Chapter -1

Introduction

1.1 DIABETES MELLITUS:

The word 'diabetes' is derived from Greek word "Diabainein" which means "to pass through". It was Aretaeus who made the first complete clinical description, describing it as "a melting down of flesh and limbs into urine" and gave the name Diabetes to the disease. However, although it had been known for centuries that diabetic urine tastes sweet, it remained for Willis in 1674 to add the observation "if imbued with honey and sugar". Thus the name Diabetes Mellitus (mellitus= honey) was established.

It has been appreciated for many years that diabetes mellitus is not a single disease entity. It is better regarded as a syndrome, of which there are many causes, that is characterized by chronic hyperglycaemia due to the deficient action of insulin on target tissues (due to inadequate insulin secretion, insulin resistance or both). The deficient action of insulin is associated with disturbance of carbohydrate, fat, protein and electrolyte metabolism. The effects of diabetes mellitus include long term damage, dysfunction and failure of various organs (Alberti & Unwin, 1999).

The struggle for recognition of the role of the pancreas in diabetes was long and arduous. The fascinating, tortuous road to discovery involved a host of clinicians, chemists, physiologists, and pathologists. The tale is filled with marvelous insights as well as egregious errors, serendipity and futile labors, triumphs, and defeats. Diabetes
is a remarkable disorder, and not one very common to man. It consists of a moist and
cold wasting of the flesh and limbs into urine, from a cause similar to that of dropsy;
the secretion passes in the usual way, by the kidneys and the bladder. The patients
never cease making water, but the discharge is as incessant as a sluice let off. This
disease is chronic in character, and is slowly engendered, though the patient does not
survive long when it is completely established for the marasmus produced is rapid and
death is speedy (Schadewaldt, 1987).

1.2 HISTORICAL BACKGROUND:

The Ebers Papyrus, dating from about 1550 B.C., recommended a high-fibre diet of
wheat and ochre (Levin & Munksgaard, 1937), testifying the long history of diabetes.
This papyrus found in a grave and named after the Egyptologist, George Ebers,
contains descriptions of various diseases, including a polyuric state resembling
diabetes mellitus. It was Aretaeus of Cappadocia in the second century A.D. who used
the term 'diabetes' (which in Ionian Greek mean 'to run through', as 'a siphon') and
wrote a factual description of the conditions causing increased urine output. Despite
the clarity of his account, however, Aretaeus would not have been able to distinguish
the various non-diabetic disorders presenting with polyuria (Papaspyros, 1964).

In 1674, Thomas Willis, a physician, anatomist, and a professor of natural philosophy
at Oxford, discovered (by tasting) that the urine of diabetic persons was sweet. This
was actually a rediscovery, for unbeknownst to him, an ancient Hindu document by
Susruta in India in about 400 B.C. had described the diabetic syndrome as
characterized by a “honeyed urine” (Schadewaldt, 1987). But in India, way back in
the sixth century B.C., the Hindu physicians Charak and Sushruta named diabetes
mellitus as Madhumeha (honey disease) and defined it in no uncertain terms while
clearly differentiating it from Udakameh (water disease i.e. diabetes insipidus) and
Ikshumeh (cane sugar disease i.e. renal glycosuria). The two types of Madhumeh, one
characterized by lean constitution, dehydration, increased thirst and polyuria is due to
a congenital defect and the other is characterized by stout built, increased appetite and
due to injudicious way of life. The symptoms, signs and complications of diabetes are
also described (Ajgaonkar, 1984). Willis could not pinpoint the chemical nature of
the "sweet" substances since a variety of different chemical substances could be
equally sweet to the sense of taste (Willis, 1674). It was Matthew Dobson of
Manchester, England, who in 1776 demonstrated that diabetics actually excrete sugar
in the urine. He evaporated urine to dryness by boiling and noted that the residue, a
crystalline material, had the appearance and taste of "brown sugar" (Dobson, 1776).
A few years later another English physician John Rollo added the term mellitus
(derived from Latin root for 'honey') to distinguish it from diabetes insipidus.
Pancreatic involvement was not suspected until 1889 - The hypothetical antidiabetic
substance thought to be secreted by the Islets was named "insulin" in 1909 by de
Meyer, but authentic proof of its existence was not obtained until 1921 when
Frederick Banting and Charles Best successfully prepared pancreatic extracts
containing insulin and a new era began. Glucose stimulates the \( \beta \)-cells of the Islets to
release insulin, which then promotes glucose uptake and storage in various tissues,
considering these effects, hyperglycemia was believed to be due to insulin deficiency
and hypoglycemia due to insulin excess. However, with the advent of insulin
radioimmunoassays, it became apparent that the majority of patients with
hyperglycemia were not completely insulin dependent and, in fact, had normal or
even elevated concentration of circulatory insulin.

1.3 SEVERITY OF DIABETES:

Epidemiological estimates made by McCarty & Zimmet indicate that the number of
individuals affected by diabetes worldwide was around 110 million in 1994, with
projection reaching 175 million in the year 2000, and closed to 240 million by the
year 2010. Approximately 8% of the population in the U.S. has diabetes, with the
numbers doubling each year. This computes to nearly 16 million people diagnosed
with the disease, just considering national statistics. The American Diabetes
Association announced that diabetes accounts for 178,000 deaths (one American dies
every 3 minutes with the disease), 54,000 amputees, and 12,000-24,000 cases of
blindness annually. It is projected that by the year 2010, diabetes will exceed both
heart disease and cancer as the leading cause of death through complications. In India,
there were 19.4 million diabetics in 1995, this number will increase to 35 million in 2000 and 57.2 million in 2025, which indicates that diabetic population in India will increase by 195% at the end of 2025 (Jain, 1999). The alarming rise in the frequency of diabetics in India and similar countries has led it to be regarded as the epidemic of the 21st century. This is in part due to obesity, sedentary or changing lifestyle and changing composition of dietary food. In spite of the advances in therapeutics (Goldfine et al., 1997; Brody, 1999), diabetes still remains a major cause of morbidity and mortality in the world. Attention has been focused on key herbomineral preparations for the management of diabetes mellitus (Tomer et al., 1999; Dubey et al., 1994). The study of such medicines might offer a natural key to unlock diabetologist's pharmacy for the future.

1.4 TYPES OF DIABETES:

The classification of diabetes is based principally upon clinical symptoms and, when possible, on more specific etiologic characterization. Two major types of diabetes: (1) diabetes associated with insulin-deficiency (Type-1, Insulin Dependent Diabetes Mellitus, IDDM) and (2) diabetes associated with insulin resistance (Type-2, Non Insulin Dependent Diabetes Mellitus, NIDDM) are suggested. Other types include gestational diabetes, impaired glucose tolerance, normal glucose tolerance but substantial risk of developing diabetes, and diabetes resulting from other conditions or syndromes (Alberti & Zimmet, 1998).

A. CLINICAL CLASSES

1. Type 1 (β-cell destruction, usually leading to absolute insulin deficiency)
   - Autoimmune
   - Clinically rapidly progressive
   - Clinically slowly progressive
   - Idiopathic

2. Type 2 (may range from predominantly insulin resistance with relative insulin deficiency to a predominantly secretory defect with or without insulin resistance)
3. Other specific types

➢ Genetic defects of β-cell function
   - Chromosome 12, HNF1α (formerly MODY 3)
   - Chromosome 7, glucokinase (formerly MODY 2)
   - Chromosome 20, HNF4α (formerly MODY 1)
   - Mitochondrial DNA
   - Others

➢ Genetic defects in insulin action
   - Type A insulin resistance
   - Leprechaunism
   - Rabson-Mendenhall syndrome
   - Lipoatrophic diabetes
   - Others

➢ Diseases of the exocrine pancreas
   - Fibrocalculous pancreatopathy
   - Pancreatitis
   - Trauma/pancreatectomy
   - Neoplasia
   - Cystic fibrosis
   - Haemochromatosis
   - Others

➢ Endocrinopathies
   - Cushing’s syndrome
   - Acromegaly
   - Phaeochromolytoma
   - Glucagonoma
   - Hyperthyroidism
   - Somatostatinoma
   - Others
Introduction

➢ Drug induced or chemical induced Infections
  • Congenital rubella
  • Cytomegalovirus
  • Others

➢ Uncommon forms of immune-mediated diabetes
  • Insulin autoimmune syndrome (antibodies to insulin)
  • Anti-insulin receptor antibodies
  • Stiff man’s syndrome
  • Others

➢ Other genetic syndromes sometimes associated with diabetes
  • Down syndrome
  • Friedreich’s ataxia
  • Huntington’s chorea
  • Klinefelter’s syndrome
  • Laurence-Moon-Biedl syndrome
  • Myotonic dystrophy
  • Porphyria
  • Prader-Willi syndrome
  • Turner’s syndrome
  • Others

4. Gestational diabetes
  ➢ Impaired glucose tolerance (IGT)
    • Non obese
    • Obese
    • Impaired glucose tolerance associated with certain conditions and syndromes

B. STATISTICAL RISK CLASSES (subjects with normal glucose tolerance but substantially increased risk of developing diabetes)

1. Previous abnormality of glucose tolerance
2. Potential abnormality of glucose tolerance
1.4.1 Type 1 (IDDM):

This disease is associated with a specific and complete loss of pancreatic beta-cells, leaving islets composed of an increased number of alpha, delta and PP cells. Thus, Type 1 diabetes can be thought of as a specific beta-cytectomy.

1.4.2 Type 2 (NIDDM):

NIDDM is the most common type of diabetes. Sixty to ninety percent of such patients are obese, rarely lose weight and often exhibit hyperinsulinemia and associated insulin resistance. Table 1.1 represents the goals for optimal glycemic control (American Diabetes Association, 1994).

### Table 1.1: Goals for glycemic control in NIDDM

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Normal</th>
<th>Goal</th>
<th>Critical values for action</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fasting or preprandial plasma glucose (mg/dl)</td>
<td>&lt;115</td>
<td>&lt;120</td>
<td>&lt;80 &gt;140</td>
</tr>
<tr>
<td>Post prandial plasma glucose (mg/dl)</td>
<td>&lt;140</td>
<td>&lt;180</td>
<td>&gt;180</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (%)</td>
<td>4–6</td>
<td>&lt;7</td>
<td>&gt;8</td>
</tr>
</tbody>
</table>
Characteristic features of diabetes subtypes are summarized in **Table 1.2**

**Table 1.2: Characteristic features of diabetes subtypes**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Type 1 (IDDM)</th>
<th>Type 2 (NIDDM)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Symptoms</strong></td>
<td>Polyuria, polydipsia, fatigue, weight loss</td>
<td>Often asymptomatic in early years but may present with Type-1 symptoms especially in advanced stages</td>
</tr>
<tr>
<td>Age</td>
<td>&lt;35 (common in youth)</td>
<td>&gt;35 (frequent in adults)</td>
</tr>
<tr>
<td>Onset</td>
<td>Abrupt (days to weeks)</td>
<td>Weeks to months to years</td>
</tr>
<tr>
<td>Nutritional Status</td>
<td>Undernourished</td>
<td>Majority are overweight</td>
</tr>
<tr>
<td>Ketosis</td>
<td>Prone</td>
<td>Resistant</td>
</tr>
<tr>
<td>Insulin</td>
<td>Mandatory</td>
<td>Required in &lt; 30%</td>
</tr>
<tr>
<td>Diet</td>
<td>Mandatory</td>
<td>Controls 30 to 50% cases</td>
</tr>
<tr>
<td>Beta-cells</td>
<td>None (complete islet cell loss)</td>
<td>Varies</td>
</tr>
<tr>
<td>Islet cell antibodies</td>
<td>Yes</td>
<td>No</td>
</tr>
<tr>
<td>Family history</td>
<td>+ in 10%</td>
<td>+ in 30%</td>
</tr>
<tr>
<td>(Identical twins)</td>
<td>~ 50% concordance</td>
<td>~ 100% concordance</td>
</tr>
</tbody>
</table>
1.5 **AETIOLOGY:**

Diabetes mellitus is not a single syndrome with one origin; it must consist of a number of different conditions arising from various causes but with the majority of symptoms in common. Accordingly, it is most unlikely that one causative agent can be assigned to this disease.

Genetic, environmental (Dahlquist et al., 1989; Elsas et al., 1989) and autoimmune (Wilkin, 1990) destruction of pancreatic β-cells has been suggested to be the most common cause of IDDM. Other initiating factors include viruses (Notkins, 1980) viz. Coxsackie B4 virus (Barrett-Connor, 1985; Yoon et al., 1979), rubella virus, reovirus (Bell & Hye, 1983), and chemical toxins (alloxan and streptozotocin). The effectiveness of these diabetogenic chemical agents is highly dependent on the age, sex, weight and species of the recipient. Less common causes of IDDM are conditions that result in a reduction in the mass of islet cell tissue, such as may occur with several types of pancreatitis, pancreatic carcinoma, and pancreatectomy.

NIDDM arises as a consequence of (1) failure of insulin action due to abnormalities at the cell surface (decreased affinity of the receptor for insulin) or within the cell (post-receptor defects) (Slieker et al., 1990; Block et al., 1991) and (2) deficiency of insulin secretion (Robertson & Chen, 1977) or a combination of (1) and (2) (Jarvinen, 1995). Although the majority of such patients are insulin resistant, it is undecided whether the primary molecular defect lies within the insulin signal transduction pathway or in β-cell insulin secretion (Taylor et al., 1994; Kahn, 1994).

On rare occasions, it may also result from point mutations in the insulin gene (Tager, 1984) or in diminished expression of the insulin-responsive glucose transporter that collects glucose in cells (Strout et al., 1990). Abnormally high secretory activity of the anterior pituitary gland can obviously be regarded as one possible extra-pancreatic cause of clinical diabetes mellitus. Over activity of the secretory function of the adrenal cortex, either primary or secondary to pituitary over action, can initiate or exacerbate clinical diabetes if adrenal steroids of the corticosterone type are produced.
in excessive amount, while abnormally high thyroid activity might also contribute to the development or maintenance of a diabetic condition (Lippold & Winton, 1968).

Experimentally, surgical removal of the pancreas and lesioning of the central nervous system can induce a condition resembling human diabetes mellitus in some important respects in certain animals. Stress, infection and toxins may also provoke diabetes. Chemical agents capable of inducing diabetes (Table 1.3) (Mordes & Rossini, 1981; Joslin, 1985) permit detailed study of the biochemical, hormonal and morphological events that occur during and after the induction of a diabetic state.

**Table 1.3: Chemical Agents Capable of Inducing Diabetes**

1. Irreversible beta-cytotoxic agents
   - Alloxan
   - Streptozotocin
   - Diphenylthiocarbazine
   - Oxine-9-hydroxyquinoline
   - Vacor

2. Reversible beta-cytotoxic agents
   - 6-Aminonicotinamide
   - L-Asparaginase
   - Azide
   - Cyanide
   - Dehydroascorbic acid
   - Fluoride
   - Malonate
   - Thiazides
   - 2-deoxyglucose
   - Mannohexulose

3. Other agents
   - Anti-insulin antibodies
   - Glucagon
   - Glucocorticoids
   - Somatostatin
   - Catecholamines
Inadequate secretion of insulin results in a syndrome called diabetes mellitus. Insulin deficiency gives rise to the sequence of events depicted in Fig 1.1. Decreased carbohydrate utilization and glycogenesis results into glucose accumulation in the blood (hyperglycaemia). Increasing hyperglycaemia elevates the blood sugar above the renal threshold and glucose appears in the urine (glycosuria), as soon as the renal capacity for reabsorption of filtered glucose is exceeded, thus inducing osmotic increased urination (polyuria). Polyuria and glycosuria in turn lead to increased hunger (polyphagia) and thirst (polydipsia) and an accelerated production of glucose from non glucose precursors within the organisms, thereby leading to mobilization and catabolism of proteins (aminoacidemia) and fats, expressed in drastic weight loss and physical wasting. Accelerated mobilization of fat indirectly leads to increased triglycerides concentration (lipemia), and their partial hepatic oxidation to ketone bodies, in excess of the capacity to the organism to oxidize them completely, results in accumulation (ketonemia) and spilling over in urine (ketonuria). This in turn necessitates massive cation excretion, which further adds to the state of dehydration. Dehydration leads to hemoconcentration with ultimate renal and circulatory failure resulting in coma and death.

Adenomas of the Islets of Langerhans give rise to overproduction of insulin causing Hypoglycaemia- some times called “insulin shock” this happens suddenly if a person using insulin eats too little food, doesn’t eat soon enough, and takes too much insulin, or exercises too much. Symptoms like inappropriate responses, crankiness, confusion, lack of coordination, drowsiness, pale complexion, perspiration, headache, trembling, sudden hunger and dizziness may rapidly appear; one or more of these may suddenly occur. The condition must be treated quickly with sugar or sugary foods because hypoglycaemia can lead to unconsciousness. If a person becomes unconscious, honey or syrup should be rubbed inside the person’s cheek, where it can be absorbed without risk of choking. If the person still does not
Fig 1.1: Pathophysiology of diabetic acidosis (Metabolic and Endocrine physiology Year Book Medical Publishers, 1962, Chicago).
respond in 10 to 15 minutes, glucagon, a hormone that raises blood sugar, may need to be injected.

1.7 **INSULIN THERAPY:**

1.7.1 **Type 1 (IDDM):**

Insulin is a major protein hormone secreted by the β-cells of the pancreas and is important for the control of diabetes (Gowthamarajan & Kulkarni, 2003). Diabetes mellitus is treated by insulin administration. But regular insulin is so rapidly taken up from the subcutaneous spaces where it is injected that small frequent administrations are necessary to avert a precipitous fall in the blood sugar. To obviate frequent administration, the other forms of insulin are usually used after equilibrium had been re-established. In most cases, one injection a day suffices. Sometimes this regime must be supplemented with regular insulin at meal times.

One should bear in mind that insulin is the primary hormone responsible for controlling the storage and utilization of cellular nutrients in all mammalian cells (Kahn & White, 1988). Both Type 1 and Type 2 patients show lower postprandial glucose levels under the influence of insulin (Shalev, 1999). The actions of insulin are traditionally classified into three groups, based on their kinetics. The immediate or rapid effects of insulin that appear within seconds or minutes include activation of glucose (Standley & Rose, 1994) and ion transport systems (Gupta et al., 1989), enzyme activation (phosphorylation or dephosphorylation) (Czech et al., 1988) and activation of phospholipid signaling systems (Farese & Cooper, 1989). The intermediate effects, such as induction of ornithine decarboxylase and tyrosine aminotransferase (Roger & Fellows, 1980), occur within 3-6 hours and result from regulation of gene expression. The long-term effects of insulin require hours to several days and include protein synthesis (Manchester and Young 1958; Morgan et al., 1972) and stimulation of cell proliferation and differentiation. These effects may also be seen in both nondiabetic and diabetic smooth muscles from experimental animals. A new rapid acting insulin analog [Lys (B28), Pro (B29)]-human insulin (LYSPRO), a technique of transplantation of pancreas and, or islet cells have been
developed (Howey et al., 1994). A number of alternatives to injecting insulin are being investigated including insulin pump, oral insulin (Meyerhoff et al., 1999), inhaled insulin (Saudek, 1997; The pink sheet, 1997) and gene therapy (Goldfine et al., 1997; Brody, 1999). Most recently, it has been published that orally administered, insulin loaded amidated pectin hydrogel beads sustain plasma concentrations of insulin in STZ diabetic rats (Musabayana et al., 2000).

1.7.2 Type 2 (NIDDM):

Recently, the combination of oral antidiabetic drugs with insulin has been found to be clinically useful for the treatment of Type 2 diabetes (Simpson et al., 1990; Hayward et al., 1997). HbA1c levels were decreased by 0.9% versus control, after 1 year of such treatment. The some of drugs for management of NIDDM are summarized in Table 1.4.

### Table 1.4: The Drugs for management of NIDDM

<table>
<thead>
<tr>
<th>Functional Class</th>
<th>Drugs</th>
</tr>
</thead>
</table>
| 1. Insulin secretagogues | Sulphonylureas  
Non Sulphonylureas  
Other agents  
GLP-1  
Amylin Antagonists |
| 2. Insulin sensitizers | Biguanides  
Thiazolidinediones  
Anti- obesity drugs  
B3 Adrenoceptor agonists |
| 3. Inhibitors of Gastro intestinal glucose absorption (Pramlintide) | Glucosidase inhibitors  
Amylin analogues |
| 4. Inhibitors of Intermediary metabolism | Antilipolytic and antihyperlipidemic agents, Fatty acid oxidation inhibitors |
| 5. Insulin mimetic drugs | Insulin analogues IGF-1  
Vanadium salts |
Currently available oral antihyperglycaemic agents include sulphonylureas, biguanides, glucosidase inhibitors and thiazolidinediones. These agents, which exhibit different modes of action (Fig 1.2), may be used as monotherapy or in various

Fig 1.2: Sites of action of oral antihyperglycaemic drugs
combinations. Although NIDDM is responsive to the treatment with oral antidiabetic drugs, many patients respond to therapy with diet and exercise alone. After diet failure, patients can be treated with one of the four available oral antihyperglycaemic drugs before considering the use of insulin. Various aspects of the management of type 2 diabetes have been taken into consideration by a European Type 2 Diabetes Policy Group (Alberti et al., 1994). Largely diffused, and regularly updated, it contains sections dealing with the diagnosis of the disease, targets for control, the principles of management, the management of intercurrent illness, and the various conditions that are associated with or that complicate Type 2 diabetes. For blood glucose control, five classes of drugs are now available in the management of type 2 diabetes and can be used alone or in combination: sulfonylureas, biguanides (mainly metformin), α-glucosidase inhibitors (mainly acarbose), thiazolidinediones (troglitazone, available in some countries) and insulin (Scheen & Lefebvre, 1998). Fig 1.3 summarizes the stepwise treatment of Type 2 diabetes that is frequently adopted today in developing countries.

Diabetes Mellitus is a contributor to considerable morbidity in the form of metabolic complications, vision disorders, neuropathy, kidney disease, peripheral vascular disease, ulcerations, amputations, heart disease, stroke, digestive disease, infection, oral complications and depression. The associated mortality rate has been estimated at 5.5% of total patient annually, and the disease is known to reduce life expectancy between 5-10 years (Chawrai & Jain, 2004).

1.8 COMPLICATIONS OF DIABETES:

Diabetes mellitus is a metabolic disorder with characteristic of hyperglycemia and insufficiency of secretion or action of endogenous insulin. Although the etiology of diabetes is not well defined, genetic components, viral infection, autoimmune disease, and environmental factors have been implicated in the disease (Kataoka et al., 1983; Paik et al., 1982). Evidence showed that the chronic elevation of plasma glucose causes many of the major complications of diabetes, including nephropathy, retinopathy, neuropathy, and macrovascular and microvascular damage (Brownlee, 2001; Cullen et al., 1999).
Diagnosis

Non-drug treatment

Patient selection

Nonobese
Obese insulin-resistant
Postprandial hyperglycemia

First drug selection

Sulphonylurea
Metformin
Troglitazone
Acarbose

Combined oral Therapy

Combined insulin-oral therapy

Failure of oral treatment

Insulin

Sulphonylurea if residual insulin secretion
Metformin if weight excess
Troglitazone if insulin resistance
Acarbose if glucose instability

Fig 1.3: A guide to the selection of oral antihyperglycemic drugs
Various short and long term complications may occur in diabetic patients. Ketoacidosis a metabolic disease is an acute complication and occurs in the absence of appropriate treatment (Foster, 1984). Development of potent antidiabetic substances (Joost, 1985), newer therapeutic (Carpenter & Bodansky, 1990) and preventive modalities (Elliot et al., 1993), ultra pure recombinant human insulin (Heinemann et al., 1990) and new methods of insulin delivery (Merouze, 1983; Houtzagers, 1989), has greatly reduced the mortality rates due to diabetes mellitus and its acute complications. In spite of these significant developments in antidiabetic therapy, diabetic complications, chiefly seen in long-term diabetes, continue to be seriously deleterious.

Diabetes Mellitus is one of the challenges faced by modern medicine. Subcutaneous insulin and oral hypoglycaemic pills therapy has rationalized and simplified the treatment of diabetes. However, the synthetic hypoglycaemic agents can produce serious side effects (Table 1.5) including hematological, cutaneous and gastrointestinal reaction, hypoglycaemic coma and disturbances of liver and kidney (Reynolds, 1997). Several animal and human studies have described about the role of hyperglycaemia in the development of secondary complications of diabetes but they have not fully defined the mechanism through which excess glucose results in tissue damage. It is also not known, whether these complications could be prevented and what degree of control was necessary. Recently it has been indicated that the generation of reactive oxygen species (oxidative stress) might play an important role in the etiology of diabetic complication (Evans et al., 2002).
### Table 1.5: Side effects of synthetic hypoglycaemic agents

<table>
<thead>
<tr>
<th>Oral Hypoglycemic agents</th>
<th>Preparations</th>
<th>Action</th>
<th>Adverse Effects</th>
</tr>
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<tbody>
<tr>
<td><strong>INSULIN SECRETAGOGUES</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sulfonylureas</td>
<td>glimepride</td>
<td>Stimulate</td>
<td>Hypoglycaemia,</td>
</tr>
<tr>
<td></td>
<td>(Amaryl),</td>
<td>insulin</td>
<td>weight gain</td>
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<tr>
<td></td>
<td>glipizide</td>
<td>secretion</td>
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<td></td>
<td>(Glucotrol),</td>
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<tr>
<td></td>
<td>glyburide</td>
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<td></td>
<td>(Diabeta,</td>
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<td></td>
<td>Micronase)</td>
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<tr>
<td>Meglitinides</td>
<td>nateglinide</td>
<td>Stimulate</td>
<td>Hypoglycemia,</td>
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<tr>
<td></td>
<td>(Starlix)</td>
<td>insulin</td>
<td>weight gain</td>
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<tr>
<td></td>
<td>repaglinide</td>
<td>secretion</td>
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<td></td>
<td>(Prandin)</td>
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<tr>
<td><strong>INSULIN SENSITIZERS</strong></td>
<td>metformin</td>
<td>Suppresses</td>
<td>Nausea, metallic</td>
</tr>
<tr>
<td>Biguanides</td>
<td>(Glucophage)</td>
<td>hepatic</td>
<td>taste, bloating,</td>
</tr>
<tr>
<td></td>
<td></td>
<td>glucose</td>
<td>diarrhea, anorexia,</td>
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<td>output,</td>
<td>contraindicated if</td>
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<td></td>
<td></td>
<td>increase</td>
<td>renal function is</td>
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<td></td>
<td></td>
<td>glucose</td>
<td>impaired</td>
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<td></td>
<td></td>
<td>uptake</td>
<td></td>
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<td>Thiazolidinediones</td>
<td>pioglitazone</td>
<td>Increase</td>
<td>Weight gain, increase</td>
</tr>
<tr>
<td></td>
<td>(Actos),</td>
<td>insulin</td>
<td>in low density</td>
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<tr>
<td></td>
<td>rosiglitazone</td>
<td>sensitivity</td>
<td>lipoprotein</td>
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<tr>
<td></td>
<td>(Avandia)</td>
<td></td>
<td>cholesterol; fluid</td>
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<td></td>
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<td></td>
<td>retention, drug</td>
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<td></td>
<td></td>
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<td>interactions, possible</td>
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<td></td>
<td></td>
<td></td>
<td>hepatotoxicity</td>
</tr>
<tr>
<td><strong>INHIBITORS OF CARBOHYDRATE DIGESTION</strong></td>
<td>acarbose</td>
<td>Reduced</td>
<td>Flatulence, diarrhea,</td>
</tr>
<tr>
<td>Alpha-glucosidase inhibitors</td>
<td>(Precose),</td>
<td>glucose</td>
<td>cramps</td>
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<td></td>
<td>miglitol</td>
<td>absorption</td>
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<td>(Glyset)</td>
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1.8.1 Free radical oxidative stress and diabetes:

Free radicals are atoms or molecules that have one or more unpaired electrons in their atomic structures and are highly reactive. The susceptibility of a given organ or organ system to oxidative stress is a function of the balance between prooxidant factors and those scavenging these factors. Oxidative damage can therefore be the consequence of raised free radical production, insufficient antioxidant potential, or both (Cross et al., 1987). The nonenzymatic, free radical-mediated oxidation of biological molecules, membranes, and tissues is associated with a variety of pathological events: cancer, aging and as recently suggested, diabetes (Baynes, 1991).

Accumulating evidence suggests that oxidative cellular injury caused by free radicals contributes to the development of diabetes mellitus (Bambolkar & Sainani, 1995). Reactive oxygen species generated in the cells are scavenged by antioxidant enzymes (Genet et al., 2002). Moreover, diabetes also induces changes in the tissue content and activity of the antioxidant enzymes (Asayama, 1989).

Free radicals are formed disproportionately in diabetes by glucose oxidation, nonenzymatic glycation of proteins, and the subsequent oxidative degradation of glycated proteins. Abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to damage of cellular organelles and enzymes, increased lipid peroxidation, and development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus (Martim et al, 2003). Increased oxidative stress is a widely accepted participant in the development and progression of diabetes and its complications (Cariello, 2000). This hypothesis is supported by the evidence that many biochemical pathways strictly associated with hyperglycaemia (glucose autoxidation, polyol pathway, prostanoid synthesis, protein glycation) can increase the production of free radicals (Fig 1.4). Diabetes is usually accompanied by increased production of free radicals (Baynes, 1999) or impaired antioxidant defences.
Fig 1.4: Possible links between hyperglycemia-induced oxidative stress and diabetic complications. Solid lines indicate experimentally established links. A genetic predisposition through reduced antioxidant status may be present (dashed lines). NCV, nerve conduction velocity; VSMC, vascular smooth muscle cells.
Lipid peroxidation occurs mainly in membranes, where the content of unsaturated fatty acids is relatively high. Peroxidation of membrane lipid arising out of oxidative damage in intact cells result in decrease fluidity, inactivation of membrane bound enzymes and receptors and change in nonspecific ion permeability. Cellular antioxidant defense system such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx) and food derived antioxidants such as α-tocopherol and β-carotene are considered to play a significant role in quenching reactive oxygen species (ROS) thereby displaying a modulatory role in many of the disease conditions. Oxidative stress is implicated in the etiopathogenesis of a variety of human disease (Frei, 1994; Peternans, 1997; Beck & Levender, 1998; Domenico et al., 1998). Several studies have given a lot of evidence of increased oxidative stress with depleted antioxidant enzymes and vitamins, in both Type-1 and Type-2 diabetes mellitus (Beck, 2000; Treitinger et al., 2000). Now oxidative stress is acknowledged as a pathogenesis mechanism in diabetic complications like diabetic retinopathy, nephropathy, microangiopathy (Cariello, 2000).

In general, studies on lipid peroxidation are consistent with studies on glycoxidation of proteins in diabetes; i.e. increased oxidation of both lipids and proteins is associated with the development of complications. This crossover between the oxidative chemistry of lipids and proteins is reminiscent of experiments discussed earlier in which glycation of proteins causes oxidation of associated lipids (Hunt et al., 1990; Hicks et al., 1988) and enhances the generation of fluorescence during oxidation of proteins (Fujimori, 1989). Thus, increased glycation of collagen and plasma proteins in diabetes may stimulate the oxidation of lipids, which may in turn stimulate autoxidative reactions of sugars, enhancing damage to both lipids and proteins in the circulation and the vascular wall, continuing and reinforcing the cycle of oxidative stress and damage.

1.8.2 Oxidative stress and insulin resistance:

Reactive oxygen species (ROS) and oxidative stress can lead to the activation of multiple serine kinase cascades in vitro. The insulin signaling pathway provides a
number of potential targets of these activated kinases, including the insulin receptor (IR) and the family of insulin receptor substrate (IRS) proteins. For IRS-1 and -2, an increase in serine phosphorylation decreases the extent of tyrosine phosphorylation and is consistent with the attenuation of insulin action (Fig 1.5).

Fig 1.5: Possible sites of action to account for the protective effects of antioxidants against oxidative stress-induced insulin resistance.

A variety of stimuli increase ROS production and oxidative stress. This results in the activation of multiple stress-sensitive serine/threonine kinase signaling cascades. Once activated, these kinases are able to phosphorylate multiple targets, such as the IR and IRS proteins. Increased phosphorlation of IR or IRS proteins on discrete serine or threonine sites decreases the extent of insulin-stimulated tyrosine phosphorylation. Consequently, the association and/or activities of downstream signaling molecules
(e.g. PI-3 kinase) are decreased, resulting in reduced insulin action. (Evans et al., 2003).

1.8.3 Oxidative stress and β-cell dysfunction:

β-cells are responsible for sensing and secreting insulin in response to glucose stimulation. These cells are sensitive to reactive oxygen species because they are low in antioxidant enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (Tiedge et al., 1997). Oxygen stress generated by short exposure of β-cells preparations to \( \text{H}_2\text{O}_2 \) increases production of p21, an inhibitor of cyclin-dependent kinase, decreases insulin mRNA, cytosolic ATP, and calcium flux in cytosol and mitochondria, and cause apoptosis. Insulin secretion stimulated by glucose or methyl succinate can be inhibited shortly, whereas the response to \( \text{K}^+ \) remains normal. These results suggest that the mitochondrial processes involved in glucose-mediated insulin secretion are particularly affected by oxidative stress (Ling, 2003).

1.8.4 Diabetes: oxidative stress everywhere:

Evidence that Oxidative stress (OST) is present in diabetes originates from the frequent observation that both reactive oxygen species and antioxidants are increased. The latter is logically rather seen in early stages of diabetes and should be interpreted as a tentative compensation of cells against increasing oxidative stress (Turk et al., 2002; Zobali et al., 2002). According to tissue and cell type, the nature of antioxidant elevation may vary, indicating specificities which, again, be important for therapeutic interventions. Countless publications exist showing the existence of various indicators of OST \textit{in vitro} and this has finally led to a lively ongoing debate about the pertinence and relevance of parameters such as TBARS, malondialdehyde, isoprostanes or nitrotyrosines (De Zewart et al., 1999). Oral intake of high glucose in animals increases TBARS and reduces the activity of hepatic enzymes susceptible to thiol group oxidation (Folmer et al., 2002). In humans, OST is also seen in postprandial periods in normal individuals but diabetic patients are unable to compensate for the increased ROS (Ceriello et al., 1998). This increase may be attributed to acute effects
of high glucose and/or lipids. Increased OST is also found in the basal state in both types of diabetes, some studies suggest that it is much more pronounced in Type 2 than in Type 1 diabetes (Seghrcuchni, 2002). Type 2 diabetics exhibit increases in TBARS and reduction in catalase activity but, surprisingly correlation was found in a recent study between TBARS and level or duration of hyperglycaemia (Turk et al., 2002). Plasma glutathione (GSH) levels are decreased and oxidized purines increase, illustrating DNA damage (Dincer et al., 2002). In blood vessels, increased levels of superoxide have been recorded in both arterial and venous segments (Guzik et al., 2002).

Data on antioxidants are variable since, according to the origin of the insult and tissue/cell type, antioxidants may even be increased (Paget et al., 1998; Costa et al., 2002). In view of data with insulin and some lipid fractions, it is likely that normoglycaemic insulin-resistant subjects may already exhibit oxidative stress. Indeed obesity, characterized by hyperinsulinemia and dyslipidemia, is accompanied by elevated OST (Ciccone et al., 1999). Interestingly, even in a healthy population, variations in insulin sensitivity are related to lipid hydroperoxide levels and reduced catalase and vit E levels (Facchini et al., 2000). Again in the general population, various markers of glucose metabolism and of insulin resistance were associated with oxidative stress (Trevisan et al., 2001). It has been suggested that low vitamin E levels are better predictors of diabetes than age, BMI or smoking (Salonen et al., 1995). Thus, oxidative stress may be a very early event in the long history of diabetes, similar to what is seen for functional microvascular defects (Wiensperger, 2002). That oxidative stress can precipitate diabetes development is suggested by an experiment showing the appearance of fasting hyperglycaemia within days after administration of a prooxidant to insulin resistant, obese Zucker rats (Laight et al., 2000). It is therefore conceivable that chronic exposure of insulin resistant tissues to oxidative stress generated for example by the daily iterative postprandial periods might constitute an important factor in the etiology of diabetes. In this scenario diabetes, by virtue of adding hyperglycaemia may essentially exacerbate a preexisting situation, as observed for functional microperfusion.
1.9 ANTIOXIDANTS AND DIABETES:

Chemical compounds and reaction capable of generating potential toxic oxygen species/free radicals are referred to as pro-oxidants. On the other hand, compounds and reactions disposing off these species, scavenging them, suppressing their formation or opposing their actions are called anti-oxidants. In a normal cell, there is an appropriate pro-oxidant: antioxidant balance (Irshad & Chaudhari, 2000). The cells maintain a variety of defenses against oxygen toxicity or free radical mediated injury. Among these are arrays of enzyme, that have been evolved to deal with oxidative stress including super oxide dismutase (SOD), catalase (CAT), glutathione peroxidase etc. In eukaryotes uric acid, alpha tocopherol, ascorbic acid and beta carotene are among the compounds that function in the cell. Both prokaryotes and eukaryotes contain high levels of glutathione, a scavenger of OH. However, cells under aerobic condition are threatened with the result of ROMs (Reactive Oxygen Metabolites) that are efficiently taken care of by the powerful antioxidant system in human body. Aerobic life is characterized as continuous production of oxidants balanced by equivalent synthesis of antioxidants (Rice-Evans et al., 1993). A shift of the balance on the oxidant side may trigger a cascade of reaction leading to the formation of highly reactive cytotoxic compounds such as ROMs. The improper balance between ROMs production and antioxidant defences results in "oxidative stress", which deregulates the cellular functions leading to various pathological conditions including diabetes (Bandyopadhyay et al, 1999).

1.9.1 Endogenous Antioxidants:

The antioxidant enzymes like superoxide dismutase and catalase, glutathione peroxidase, which catalyze the reduction of oxidants in the intracellular environment are termed as endogenous antioxidants. Recombinant superoxide dismutase and catalase are available in conjugated as well as unconjugated form. Mc Cord and Fridovich's discovery of superoxide dismutase in 1969 led to the recognition of the toxic effect of superoxide radical. Superoxide dismutase catalyzes the reaction between two molecules of super oxide anion to form hydrogen per oxide and molecular oxygen.
Eukaryotes contain typically, both Cu and Zn containing SOD and another SOD that contains manganese. The latter is compartmentalized in mitochondria, while the former is in the cytoplasm. The catalase belongs to the family of enzymes, which contains the hydroperoxidase and peroxidases. Catalase catalyses the formation of water and oxygen from H\textsubscript{2}O\textsubscript{2}.

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

The action of these two enzymes minimize the possibility of superoxide, hydrogen peroxide and transition metal ion interaction and subsequently prevents the formation of highly reactive hydroxyl radicals [Fenton reaction and Haber Weiss reaction]

Fenton reaction \[ \text{H}_2\text{O}_2 + \text{Fe}^{++} \rightarrow \text{OH}^- + \text{OH-Fe}^{++} \]

Haber Weiss reaction \[ \text{O}_2 + \text{H}_2\text{O}_2 \rightarrow \text{OH}^- + \text{OH}^- + \text{O}_2 \]

1.9.2 Exogenous Antioxidants:

They are also known as scavenging or chain breaking antioxidants. They are classified into two main classes, one water soluble like ascorbic acid (vitamin C) and other lipid soluble- alpha tocopherol (vitamin E) and \(\beta\)-Carotene. The former works as a strong direct acting reducing agent, and the latter as an antioxidant, functioning through selenium dependent glutathione peroxidase enzyme system.

The major goals of antioxidant treatment have been to reduce oxidative stress with the expectation of:

1. preventing;
2. delaying the progression; or
3. reversing (i.e. improving) the late microvascular and/or macrovascular complications of diabetes.
The potent sources of natural antioxidants are spices and herbs (Rajalashmi & Narsimhan, 1996). Phenolic components in spices and herbs have been reported to act as powerful inhibitors of lipid peroxidation. It is further reported that vegetarian diets play a preventive role in many of the chronic diseases due to their antioxidant components. Dietary components possessing antioxidant properties have been identified as vitamin C, E and beta carotene and non-nutrient components such as flavanoids and poly phenols. Thus free radicals are not widely considered to be causative of either Type-1 or Type-2 diabetes. Antioxidant action has been observed in number of medicinal plants (Miyake et al., 1997), hence they are extensively used to prepare tonics and health care medicines. Thus at this stage it has not been convincingly established that—

a) Diabetic hyperglycaemia weakens antioxidant defense.

b) Diabetic hyperglycaemia increases free radical generation.

c) Subjects with weak antioxidant defense are more prone to diabetes.

d) Subjects with abnormally high radical generation rate are more prone to diabetes.

Important progress has been made in understanding the progression of the pathogenesis of diabetes mellitus. In the last few years, revolutionary changes in the therapy of diabetes mellitus have been described. Drugs like insulin, sulfonylureas, biguanides have rationalized and simplified the treatment of diabetes. But none of them have been unequivocally successful in maintaining euglycemia and in avoiding late stage complications of diabetes. New drugs are being tried to reduce glucose absorption from gut and to prevent other diabetic complications. In spite of the advances in therapeutics, diabetes still remains a major cause of morbidity and mortality in the world. The researches were tempted to key herbomineral preparations for the management of diabetes mellitus. World ethnobotanical information about medicinal plants reports almost 800 plants used in the control of diabetes mellitus (Alarcon et al., 1998), but only a small number of these have received scientific and
medical evaluation to assess its efficacy. However, traditional herbal remedies, which are naturally free from side effects, are still in use by diabetic patients (Mutalik et al., 2003) and may, therefore, provide new avenues in the search for alternative hypoglycaemic drugs.

Under the conflict back ground, about the role of antioxidants in diabetes, we have under taken the present study to examine the effect of plant Coccinia indica and recently used antioxidant-Alpha Lipoic acid on a well characterized animal model of diabetes.