result of gastrointestinal side effects. These gastrointestinal adverse effects are dose-dependent, with 30-45% of patients experiencing nausea, vomiting or less frequently diarrhoea (Amori, 2007). Claims on prevention of functional beta cell decline are based only on in vitro and animal data. A major issue is cost and the lack of data on long-term effectiveness and safety.

A different path aimed at exploiting the incretin system has been the development of agents that inhibit the action of the DPP-4 enzyme, with two pioneers, sitagliptine and vildagliptine. By inhibiting DDP-4, these products expand the life of natural incretins. These products are taken orally and very few side effects (mostly an increase in upper respiratory infections) have been reported until now. Neither weight gain, nor gastrointestinal side effects, nor hypoglycaemia were observed. The glucose lowering potential is comparable to other oral agents (0.5-1.5% depending on starting value) (Drucker, 2007). Long term studies on durability of glucose lowering effects or diabetes complications are lacking. The major hurdle in using these drugs, next to absence of long term data, is their cost.

*Insulin in type 2 diabetes :*

Due to the progressive nature of the disease, most patients with type 2 diabetes will eventually require insulin to achieve and maintain glycaemic control. Current American Diabetes Association (ADA) – European Association for the Study of Diabetes (EASD) guidelines (Nathan, 2008) suggest adding one bedtime dose of long-acting insulin to oral agents (OAD) when HbA1c is not on target. Basal insulin added to existing OAD is an easy way to initiate insulin therapy in type 2 diabetic patients and achieves HbA1c below 7% in many patients. An important hurdle to adding basal insulin to OAD is the occurrence of weight gain and even more importantly, (nocturnal) hypoglycaemia. The advent of insulin glargine, and more recently detemir, has revolutionized the concept of basal insulin therapy. In patients with a normal fasting glycaemia, but with mainly a problem of postprandial hyperglycaemia, the use of prandial insulins or premixes is more appropriate. A common feature exists for all insulins: the need for titration and intensification. However, the hassle of injecting insulin, the hypoglycaemia risk, the weight gain and the fear of injecting in many type 2 patients have lead to
insulin being initiated too late and not being titrated or intensified properly. Using insulin analogues will allow intensification to occur with fewer side effects (hypoglycaemia, weight gain) and, especially, more comfort. At present, however, data on the effects of analogue insulins on long term diabetes complications are lacking. The drop in HbA1c that can be achieved by insulin regimens is only limited by the occurrence of hypoglycaemia. Installing and intensifying insulin therapy is intricately linked to intensive diabetes education and self-monitoring of blood glucose levels by the patients.

➢ **Issues in development of new chemical entities for treatment of diabetes:**

By many accounts, the pharmaceutical industry is experiencing a severe decline in research productivity. More and more capital is being invested in research and development, but the rate at which new drugs are introduced to the market is failing to keep pace. According to the FDA, new compounds entering Phase I development today have only an 8% chance of reaching the market versus a 14% chance 15 years ago. And the Phase III failure rate has risen to 50% versus 20% just ten years ago (Tempio, 2008). This issue is particularly apparent in the field of diabetes.

Moreover, in 2008, FDA introduced a new guideline for clinical development of anti-diabetes agents. This new guideline stipulates a minimum number of test subjects to be enrolled and a minimum duration of the evaluation period on pivotal clinical trials.

The reasons for the high safety demands of novel anti-diabetes treatments involve

a) A rapidly increasing size of the target population,

b) A decrease in the average age at diagnosis and

c) The life-long requirement for therapy of subjects once obtaining the diagnosis.


 CHAPTER 1

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➢ *Therapeutic algorithms*

Any approach to treatment of type 2 diabetes must combine education, diet, exercise, and management of multiple risk factors. Control of hypertension and dyslipidemia is essential. Blood pressure of less than 130/85 mm/Hg and a low density lipoprotein cholesterol level below 130 mg/dL (low density lipoprotein cholesterol, 100 mg/dL if coronary artery disease is present) are a suggested standard of care (American Diabetes Association, 1998). The degree of glycemic control recommended will vary depending upon age, education, and complicating risk factors. In otherwise healthy individuals, near normalization of the glycosylated hemoglobin level is recommended (Gaster, 1998), and in all cases a HbA1c level above 8.0% demands therapeutic intervention. In those patients in whom insulinopenia is the likely cause of hyperglycemia manifested by lean body weight, younger age, and enhanced insulin sensitivity, a sulfonylurea or other β-cell secretagogue would be favored, whereas those patients who are likely to be insulin resistant with coexistent features of hypertension, hyperlipidemia, and obesity would more likely respond to an insulin-sensitizing agent, either metformin or troglitazone. If HbA1c values continue to exceed 8%, a second agent may be added, either a secretagogue or another insulin-sensitizing agent depending upon patient characteristics, and if postprandial hyperglycemia persists, an α-glucosidase inhibitor may be added. Ultimately, insulin therapy may become necessary either early in the course of the disease to establish control or later in the disease course as β-cell failure ensues (Roman, 1997). The addition of bedtime insulin to sulfonylureas may offer some interim protection, and preliminary studies with insulin and the insulin-sensitizing drugs have shown promising results in delaying β-cell failure (Schwartz, 1998). Whether such combinations will provide long term benefit remains to be determined. In just a few years in the United States, pharmacotherapy for hyperglycemia has greatly expanded, allowing many patients whose diabetes was formerly treated by insulin alone to be controlled with oral agents. Therapies will continue to evolve as insights into molecular mechanisms further expand our therapeutic horizon. Diabetologists and endocrinologists will play an essential part in the goal of diagnosing all type 2 diabetic individuals at an earlier stage and treating them as an attempt to minimize the burden of diabetes associated complications worldwide.
Perspectives for Globalized Natural Medicines serving leads for potential hypoglycemic agents

The research and development drive in the pharmaceutical sector is focused on development of new drugs, through innovative/indigenous processes for known drugs and development of plant-based drugs through investigation of leads from the traditional systems of medicine. In addition, many nutraceuticals are being consumed in unregulated markets for supposed benefits in health care and improvement of quality of life. The US Congress has fuelled the rapid growth of nutraceuticals with the passage of the Dietary Supplement Health and Education Act (DSHEA) in 1994. Globally, there have been efforts to examine quality and regulate the growing trade of herbal drugs and traditional medicine. Natural medicines provide valuable resources to meet the requirements for global health care at affordable prices. Therefore, safety and efficacy need to be proven in a comparable manner to conventional drugs. Evidence-based natural and western medicine may merge to a “one-world medicine” for the sake of all patients in industrialized and developing countries.

However, natural medicines seem to be barely able to provide convincing alternatives to conventional western medicine for global health-care. Some reasons are as follows:

a) The knowledge of shamans and traditional healers is getting lost, since their oral traditions being handed down from generation to generation for thousands of years seem to be extinguished in modern times.

b) The traditional use of medicinal plants needs to be systematically investigated and standardized.

c) Overharvesting of medicinal plants from the wild presents a severe problem of preserving many plant species endangered to be extinguished.

d) Global climate change may affect both the growths of medicinal plants as well as their constituents (Cavaliere, 2009)

Several medicinal plants have found potential use as hypoglycemic in the Indian system of medicines, including Ayurveda (Table 1.3). The use of herbs as hypoglycemics is a major avenue in Indian perspectives particularly for treating
diabetes, which require to be explored more effectively as there are so many literatures available on these aspects (Vaidya, 2008).

Various plant species from Indian biosphere, having potent hypoglycemic activity are described in the following section.

Table 1.3: Selected Indian medicinal plants with blood glucose lowering activity

<table>
<thead>
<tr>
<th>Name of the plant</th>
<th>Reported mechanism of action</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Acacia arabica</em> (Lam.) Muhl.</td>
<td>Acts through release of insulin from pancreatic beta cells, which accounts for the hypoglycemic activity (Singh, 1975; Wadood, 1989)</td>
</tr>
<tr>
<td>Common name: Babul [Family: Fabaceae]</td>
<td></td>
</tr>
<tr>
<td><em>Aegle marmelos</em> (L.) Correa</td>
<td>Increases utilization of glucose; either by direct stimulation of glucose uptake or via the mediation of enhanced insulin secretion (Sachdewa, 2001a) and also decreases the elevated glucose and glycosylated hemoglobin levels (Kamalakkanan, 2003)</td>
</tr>
<tr>
<td>Common name: Wood apple [Family: Rutaceae]</td>
<td></td>
</tr>
<tr>
<td><em>Allium cepa</em> L. Common name: onion [Family: Liliaceae]</td>
<td>Lowers blood glucose level and has potent antioxidant activity, which may account for the hypoglycemic potential (Augusti, 1973)</td>
</tr>
<tr>
<td><em>Allium sativum</em> L. Common name: garlic [Family: Alliaceae]</td>
<td>Has strong antioxidant activity and rapid reactivity with thiol containing proteins responsible for the hypoglycemic property (Rabinkov, 1998)</td>
</tr>
<tr>
<td><em>Artemisia pallens</em> Wall. ex DC. Common Name: Davana [Family: Compositae]</td>
<td>Inhibits glucose re-absorption or increase in peripheral glucose utilization (Subramaniam, 1996)</td>
</tr>
<tr>
<td><strong>Andrographis paniculata</strong> Nees</td>
<td>Prevents glucose absorption from gut (Borhanuddin, 1994; Yu, 2003). Has hypotriglyceridemic effect and antioxidant activity, which may be responsible for beneficial effect in the diabetic state (Zhang, 2000 a,b)</td>
</tr>
<tr>
<td><strong>Azadirachta indica</strong> A.Juss.</td>
<td>Inhibits action of epinephrine on glucose metabolism, resulting in increased utilization of peripheral glucose (Chattopadhyay, 1987; Chattopadhyay, 1996) and exhibits hypoglycaemic activity without altering the serum cortisol concentration (Chattopadhyay, 1999; Gholap and Kar, 2004)</td>
</tr>
<tr>
<td><strong>Biophytum sensitivum</strong> (L.) DC.</td>
<td>Stimulates pancreatic beta cells to release insulin (Puri, 1998)</td>
</tr>
<tr>
<td><strong>Beta vulgaris</strong> L.</td>
<td>Lowers blood glucose level (Yoshikawa, 1996)</td>
</tr>
<tr>
<td><strong>Brassica juncea</strong> (L.) Czern.</td>
<td>Increases the concentration of hepatic glycogen and glycogenesis and suppressed the activity of glycogen phosphorylase and gluconeogenic enzymes, lead to reduction in glycogenolysis and gluconeogenesis (Khan, 1995)</td>
</tr>
<tr>
<td><strong>Boerhavia diffusa</strong> L.</td>
<td>Increases plasma insulin levels and improves glucose tolerance, produces significant antioxidant activity (Pari, 2004; Satheesh, 2004)</td>
</tr>
<tr>
<td><strong>Cassia auriculata</strong> L.</td>
<td>Suppresses enhanced gluconeogenesis during diabetes and enhance utilization of glucose through increased glycolysis (Pari, 2002; Latha, 2003) in addition to pronounced alpha-glucosidase inhibitory actions resulting in a significant and potent lowering of blood glycemic response (Latha 2003; Abesundara, 2004)</td>
</tr>
<tr>
<td><strong>Caesalpinia bonduc</strong> (L.) Roxb.</td>
<td>Increases the release of insulin from pancreatic cells (Sharma, 1997)</td>
</tr>
</tbody>
</table>
### Chinese Cinnamon
[Family: Caesalpiniiaceae]

**Cajanus cajan** (L.) Millsp.
Common name: Pigeon pea [Family: Fabaceae]

Lowers plasma glucose level (Amalraj, 1998a)

### Citrullus colocynthis (L.) Schrad. Common name: Bitter apple [Family: Cucurbitaceae]

Exerts an insulinotropic effect (Abdel-Hassan, 2000)

### Coccinia indica Wight & Arn.
Common name: Ivy gourd [Family: Cucurbitaceae]

Suppresses glucose synthesis, through depression of the key gluconeogenic enzymes glucose-6-phosphatase and fructose-1,6-bisphosphatase and enhances glucose oxidation by shunt pathway through activation of its principal enzyme glucose-6-phosphate dehydrogenase (Shibib, 1993).

Also has an insulin secretagogue effect and acts like insulin by correcting elevated enzymes in glycolytic pathway (Kamble, 1998)

### Casearia esculenta Roxb.
Common name: Carilla Fruit [Family: Flacourtiaceae]

Exhibits significant reduction in blood glucose level, a decrease in the activities of glucose-6-phosphatase and fructose-1,6-bisphosphatase and an increase in the activity of liver hexokinase, resulting in potent hypoglycemic activity (Prakasam, 2002)

### Catharanthus roseus (L.) G. Don Common name: Madagascar periwinkle [Family: Apocynaceae]

Increases metabolization of glucose (Singh, 2001) and enhances secretion of insulin either from the beta cells of Langerhans or through extrapancreatic mechanism (Nammi, 2003)

### Camellia sinensis Kuntze. Common name: Green tea [Family: Theaceae]

Epigallocatechin gallate, present in tea increases insulin activity and prevents oxidative damages, responsible for the hypoglycemic activity (Anderson, 2002)

### Enicostemma littorale Blume Common name: Nahi [Family: Gentiiaceae]

Enhances glucose-induced insulin release from isolated rat pancreatic islets, mediated through K (+)-ATP channel-dependent pathway (Maroo, 2002)
<table>
<thead>
<tr>
<th><strong>Eugenia jambolana</strong> Lam. (syn. <em>Syzygium cumini</em> L.)</th>
<th>It enhances serum insulin activity (Sharma, 2003) and exhibits normoglycemia and better glucose tolerance (Ravi, 2004)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Common name: Indian black berry [Family: Myrtaceae]</td>
<td>Stimulates insulin secretion from beta cells of islets of langerhans (Achrekar, 1991; Cherian, 1992; Augusti 1994) and inhibits insulin degradative processes (Kumar, 1989)</td>
</tr>
<tr>
<td><strong>Ficus bengalensis</strong> L.</td>
<td>Stimulates insulin secretion from pancreatic beta cells and increases utilization of glucose, either by direct stimulation of glucose uptake or via the mediation of enhanced insulin secretion (Sachdewa, 2003)</td>
</tr>
<tr>
<td><strong>Hibiscus rosa-sinensis</strong> L.</td>
<td>Reduces insulin resistance (Kusano, 2000) and possibly acts by maltase inhibition (Matsui, 2002)</td>
</tr>
<tr>
<td>Common name: China Rose [Family: Malvaceae]</td>
<td>Possibly acts through intestinal reduction of the absorption of glucose (Aderibigbe, 1999) as well as pancreatic and extrapancreatic mechanisms (Muruganandan, 2005)</td>
</tr>
<tr>
<td><strong>Helicteres isora</strong> L. Common name: Screw tree [Family: Sterculiaceae]</td>
<td>May act by increasing hepatic glycogen (Rao, 1999)</td>
</tr>
<tr>
<td><strong>Ipomoea batatas</strong> (L.) Lam.</td>
<td>Possibly acts through stimulation of the release of insulin and/or by a direct insulin-like action due to the presence of trace elements like manganese, zinc, etc. (Akhtar, 1990)</td>
</tr>
<tr>
<td><strong>Mangifera indica</strong> L. Common name: Mango [Family: Anacardiaceae]</td>
<td></td>
</tr>
<tr>
<td><strong>Murraya koenigii</strong> (L.) Spreng. Common name: curry-leaf tree [Family: Rutaceae]</td>
<td>Increases glycogenesis and decreases glycogenolysis and gluconeogenesis (Khan, 1995)</td>
</tr>
<tr>
<td><strong>Punica granatum</strong> L. Common name: Pomegranate Family: Punicaceae</td>
<td>Inhibits intestinal alpha-glucosidase activity, leading to antihyperglycemic property (Li, 2005)</td>
</tr>
<tr>
<td><strong>Salacia reticulata</strong> Wight. Common name: Salacia Family: Celastaceae</td>
<td>Inhibits alpha-glucosidase activity (Karunanayake, 1984; Yoshikawa, 1998)</td>
</tr>
<tr>
<td><strong>Salacia Oblonga</strong> Wall. Family: Celastaceae</td>
<td>Acts through inhibition of alpha-glucosidase activity (Matsuda, 1999)</td>
</tr>
<tr>
<td><strong>Swertia chirayita</strong> (Roxb. ex Fleming) H. Karst. Common name: Indian Gentian Family: Gentianaceae</td>
<td>Stimulates insulin release from islets of Langerhans by depleting aldehyde-fuchsin stained beta-granules and immunostained insulin (Saxena, 1993)</td>
</tr>
<tr>
<td><strong>Scoparia dulcis</strong> L. Common name: Sweet Broomweed Family: Scrophulariaceae</td>
<td>Suppresses glucose influx into the polyol pathway leading to increased activities of antioxidant enzymes and plasma insulin and decreases activity of sorbitol dehydrogenase. Also potentiates insulin release from pancreatic islets (Latha, 2004)</td>
</tr>
</tbody>
</table>
1.2.9 Phytoconstituents with hypoglycemic potentials

Compounds with different structures but with the same therapeutic activity isolated from different plant species act as active moieties for the treatment of various diseases. Some of these active principles originate from edible plants and their inclusion in the diet would undoubtedly be of some value because of their hypoglycemic potential. Several phytomolecules including flavonoids, alkaloids, glycosides, saponins, glycolipids, dietary fibres, polysaccharides, peptidoglycans, carbohydrates, amino acids and others obtained from various plant sources have been reported as potent hypoglycemic agents.

- **Alkaloids**

Various alkaloids have been isolated from numerous Indian medicinal plants and investigated for their possible hypoglycemic activity in different animal models. Berberine is known to have potent hypoglycemic activity. It is obtained from *Tinospora cordifolia* (Singh et al., 2003). The mode of its antihyperglycemic activity was investigated in the Caco-2 cell line. Berberine effectively inhibited the activity of disaccharidases in Caco-2 cells, decreased sucrase activity after preincubation with Caco-2 cells for 72 h but failed to produce any significant effect on gluconeogenesis and glucose consumption of Caco-2 cells, suggesting that the antihyperglycemic activity of berberine is at least partly due to its ability to inhibit alpha-glucosidase and decrease glucose transport through the intestinal epithelium (Pan, 2003). Alkaloids like catharanthine, vindoline and vindolinine, obtained from *Catharanthus roseus* also lower blood sugar level (Chattopadhyay, 1999).

- **Imidazoline compounds**

Certain imidazoline compounds are known to have a stimulatory action on insulin secretion by activation of imidazoline I (3) binding sites in the pancreatic beta cell. Beta-carbolines, having activity at imidazoline sites been studied for their effects on insulin secretion. Harmane, norharmane and pinoline, the beta-carbolines were found to increase insulin secretion two-to three-fold from isolated human islets of Langerhans. Harmane and norharmane obtained from *Tribulus terrestris* L. and may account for the hypoglycemic property of the plant (Nadkarni, 1976; Kirtikar, 1993). Harmane stimulates insulin secretion in a
glucose-dependent manner. The results strongly substantiated the claim of betacarbolines as potent insulin secretagogues (Cooper, 2003).

- **Polysaccharides**
  Various Indian hypoglycemic plants like *Aloe vera, Ocimum sanctum, Alpinia galanga* are found to contain polysaccharides. A protein-bound polysaccharide, isolated from water-soluble substances of pumpkin was investigated for hypoglycemic activity in various doses (500 and 1000 mg/kg body weight) in alloxan diabetic rats. The results indicated that the polysaccharides increased the levels of serum insulin, reduce the blood glucose levels and improve tolerance of glucose (Quanhong, 2005).

- **Flavonoids**
  Flavonoids represent another beneficial group of naturally occurring compounds with hypoglycemic potentials. These are widely distributed in plant kingdom and exhibit distinctive pharmacological properties. The flavonoids can be widely classified into different categories like flavanols, flavones, catechins, flavanones, etc. Some flavonoids have hypoglycemic properties because they improve altered glucose and oxidative metabolisms of diabetic states. Quercetin is an important flavonoid known to possess a vast array of pharmacological activities. Intraperitoneal administration of quercetin to normal as well as streptozocin-induced diabetic rats resulted in marked reduction in plasma glucose level of diabetic animals while the glucose level of the normoglycemic rats remained unaltered. Quercetin also suppressed the glucose level in diabetic rats in glucose tolerance tests, reduced plasma cholesterol and triglycerides significantly and increased their hepatic glucokinase activity probably by enhancing the insulin release from pancreatic islets of the diabetic rats (Vessal, 2003). Some flavonoid molecules like quercetin, naringenin, chrysin significantly enhanced the insulin release from isolated rat islets of langerhans in presence of 20 mmol glucose/l. Quercetin exerted its stimulatory effect on insulin release partly by changing Ca2+ metabolism (Hii, 1985). Effect of citrus bioflavonoids, hesperidin and naringin, on blood glucose level, hepatic glucose-regulating enzymes activities, hepatic glycogen concentration, and plasma insulin levels was investigated in male C57BL/KsJ-db/db mice, an animal model for Type 2
diabetes. Supplementation of the citrus flavonoids (0.2 g/kg diet) in the diet significantly reduced the blood glucose level as well as increased hepatic glucokinase activity and glycogen concentration in diabetic rats. Naringin also markedly lowered the activity of hepatic glucose-6-phosphatase and phosphoenol pyruvate carboxykinase and the plasma insulin, C-peptide, and leptin levels in the diabetic mice were significantly increased as a result of supplementation. The findings suggested that hesperidin and naringin both play important roles in preventing the progression of hyperglycemia, partly by increasing hepatic glycolysis and glycogen concentration and/or by lowering hepatic gluconeogenesis (Jung, 2004). The soy isoflavones genistein or daidzein were investigated for their possible hypoglycemic activity in male and female obese Zucker rats, a model of Type II diabetes. Proanthocyanidins, the flavonoids with an oligomeric structure, are found to improve the pathological oxidative state of a diabetic situation. An extract of grape seed proanthocyanidins administered orally to streptozotocin-induced diabetic rats produced significant antihyperglycemic activity possibly by its insulinomimetic activity. It also stimulated glucose uptake in insulin sensitive cells in vitro (Pinent, 2004). Another flavonoid glycoside Kaempferitrin (Kaempferol-3,7-O-(alpha)-l-dirhamnoside) was found to have an acute lowering effect on blood glucose in diabetic rats and stimulated the glucose uptake, as efficiently as insulin in muscle from normal rats in vitro, suggesting that blood glucose lowering activity of the compound attributed to altered intrinsic activity of the glucose transporter (Jorge, 2004). The hypoglycemic activity of the compound was also studied along with its antioxidant potential in normal and in alloxan-induced diabetic rats. Oral administration of the compound (200 mg/kg) significantly reduced the blood glucose level in normal rats 1 h after treatment and the antihyperglycemic activity in diabetic rats was observed at all doses tested (50, 100, and 200 mg/kg) throughout the period of the study. Green tea flavonoid, epigallocatechin gallate is reported to have glucose lowering effects in animals. It was found to decrease hepatic glucose production and increased tyrosine phosphorylation of the insulin receptor and insulin receptor substrate-1 (IRS-1) like insulin. It also reduces phosphoenolpyruvate carboxykinase gene expression in a phosphoinositide 3-kinase-dependent manner and mimics insulin by increasing phosphoinositide 3-kinase, mitogen-activated protein kinase, and p70
(s6k) activity. These findings suggest that epigallocatechin gallate is an important hypoglycemic agent (Waltner-Law, 2002). Another flavonoid molecule, (-)-epicatechin (VII), has been reported to possess insulin-like activity (Chakravarthy, 1981). It was suggested that the molecule protected the experimental albino rats against the diabetogenic actions of alloxan. This flavonoid molecule mimics insulin in its effect on erythrocyte membrane acetylcholinesterase (AChE) and has a pronounced insulin-like effect on erythrocyte membrane-bound AChE in Type 2 diabetic patients (Rizvi, 1995; Rizvi, 2001).

• **Dietary fibers**

The role of dietary fibers in diabetes has been studied by several workers. Long term dietary treatment with increased amounts of fiber-rich, low-glycaemic index natural foods improves blood glucose levels and reduces the number of hypoglycemic events in type I diabetic patients (Nandini, 2003). The hypoglycemic and anti-hyperglycemic actions of fenugreek have been attributed both to gastrointestinal effects of local dietary fibre (Madar, 1988). A study investigated the effect of dietary carbohydrate and fiber on mucosal insulin receptors in order to correlate changes in cellular proliferation with hormonal responsiveness. It revealed that insulin binding was significantly affected by the consumption of dietary fiber (MacDonald, 1991).

• **Saponins: triterpenoid and steroidal glycosides**

Triterpenoid and steroidal glycosides, referred to collectively as saponins, are bioactive compounds present naturally in many plants and known to possess potent hypoglycemic activity (Rao, 2000). Charantin, a steroidal saponin, obtained from *Momordica charantia* is known to have an insulin-like activity, responsible for its hypoglycemic effect (Ng, 1986a). Charantin stimulates the release of insulin and blocks the formation of glucose in the bloodstream, which may be helpful in the treatment of diabetes, particularly in noninsulin-dependent diabetes. Lactucain C and furofuran lignan, lactucaside, obtained from *Lactuca indica* found to produce significant hypoglycemic activity (Hou, 2003). Beta-Sitosterol, a steroid obtained from *Azadirachta indica*, may be responsible for its hypoglycemic property. Andrographolide, another diterpenoid lactone, obtained from...
Andrographis paniculata Neeswas is found to possess significant hypoglycemic activity (Yu, 2003). Gymnemic acid IV, obtained from leaves of Gymnema sylvestre R. exhibits potent hypoglycemic activity in experimental animals models of diabetes.

- **Ferulic acid**

  It is 4-hydroxy-3-methoxycinnamic acid found in the leaves and seeds of many plants like Curcuma longa L. Oral administration of ferulic acid at low dose produced significant hypoglycemic activity in both types of diabetes as evident from a study on streptozotocin-induced diabetic mice and KK-Ay mice. The study suggested potent antioxidant activity of the compound in addition to its blood glucose lowering activity in experimental hyperglycemia in animals (Ohnishi, 2004). An *in vitro* study utilising rat pancreatic RIN-5F cell suggested that amide compounds, derived from ferulic acid have stimulatory effects on insulin secretion (Nomura, 2003).
CHAPTER 1

INTRODUCTION

Figure 1.5: Structures of phytoconstituents with hypoglycemic potentials

Ferulic acid

Vindoline

β-Carboline

Pinoline

Harmane

Quercetin

β-sitosterol

Amylose: α-1,4 glucosidic bonds
1.3 *In vivo* ANIMAL MODELS OF DIABETES MELLITUS

Diabetes can be induced by pharmacologic, surgical or genetic manipulations in several animal species. Most experiments in diabetes are carried out on rodents, although some studies are still performed in larger animals. The classical model employed by Banting and Best was pancreatectomy in dogs (Bliss, 2000). Currently, the murine model is one of the most used due to the availability of over 200 well-characterized inbred strains and the ability to delete or over-express specific genes through knock-out and transgenic technologies (Rees, 2005; Masiello, 2006).

1.3.1 Pharmacological induction of diabetes in animals by chemical agents

The majority of studies published in the field of ethnopharmacology between 1996 and 2006 employed these models. Streptozotocin (STZ, 69%) and alloxan (31%) are by far the most frequently used drugs and this model has been useful for the study of multiple aspects of the disease. Both drugs exert their diabetogenic action when they are administered parenterally: intravenously, intraperitoneally or subcutaneously. The dose of these agents required for inducing diabetes depends on the animal species, route of administration and nutritional status. According to the administered dose of these agents, syndromes similar to either type1, type2 diabetes mellitus or glucose in tolerance can be induced (Lenzen, 1996; Mythili, 2004). Protocols are available indicating the critical pH and type of buffer employed as well as the preparation of the solution of either alloxan or streptozotocin on the day of the experiment (Yu, 2000; Gupta, 2005a,b; Lei, 2005; Babu, 2006; Miranda, 2006).

The cytotoxic action of these diabetogenic agents is mediated by reactive oxygen species, but both drugs differ in their mechanism of action (Federiuk, 2004; Lei, 2005).

Differently from pancreatectomy, chemical induction of diabetes offers the advantage of preservation of both exocrine and endocrine cell populations other than β - cells, thus resembling the situation in human diabetes. Furthermore, the good conditions of the animals after chemical induction of diabetes do not require particular welfare measures and allow studies on the effects of high fat diet that cannot be carried out in pancreatectomized animals.
A. Alloxan

Alloxan (2,4,5,6-tetraoxohexahydropyrimidine) is a derivative of uric acid. The monohydrate is the commonly employed form of the drug. Alloxan is freely soluble in water and is slightly acid in solution (pK 6.6). Below pH 3, alloxan in solution is fairly stable at room temperature. At pH 7 the solution must be kept below 4°C to prevent conversion to alloxanic acid. The unique capability of alloxan to selectively destroy the pancreatic beta cells was first described by Dunn and associates in 1943 (Dunn, 1943). Although the evidence is overwhelming that alloxan causes diabetes by a direct toxic effect on the beta cells, the site of alloxan action and the exact mechanism of its toxicity are not completely understood. A number of studies have shown that alloxan disrupts the integrity of the beta cell plasma membrane. The site at which alloxan interacts with the cell membrane is uncertain. Some evidence indicates that alloxan acts at the site for sugar transport into the cell. Alloxan inhibits D-glucose-stimulated insulin release from islets in vivo and in vitro (Howell, 1976; Tomita, 1974). In addition, the diabetogenic action of alloxan is blocked by pretreatment with both metabolizable (D-glucase) and non metabolizable (3-O-methyl-D glucose, 2-deoxy-Dglucose) sugars which share a common transport mechanism through the plasma membrane (Kass, 1945; Watkins, 1973). Since transportable sugars block the effect of alloxan and since glucose transport is probably related to insulin release, it is reasonable to suspect that alloxan acts at the transport site. On the other hand, there is also evidence to suggest that alloxan acts at a gluco receptor site responsible for insulin release which is separate from the transport site. Finally, alloxan may bind at a location separate from either the transport or gluco receptor site (Scheynius, 1971).

Alloxan has been shown to be incorporated into beta cells where it undergoes some type of decomposition (Weaver, 1978). Boquist (Boquist, 1980) has postulated that alloxan acts by inhibiting a mitochondrial transport system for inorganic phosphate leading to a fall in intracellular pH and cell death. It has also been proposed that alloxan leads to mitochondrial dysfunction and interferes with intracellular glucose oxidation (Borg, 1981).
The chemical mechanism through which alloxan exerts its cytolytic effects is unknown. The generally held view has been that alloxan interacts with sulfhydryl-containing cellular components, for example a critical SH-containing enzyme. There is abundant evidence that \textit{in vivo} interaction with SH groups is an important part of alloxan toxicity (Rerup, 1970) and \textit{in vitro} studies have demonstrated that a number of SH-binding reagents can mimic the effects of alloxan on beta cell permeability and glucose-induced insulin release (R-Candela, 1963).

In the body, alloxan can be reduced to dialuric acid and reoxidized. Free radicals are regenerated in this process and could be cytotoxic to islet protein, lipids, and nucleic acids (Houee, 1981). Chemicals which can act as scavengers of free radicals have been shown to protect against alloxan diabetes and pretreatment of islets with the endogenous free radical scavenger superoxide dismutase is likewise protective (Fischer, 1980). In addition, it has recently been shown that alloxan administration leads to a decrease in islet superoxide dismutase activity (Crouch, 1981). The sulfhydryl and free radical theories of alloxan action are not mutually exclusive; membrane SH groups may be the target of free radicals generated during the reduction and reoxidation of alloxan. One of the most intriguing features of alloxan toxicity is its high degree of selectivity for the beta cell. Alloxan does not seem to be selectively accumulated in beta cells in preference to other tissues (Coleman, 1982). Recently it has been suggested that the beta cells are uniquely sensitive to alloxan because their plasma membrane contains ionized SH groups related to insulin release which are not found in other tissues (Watkins, 1979). Alloxan may be administered by a number of routes, but the intravenous one is the most effective and logical. The diabetogenic dose is approximately 40-45 mg/kg. Alloxan has been shown to cause diabetes in dogs, cats, sheep, rabbits, rats, mice, monkeys, fish, turtles, birds, and some, but not all, hamster species.
B. Streptozotocin induced models – the preferred models

Streptozotocin (STZ) (2-deoxy-2-(3-methyl-3-nitrosoureia) 1-d-glucopyranose, is a broad spectrum antibiotic produced by *Streptomyces achromogenes* (Alan M. Preston, 1985) and was first discovered in 1960 (Vavra, 1960). Structurally it is an N-nitroso derivative of glucoseamine and possesses at least four major biological properties:

a) Antibacterial,
b) Antitumoral,
c) Oncogenic and
d) Diabetogenic.

STZ injection in animals is associated with profound alterations in hormone, trace metal, enzyme and lipid metabolism. The drug consists of a methyl nitrosourea side chain linked to the C\textsubscript{2} position of D-glucose. The solid is unstable and must be kept frozen. In aqueous solution, streptozotocin decomposes rapidly at neutral pH and its optimum stability in solution is at pH 4.

As is the case with alloxan, streptozotocin causes diabetes by a direct toxic effect on the pancreatic beta cell. Also like alloxan, the exact site of its interaction with the beta cell is open to speculation. Some evidence suggests that the plasma membrane of the beta cell is damaged by streptozotocin, leading to morphologic and permeability changes similar to those seen after alloxan administration (Ravazzola, 1976). Because streptozotocin contains a glucose moiety, it is attractive to speculate that it binds with a glucose receptor on the plasma membrane. Streptozotocin does in fact block glucose stimulated insulin release (Kazumi, 1979). In addition, some transportable sugars block the diabetogenic action of streptozotocin (Ganda, 1976). Third, substitution of the glucose moiety in the streptozotocin molecule with a different sugar destroys its ability to induce diabetes (Bannister, 1972). In addition to possible effects on the beta cell plasma membrane, it is widely believed that streptozotocin acts intracellularly. Within the cell, streptozotocin is thought to decrease levels of nicotinamide adenine dinucleotide (NAD) (Bryan, 1977). Whether streptozotocin reduces intracellular NAD levels by interfering with its synthesis or increasing its degradation is not entirely
established (Hinz, 1973; Schein, 1973). Pretreatment of islets with exogenous nicotinamide blocks the diabetogenic effects of streptozotocin (Schein, 1973). It has been suggested that streptozotocin may be an oxidant and interact with sulfhydryl (SH) groups in a manner similar to that proposed for alloxan. Streptozotocin has been shown to decrease glutathione (GSH) levels in both red cells and pancreatic beta cells (Sima, 1982). The possibility that free radicals are responsible for streptozotocin-induced beta cell toxicity has also been entertained, although the case is not as well made as for alloxan. Streptozotocin does inhibit the endogenous free-radical-scavenger superoxide dismutase in red cells (Crouch, 1978). However, experiments in which superoxide dismutase has been preadministered to block streptozotocin diabetes have yielded conflicting results (Gold, 1981). The basis for the relatively specific toxicity of streptozotocin against beta cells is unknown. Streptozotocin has been shown to selectively accumulate in the beta cells of some species, but this has not been the case in other species (Tjalve, 1976). It does seem likely that the specificity of streptozotocin is related to its glucose component; one proposal holds that streptozotocin binds to the beta cell on the basis of its glucose moiety, whereupon the nitrosourea portion of the molecule is split off, enters the cell, and is cytotoxic. This possibility is made more plausible by the fact that N-nitrosomethylurea itself can induce beta cell necrosis, although not as effectively as streptozotocin (Gunnarsson, 1974).

The diabetogenic dose of streptozotocin is approximately 65 mg/kg body weight. In rodents, diabetes may also be induced by multiple subdiabetogenic doses. Streptozotocin causes diabetes in many species, including dogs, cats, pigs, monkeys, rabbits, rats, mice, hamsters, and guinea pigs. In rodents, the effectiveness of streptozotocin decreases with increasing age of the animals (Masiello, 1979).

➤ **Neonatal rats treated with streptozotocin (n-STZ rats):**

Neonatal Wistar rats treated with 90–100 mg/kg b.w. STZ (n-STZ) on the day of birth or 2 days after birth (Weir, 1981) undergo a transient hyperglycaemia followed by a rapid spontaneous remission until about 6–8 weeks of age.
Thereafter, non-fasting chronic hyperglycaemia develops with plasma glucose concentrations usually ranging 170–200 mg/dl for rats injected at birth and 200–350 mg/dl for rats injected at 2 days of age. The remission of initial STZ-induced hyperglycaemia is accompanied by partial $\beta$-cell and insulin stores reconstitution (Weir, 1981), most of new $\beta$-cells originating from undifferentiated duct cells. The residual $\beta$-cell mass, which is reduced to approximately 20% upon STZ administration, is finally recovering up to 50% as a consequence of $\beta$-cell regeneration and/or neogenesis. The moderate diabetic state of adult n-STZ rats does not affect body weight gain nor requires insulin treatment. However, in these rats, insulin responsiveness to glucose and tolbutamide is lacking, that is not justified by the 50% reduction of $\beta$-cell mass per se, but might be dependent on an incomplete differentiation of the newly formed $\beta$-cells after the initial STZ-induced loss (Portha, 2007 b).

**Streptozotocin-nicotinamide-treated adult rats (STZ-NA rats)**

On the basis of previous knowledge that suitable doses of nicotinamide (NA) could exert a partial protection against the $\beta$-cytotoxic effect of streptozotocin (STZ), a new experimental diabetic syndrome in adult rats that appears closer to human type 2 diabetes than other available models (e.g. neonatally STZ-injected rats), with regard to insulin responsiveness to glucose and sulphonylureas is established (Masiell, 1998 b). In 2-month-old Wistar rats, a dose of 200–230 mg/kg b.w. nicotinamide, given intraperitoneally 15 min before streptozotocin administration (60 mg/kg i.v.), yields a maximum of animals (75–80%) with 40% reduction of pancreatic $\beta$-cell mass (and no change in $\beta$-cell mass) and moderate stable non-fasting hyperglycaemia (150–180 mg/dl). Interestingly, the remaining 20–25% treated animals either become severely diabetic within 2–3 weeks or remain normoglycaemic, yet glucose-intolerant. Two to 3 weeks after diabetes induction, intravenous glucose tolerance tests reveal clear abnormalities in glucose tolerance and insulin responsiveness, which are reversed by tolbutamide administration. These features remain unchanged for a long time after induction of diabetes. In the isolated perfused pancreas of STZ-NA rats, insulin response to glucose elevation is clearly present, although significantly reduced with respect to controls. Moreover, the insulin response to tolbutamide is similar to that observed
in normal pancreases. In islets isolated from STZ-NA rats, compensatory adaptations occur. Indeed, glucose oxidation and utilization are increased when expressed per islet DNA content and insulin release is stimulated by intermediate glucose concentrations and potentiated in presence of free fatty acids more than in controls. A moderate increase of β-cell neogenesis in the pancreas of STZ-NA rats without evidence of significant β-cell replication, has been observed within a few weeks after treatment (Novelli, 2001), but extensive studies on this topic have not been conducted yet in this model. Thus, the STZ-NA-induced diabetic syndrome with decreased β-cell mass and preserved insulin responsiveness to glucose and tolbutamide, may provide a particularly advantageous tool for pharmacological investigations of not only new insulinotropic agents, but also factors stimulating β-cell growth, such as glucagon-like peptide 1 (GLP-1)/exendin-4. These studies would clarify whether such promising growth-stimulating factors that are currently tested in neonate or very young animals, are also able to promote β-cell growth in adult animals with reduced β-cell mass. Furthermore, the STZ-NA model appears very suitable for longitudinal studies aiming at assessing whether a reduced β-cell mass is able to cope with increased insulin demand induced by high-fat diet/obesity/insulin resistance and what are the mechanisms underlying the compensation and the subsequent expected failure of insulin secretory function.

1.3.2 Diabetogenic Action of Streptozotocin

STZ induces diabetes in virtually all laboratory animals tested. These include the rat, mouse, hamster, dog, monkey, guinea pig and rabbit (Weir, 1975; Portha, 2007a; Rakieten, 1963; Junod, 1967; Brodsky, 1969; Pitkin, 1970). Mode of action is through cytotoxic damage to target cells with STZ having a particularly strong affinity for the β-cells of the islet of Langerhans (Lazarus, 1972; Like, 1976). To overcome this cytotoxic damage, STZ is often administered in several small doses (Matthews, 1972) which produces a delayed experimental diabetes but increases the survival rate of injection animals. Like and Rossini further established that this delayed effect of STZ was associated with lymphatic infiltration of the pancreatic cells suggesting a cell-mediated immune reaction against the β-cells. This hypothesis has been supported in recent years (Rossini,
1978; Paik, 1980, McEvoy, 1984) providing strong evidence that T-cell mediated auto-immune mechanisms are stimulated during the induction of diabetes after STZ injections in multiple small doses. Long term work with STZ-treated rats has demonstrated that even very low doses which do not lead to apparent diabetes can still cause some problems with glucose tolerance 16 weeks after i.v. injection (Tancrede, 1983). There are considerable differences among species in susceptibility to the diabetogenic action of STZ as well as a sex difference with males being more prone than females to development of diabetes. Induction of STZ diabetes in mice was found to be potentiated by androgens and inhibited by estrogens (Paik, 1982).

1.3.3 Mechanism of Action of Streptozotocin

STZ is characterized by its diabetogenic and oncogenic activities in animals and the presence of glucose in its chemical structure may facilitate its uptake into the β-cells of the pancreas (Schein, 1973). The coupling of the alkylating agent, methyl nitroso urea (MNU) to the glucose moiety allows 3.8 times the cellular uptake of MNU alone and is undoubtedly responsible for the antitumor and oncogenic activities of STZ (Smulson, 1977). The diabetogenic effect has long been associated with a depletion of cellular nicotinamide adenine dinucleotide (NAD and NADH) levels and impaired NAD synthesis from nicotinamide (Schein, 1968). The observation of reduced pyridine content was made using mouse liver and rat pancreatic islets (Ho, 1972). More recent studies have also shown that STZ inhibits proinsulin synthesis and insulin release in rat islets in a time and concentration-dependent manner (Maldonato, 1976). Although the exact mechanism of action of STZ remains to be elucidated a very attractive hypothesis has been offered by Okamoto and coworkers (Okamoto, 1981; Okamoto, 1983; Uchigata , 1982 ) who present a unifying concept as to the oncogenic and diabetogenic effects of STZ. These workers suggest that the principle action of STZ on pancreatic B cells is induction of lesions to the DNA strand (Yamamoto, 1981). This injury induces DNA repair involving the action of polyadenosine diphosphoribose (poly (ADPrib)) synthase which uses cytosolic NAD as a substrate. It is thought that the lowered concentration of NAD following this sequence of events may be the final step in the destruction of the β-cell.
Sandler and Sweene (Sandler, 1983) using autoradiographic techniques have recently confirmed that STZ induces DNA repair synthesis in mouse pancreatic islets in vitro. Before this hypothesis is universally accepted however, caution should still be exercised when extrapolating from one tissue system or from one species of animal to another in view of the diverse and multiple actions of STZ (Robbins, 1980). The vast majority of literature on STZ has come from short-term experiments employing individual protective agents. Recently, however, investigators have begun testing the effects of more complex dietary components such as carbohydrate, protein and fat on modification of STZ-induced diabetes. Schmidt et al., (Schmidt, 1980) demonstrated marked improvement in the diabetic state of rats fed either a low carbohydrate: high protein diet (6%: 63%) or a low carbohydrate: high fat diet (5%: 75%) compared to rats fed a standard diet (68% carbohydrate, 20% protein, 12% fat). These diets were fed for 43 weeks after rats had been injected with STZ. Similar beneficial results were noted in terms of blood glucose lowering and increased insulin synthesizing capacity of β-cells was observed in rats fed an intermediate level of carbohydrate and high protein (27%: 50%). Amelioration of some secondary changes such as basement membrane thickening and damage to retina accompanied this improved diabetic state (Siegel, 1980). Eizirik and Migliorini (Eizirik, 1984) compared a carbohydrate free: high protein diet to a standard diet in STZ injected rats, however these workers fed the diets 3 weeks prior to STZ treatment. Results indicated reduced mortality and decreased severity of diabetes as judged by several parameters including plasma glucose, serum and pancreatic insulin as well as morphology of islet cells in rats fed the experimental diet. Hence, similar to nicotinamide, vitamin E, etc. feeding a low carbohydrate: high protein diet suggests that a partial protection against the diabetogenic activity of the drug occurs as well as a subsequent improvement of the induced diabetic state.

1.3.4 Biochemistry of Chemically Induced Diabetes

The effects of alloxan and streptozotocin on glucose homeostasis are sufficiently similar (except for few minor differences) to allow them to be discussed together in regard to the biochemistry of chemically induced diabetes.
• **Blood glucose:** After the administration of either alloxan or streptozotocin there is a characteristic triphasic response in blood glucose. In the first 2 hr, blood glucose rises. This transient hyperglycemia is thought to be due to sudden breakdown of liver glycogen and can be attenuated or abolished by fasting prior to the administration of the diabetogenic agent (Rice, 1980). Plasma insulin levels are low during the hyperglycemic phase (Lundquist, 1967).

The reason for the breakdown of liver glycogen during this phase is unknown, but may be a secondary effect of epinephrine release. The second phase, starting at about 6 hr, is a hypoglycemic one which may be severe enough to lead to death. The hypoglycemia is due to a sudden outpouring of insulin from dying beta cells. Plasma insulin levels are very high during this phase and the hypoglycemic response can be blocked by anti-insulin serum. Hypoglycemia is more pronounced in fasted animals, and, therefore, alloxan and streptozotocin should usually be administered to fed animals to avoid mortalities during the phase of low blood sugar. The third phase begins at about 10-12 hr and is the phase of permanent hyperglycemia. At this point, plasma insulin levels have fallen to low levels where they remain for months. By the time plasma glucose rises, viable beta cells and plasma insulin are already at very low levels (Jakobsen, 1976). Although the blood glucose exhibits a triphasic response after both alloxan and streptozotocin, the changes occur about 1 hr later with streptozotocin. At 48 hr, however, the ultimate blood glucose levels are similar. The delay in blood sugar response to streptozotocin corresponds with a delay in morphologic changes in the beta cells compared to alloxan. In the chronic diabetic state, hyperglycemia is constant and blood glucose levels in the range of 400 mg% or more can be expected after standard diabetogenic doses of alloxan and streptozotocin. Blood sugar levels tend to be higher after alloxan when compared to equimolar doses of streptozotocin. Ketosis is more common in alloxan diabetes. Oral glucose tolerance tests in both models demonstrate an elevated basal glucose, an excessive rise in glucose, and a delayed return to baseline. In long-term studies extending more than a few months, the blood sugar may start to fall. The regeneration of beta cells and the development of islet cell
adenomas in chronic streptozotocin diabetes is well described and probably represents an unbridled regenerative response to chronic hyperglycemia. The reported incidence of islet cell adenomas in animals with chronic streptozotocin diabetes at 1 yr varies from 5 to 99% but is clearly high enough to limit the effectiveness of the model to studies of approximately 6 months duration in rodents. The islet cell tumors which occur after streptozotocin administration contain large amounts of insulin and can secrete enough insulin to reverse the diabetes (Yoshino, 1981). There is much less information in the literature about reversal of hyperglycemia and development of islet cell tumors in alloxan diabetes. In few reports, no islet tumors were seen after 6 months, but reversal of diabetes has been reported as soon as 3 months after injection; the risk of reversal rises progressively and was reported as 100% at 2 years in one study. Occasional islet cell tumors in long-term alloxan-diabetic rats, appear to be much rarer than in streptozotocin diabetes.

- **Insulin:** In the first 24 hr after the administration of alloxan or streptozotocin, plasma insulin displays a triphasic response corresponding to the changes in plasma glucose. Plasma insulin is modestly depressed during the initial hyperglycemic phase, then rises dramatically in the hypoglycemic phase associated with beta cell necrosis, and finally falls to very low levels. Depressed levels of plasma insulin are the hallmark of the chronic diabetic state induced by alloxan or streptozotocin. Glucose-stimulated insulin release is abolished or severely attenuated in chemically induced diabetes from the onset (Kazumi, 1979). The ability to secrete insulin in response to amino acids is also lost (Tomita, 1980). Pancreatic content of insulin in chronic chemically induced diabetes is invariably low.

- **Glucagon:** The role of glucagon in chronic alloxan or streptozotocin diabetes is not entirely clear. Data in the literature are conflicting, probably because glucagon levels vary with the severity of the induced diabetes and because results vary depending on whether the intact animal, perfused pancreas, or isolated islets are studied. Nevertheless, chronic chemically induced diabetes should be thought of in general as a state of glucagon excess, much the same as in human insulin-dependent diabetes. Plasma levels
of glucagon during the first day after alloxan or streptozotocin exhibit a triphasic response corresponding to changes in glucose and insulin, rising in the first 2 hr, falling from 6 to 10 hr, and finally rising again at the end of 24 hr. In chronic chemically induced diabetes, plasma levels of glucagon are usually increased although they may be normal if the diabetes is mild. Pancreatic content of glucagon is probably increased and basal glucagon secretion in the perfused pancreas is increased (Weir, 1975). Normally, glucagon release is inhibited in response to elevation in ambient glucose concentration. This inhibition is abolished in alloxan and streptozotocin diabetes. In the chemically induced diabetic animal, oral glucose actually leads to a paradoxical rise in glucagon secretion. This suggests a defect in the A-cell glucoreceptor, a theory supported by the fact that alloxan-diabetic animals were also shown not to be able to release glucagon in response to hypoglycemia (Lykkelund, 1979). This issue is clouded, however, by a report of a normal glucagon response to hypoglycemia in the perfused pancreas of streptozotocin-treated rats. Glucagon secretion in response to amino acids in chemically induced diabetes has been variously reported as low, normal, or increased (Pagliara, 1975). The response to isoproterenol and calcium is apparently preserved. Further studies are necessary to delineate the exact role of glucagon in chemically induced diabetes. It is difficult to sort out, for example, which effects are secondary to direct alpha-cell toxicity of alloxan and streptozotocin and which are secondary to insulin deficiency. Nevertheless, like human diabetes, a derangement in glucagon response, especially to glucose, appears to play an important part in this type of experimental diabetes.

- **Somatostatin.** The physiology of somatostatin in chemically induced diabetes has only recently been examined, but it appears clear that chronic alloxan and streptozotocin diabetes are characterized by a relative somatostatin excess. Most of the evidence indicates that alloxan and streptozotocin have no acute effects on somatostatin. In the first few days after the administration of either drug, D cells appear unaffected and plasma and pancreatic levels of somatostatin are normal (Patel, 1978). However, after
about 2 weeks of chemically induced diabetes, peripheral and portal venous and pancreatic tissue levels of somatostatin begin to rise progressively (Schusdziarra, 1978). Somatostatin release in response to glucose and amino acids is excessive (Schein, 1968). At this point, the number of pancreatic D cells appears to be increased. It seems likely that the increased somatostatin activity in chemically induced diabetes is a reaction to the chronic state of hyperglycemia, hypoinsulinemia, and hyperglucagonemia. This concept is supported by the slow development of hypersomatostatinemia in chemically induced diabetes in contrast to the rapid onset of changes in insulin and glucagon, by the fact that somatostatin levels are inversely related to the severity of the insulin deficiency, and by the fact that insulin administration reduces the elevated blood somatostatin levels (Kazumi, 1980).

Recent advances and prospects in β-cell measurement and marker genes

Biomedical researchers are currently exploring potential of stem cell therapies for treating diabetes. Methods to differentiate adult stem cells from umbilical cord blood-derived mesenchymal cells (UCB-MSC), Wharton’s jelly-derived mesenchymal stem cells (WJ-MSC) and amniotic epithelial stem cells (AE-SC) into insulin-producing cells are established. Serum-free protocols are developed to help in the differentiation of cells into definitive endoderm, pancreatic foregut, pancreatic endoderm and, finally, pancreatic endocrine cells, which express the marker genes, sex determining region Y-box 17(SOX17), pancreatic and duodenal homeobox 1(PDX1), neurogenin 3 (NGN3), NK6 homeobox 1 (NKX6.1), insulin (INS), glucagon (GCG), and pancreatic polypeptide (PPY), respectively. Detection of the expression of the gap junction-related gene connexin-36 (CX36) using RT-PCR provides evidence for insulin-producing cell differentiation. In addition insulin and C-peptide protein can be detected by immuno histochemistry and ELISA (D'Amour, 2006 and Gao, 2008).