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3.1 INTRODUCTION TO DIABETES MELLITUS

The term diabetes mellitus describes a metabolic disorder of multiple aetiology characterized by chronic hyperglycaemia with disturbances 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 (WHO, 1999), especially the eyes, kidneys, nerves, heart and blood vessels (Pickup and Crook, 1998).

Several pathogenic processes are involved in the development of diabetes. These range from autoimmune destruction of the β-cells of the pancreas with consequent insulin deficiency to abnormalities that result in resistance to insulin action. The basis of the abnormalities in carbohydrate, fat and protein metabolism in diabetes is deficient action of insulin on target tissues. Deficient insulin action results from inadequate insulin secretion and/or diminished tissue responses to insulin at one or more points in the complex pathways of hormone action (WHO, 1999). Impairment of insulin secretion and defects in insulin action frequently coexist in the same patient, and it is often unclear which abnormality, if either alone, is the primary cause of the hyperglycemia.

The long-term effects of diabetes mellitus include progressive development of the specific complications of retinopathy with potential blindness, cataract, nephropathy that may lead to renal failure, and/or neuropathy with risk of foot ulcers, amputation, Charcot joints, and features of autonomic dysfunction, including sexual dysfunction. People with diabetes are at increased risk of cardiovascular, peripheral vascular and cerebrovascular disease. Hypertension, abnormalities of lipoprotein metabolism and periodontal disease are also often found in people with diabetes. The emotional and social impact of diabetes and
the demands of therapy may cause significant psychosocial dysfunction in patients and their families.

Epidemiology of Diabetes

Diabetes mellitus is one of the most common chronic endocrine disorders affecting millions of people worldwide and is now recognized as serious global health problem (WHO 1985, King and Rewers, 1993). The number of people with diabetes is increasing due to population growth, aging, urbanization, and increasing prevalence of obesity and physical inactivity. The world prevalence of diabetes among adults (aged 20–79 years) was estimated to be 6.4%, affecting 285 million adults, in 2010, and is projected to increase to 7.7%, and 439 million adults by 2030. Between 2010 and 2030, 69% increase is estimated in numbers of adults with diabetes in developing countries and a 20% increase is estimated in developed countries (Shaw et al, 2010). The prevalence of diabetes is higher in men than women, but there are more women with diabetes than men. The urban population in developing countries is projected to double between 2000 and 2030. The most important demographic change to diabetes prevalence across the world appears to be the increase in the proportion of people >65 years of age (Wild et al, 2004). These findings indicate that the "diabetes epidemic" will continue even if levels of obesity remain constant.

Fig 3.1: Estimated number of diabetic subjects in India. (Huizinga and Rothman, 2006)
India leads the world with largest number of diabetic subjects earning the dubious distinction of being termed the "diabetes capital of the world". According to the Diabetes Atlas 2006 published by the International Diabetes Federation, the number of people with diabetes in India, currently around 40.9 million, is expected to rise to 69.9 million by 2025 unless urgent preventive steps are taken (Huizinga and Rothman, 2006). The so called "Asian Indian Phenotype" refers to certain unique clinical and biochemical abnormalities in Indians which include increased insulin resistance, greater abdominal adiposity i.e., higher waist circumference despite lower body mass index, lower adiponectin and higher high sensitive C-reactive protein levels. This phenotype makes Asian Indians more prone to diabetes and premature coronary artery disease. At least a part of this is due to genetic factors. However, the primary driver of the epidemic of diabetes is the rapid epidemiological transition associated with changes in dietary patterns and decreased physical activity as evident from the higher prevalence of diabetes in the urban population. Even though the prevalence of microvascular complications of diabetes like retinopathy and nephropathy are comparatively...
lower in Indians. The prevalence of premature coronary artery disease is much higher in Indians compared to other ethnic groups. The most disturbing trend is the shift in age of onset of diabetes to a younger age in the recent years. This could have long lasting adverse effects on nation’s health and economy. Early identification of at-risk individuals using simple screening tools like the Indian Diabetes Risk Score (IDRS) and appropriate lifestyle intervention would greatly help in preventing or postponing the onset of diabetes and thus reducing the burden on the community and the nation as a whole.

**Etiological classification of diabetes mellitus**

A major requirement for the clinical management of diabetes is an appropriate system of classification that provides a framework within which to identify and differentiate its various forms and stages.

1. **Type 1 diabetes mellitus (formerly IDDM)**
   - Autoimmune type 1 diabetes mellitus (type 1 A)
   - Non autoimmune or idiopathic type 1 diabetes mellitus (type 1 B)

2. **Type 2 diabetes mellitus (formerly NIDDM)**

3. **Other specific types**
   - **Specific defined gene mutations**
     - *Maturity onset diabetes of the youth* (MODY)
       - MODY 1 hepatic nuclear factor $4\alpha$ gene mutations
       - MODY 2 glucokinase gene mutations
       - MODY 3 hepatic nuclear factor $1\alpha$ gene mutations
       - MODY 4 pancreatic determining factor $X$ gene mutations
       - MODY X unidentified gene mutations
     - *Maternally inherited diabetes and deafness* (MIDD)
       - Mitochondrially leucine tRNA gene mutations
       - Insulin gene mutations
       - Insulin receptor gene mutations

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Secondary to pancreatic diseases

Chronic pancreatitis
Tropical diabetes (chronic pancreatitis associated with nutritional and/or toxic factors)
Neoplasia
Pancreatectomy

Secondary to endocrinopathies

Acromegaly
Cushing's syndrome
Glucagonoma
Pheochromocytoma
Hyperthyroidism

Secondary to immune suppression

Due to infections
Congenital rubella
Cytomegalo virus
Others

Drug or chemical induced diabetes

Glucocorticoids
Diuretics
Diazoxide
Ca++-channel blockers
β2-adrenergic receptors agonists
Phenytoin
α-interferons
Heparin
Morphine
Nalidixic acid
Sulphinpyrazone
Clonidine

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Thyroid hormones
Pentamidine
Vacor

Other genetic disorders sometimes associated with diabetes
Down syndrome
Turner’s syndrome
Klinfelter’s syndrome
Prader-Willi syndrome
Wolfram syndrome
Friedreich’s syndrome
Huntington’s chorea
Porphyria

4. Gestational diabetes mellitus (GDM)

On the basis of etiology, type 1 diabetes is present in patients who have little or no endogenous insulin secretory capacity and who therefore require insulin therapy for survival. The two main forms of clinical type 1 diabetes are type 1a (about 90% of type 1 cases) which is thought to be due to immunological destruction of pancreatic β-cells resulting in insulin deficiency; and type 1b (idiopathic, about 10% of type 1 diabetes), in which there is no evidence of autoimmunity. Type 1a is characterized by the presence of islet cell antibody (ICA), anti-glutamic acid decarboxylate (anti-GAD) or insulin antibodies that identify the autoimmune process with β-cell destruction (Atkinson and Maclaren, 1994; Zimmet et al, 2004). Autoimmune diseases such as Grave’s disease, Hashimoto’s thyroiditis and Addison’s disease may be associated with type 1 diabetes mellitus (Betterle et al, 1984; Atkinson and Maclaren, 1994). There is no known etiological basis for type 1b diabetes mellitus. Some of these patients have permanent insulinopaenia and are prone to ketoacidosis, but have no evidence of autoimmunity (McLarty et al, 1990). This form is more prevalent among individuals of African and Asian Origin (Ahrén and Corrigan, 1984).
Type 2 diabetes is the commonest form of diabetes and is characterized by disorders of insulin secretion and insulin resistance (DeFronzo et al., 1997). In Western countries, the disease affects up to 7% of the population (WHO, 1994; Harris et al., 1998). Globally, it affects 5-7% of the world’s population (Harris et al., 1998; Amos et al., 1997; King et al., 1998). At least initially and often throughout their lifetime, these individuals do not need insulin treatment to survive. There are probably many different causes of this form of diabetes and it is likely that the proportion of patients in this category will decrease in the future as identification of specific pathogenic processes and genetic defects permits better differentiation among them and a more definitive subclassification. Although the specific etiologies of this form of diabetes are not known, autoimmune destruction of β-cells does not occur and patients do not have any of the other causes of diabetes.

Most patients with this form of diabetes are obese and obesity itself causes some degree of insulin resistance (Kolterman et al., 1981; Bogardus et al., 1985). Patients who are not obese by traditional weight criteria may have an increased percentage of body fat distributed predominantly in the abdominal region (Kissebah et al., 1982). Ketoacidosis seldom occurs spontaneously in this type of diabetes; when seen, it usually arises in association with the stress of another illness such as infection (Banerji et al., 1994; Butkiewicz et al., 1995; Umpierrez et al., 1995). This form of diabetes frequently goes undiagnosed for many years because the hyperglycemia develops gradually and at earlier stages is often not severe enough for the patient to notice any of the classic symptoms of diabetes (Harris, 1989; Zimmet, 1992). Nevertheless, such patients are at increased risk of developing macrovascular and microvascular complications (Moss et al., 1984; Fujimoto et al., 1987; Uusitupa et al., 1993; Kuusisto et al., 1994; Andersson and Svaardsudd, 1995). Whereas patients with this form of diabetes may have insulin levels that appear normal or elevated, the higher blood glucose levels in these diabetic patients would be expected to result in even higher insulin values had their β-cell function been normal (Polonsky et al., 1996).
Thus, insulin secretion is defective in these patients and insufficient to compensate for the insulin resistance. Insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia but is seldom restored to normal (Scarlett et al, 1982; Firth et al, 1986; Simonson et al, 1984; Henry et al, 1986; Wing et al, 1994). The risk of developing this form of diabetes increases with age, obesity and lack of physical activity (Zimmet, 1992; Harris et al, 1995). It occurs more frequently in women with prior gestational diabetes mellitus (GDM) and in individuals with hypertension or dyslipidemia and its frequency varies in different racial/ethnic subgroups (Zimmet, 1992; Fujimoto et al, 1987; Harris et al, 1995). It is often associated with a strong genetic predisposition, more so than is the autoimmune form of type 1 diabetes (Barnett et al, 1981; Newman et al, 1987). However, the genetics of this form of diabetes are complex and not clearly defined.

**Pathogenesis of Type 1 diabetes mellitus**

Understanding the pathogenesis of any disease is of prime importance when considering treatment. Recent breakthroughs in the evaluation and management of diabetes and the availability of new therapeutic regimens make it imperative that the primary care physician be aware of these advances to improve patient care.

Type 1 diabetes, also known as insulin-dependent diabetes mellitus (IDDM), is a chronic autoimmune disease that results from a complex interaction of both genetic and environmental factors (Eisenbarth, 1986). Genetic susceptibility likely is followed by humoral as well as T-cell abnormalities, even though organ dysfunction may be absent or occurring subclinically. It is well known that pancreatic tissue taken from children who have died from diabetic ketoacidosis demonstrates a mononuclear cell infiltration confined to pancreatic islet cells, predominantly CD8+ cells, that are likely involved in beta cell specific destruction, which supports the role of islet cell autoimmunity in type 1 diabetes (Conrad et al, 1994). It also has been shown that a long prodrome is present in...
exhibit a progressive loss of their ability to secrete insulin in response to physiologic secretagogues. The natural history of this disorder can be subdivided into a series of stages, commencing with genetic susceptibility and ending with disease onset (Fig 3.3). Several studies have suggested that the use of a combination of immunologic markers to islet antigens (e.g., insulin, GAD and the tyrosine phosphatase IA-2) rather than a single test gives a higher predictive value for type 1 diabetes and provides greater sensitivity without significant loss of specificity (Conrad et al, 1994; Kruglyak and Lander, 1995). Prediction of type 1 diabetes is now sufficiently accurate to allow the design of large clinical trials to study the prevention of the disease process and to provide a better understanding of disease pathogenesis in individuals at risk of developing the disease (Bingley et al, 1994; Pietropaolo et al, 1998).

Fig 3.3: Hypothetical stages of autoimmune destruction of pancreatic β cells during the natural history of type 1 diabetes mellitus. (Eisenbarth, 1996)

a. Genetic Susceptibility

The application of genome-wide scans has resulted in identification of 218 putative loci, but only linkage to human leukocyte antigen (HLA) loci appears to be unequivocal. Although excitement has been generated by the results of
genome-wide scans for type 1 diabetes, careful and rigorous replication in many populations along with association studies is necessary before any attempts are made using either positional cloning or the candidate gene approach to identify potentially elusive sequence variations that could influence genetic susceptibility (Kruglyak and Lander, 1995). Convincing evidence exists that 22 chromosomal regions are associated with and linked to type 1 diabetes. The HLA region on chromosome 6~21 (IDDMI) and the insulin gene region on chromosome 11~15 (ZDDM2) confer susceptibility to type 1 diabetes. The contribution of these 2 loci to familial inheritance is 42% for ZDDM1 and 10% for IDDA42. A rare form of autoimmune diabetes resembling type 1 diabetes occurs in autoimmune polyglandular syndrome type I, also known as autoimmune polyendocrinopathy-candidiasis-ectodermal dystrophy (APECED). This syndrome is unique among well-known autoimmune disorders in that it is caused by a single gene pair-homozygosity for defect in the AZRE (autoimmune regulator) gene which is localized on chromosome 21q22. This example demonstrates that mutation of a single gene can give rise to an array of autoimmune poly-endocrine disorders, including insulin-requiring diabetes of autoimmune origin. Similarly, autoantibodies to islet cell antigens such as GAD and islet cell antibodies can occur in patients affected by APECED, as seen in IDDM. The nature of the immune abnormalities in APECED remains elusive.

b. HLA Complex and Susceptibility

Amino acid polymorphism at position 57 on the HLA-DQP chain of the HLA class II molecule could influence interaction among the class II molecule, the peptide antigen, and the T-cell receptor, which in turn could influence the specificity of the immune response to foreign and self-antigens. However, other residues in the DQP chain as well as in the DQA chain also appear to be involved in susceptibility to type 1 diabetes (Pietropaolo and Trucco, 2000). Genetic susceptibility to autoimmune diabetes appears to be strongly conferred by HLA-DQA 1*0501-DQB 1*0201 and -DQA1*0301-DQB 1*0302 haplotypes, both of which are in linkage disequilibrium with the DR3 and DR4 alleles. In many
populations the HLA-DQB 1*0602 allele is rarely found among patients with IDDM, which suggests that this allele may play a protective role in the disease process. At present, possession of a “protective” DQB 1*0602 allele is considered a criterion for excluding first-degree relatives of diabetic patients in clinical trials such as the Diabetes Prevention Trial 1 (DPT-1). This trial has been designed to prevent by effective treatment the progression to type 1 diabetes in individuals considered at high risk of developing the disease.

c. The Insulin Gene (IDDM2) and the Biologic Significance of the Variable-Number-of-Tandem-Repeats (VNTR) Region

The central role of insulin in metabolism and blood glucose homeostasis and its unique distinction as the only known pancreatic beta cell-specific antigen make it a likely candidate for an inherited susceptibility to IDDM. A number of studies have suggested that the insulin gene (INS) VNTR region may play a biologic role in the genetic regulation of insulin expression. The proximity of this polymorphism to the INS transcriptional start site (~400 base pair upstream) makes this an attractive hypothesis. Nevertheless, the exact function of the INS VNTR region is still a subject of discussion. The insulin promoter and thymic autoantigen expression data are consistent with a proposed general hypothesis that self-tolerance to peripheral proteins develops during thymic selection of T cells; this hypothesis, however, is controversial.

d. Environmental Triggers

Even in genetically predisposed individuals, an environmental agent, such as a virus, may be required for the generation of autoimmunity. For example, the incidence of both type 1 diabetes and multiple sclerosis in a given population changes as these individuals migrate to different regions. Such observations, along with the lower than expected rate of concordance among monozygotic twins, suggest that an environmental factor may play a role in the development of autoimmune responses. In the case of type 1 diabetes, the environmental trigger could be the coxsackievirus (Kruglyak and Lander, 1995). Antigenic similarity between a foreign pathogen (e.g., a virus) and an antigen normally present on or
in the target tissue (e.g., an autoantigen) may be involved in triggering the onset of type 1 diabetes. This concept is embodied in the term "molecular mimicry." The best example of a possible molecular mimicry-induced reaction acting in diabetes etiopathogenesis is that presented by Kaufman and colleagues in which the peptide PEVKEK, an amino acid stretch of the P2-C protein of coxsackievirus B, is shared by the islet antigen GAD65 and may activate T cells to destroy GAD65-expressing cells (Kaufman et al, 1992).

e. Autoimmunity to Islet Antigens

A common characteristic of many autoimmune diseases such as type 1 diabetes is a T-cell and humoral response against multiple target antigens. As autoimmunity in type 1 diabetes progresses from initial activation to a chronic state, there is often an increase in the number of islet autoantigen targeted by T cells and antibodies. This condition is termed "epitope spreading" and involves overexpression of cytokines and other inflammatory mediators. This is a cascading process in that T cells activate additional autoreactive B cells and B cells present additional epitopes from different proteins until there is autoreactivity to numerous autoantigens. In a similar fashion, multiple novel peptides within the same autoantigen can activate T cells. Immunologic diagnosis of type 1 diabetes relies essentially on the detection of autoantibodies to islet antigens in the serum of patients (Bingley et al, 1994). Although their pathogenic significance is still unclear, such antibodies have the great advantage of serving as surrogate markers for specific autoimmune responses and now represent major markers for enrollment in primary intervention trials studying patients with type 1 diabetes.

f. Failure of Immunologic Tolerance to Self-Antigens

Tolerance to self-molecules is established and maintained through complex mechanisms in both the thymus (central tolerance) and peripheral lymphoid organs (peripheral tolerance). One attractive hypothesis is that type 1 diabetes is essentially caused by the failure of negative selection of autoreactive T cells in either the thymus or the periphery or is the result of a breakdown in
tolerance to islet cell-specific antigens (Fig. 3.4). Recent evidence suggests that molecules with tissue-restricted expression may also be expressed in the thymus (Pugliese et al, 1997). Genes encoding the IDDM-related islet autoantigens insulin, IA-2, GAD, and the neuroendocrine antigen ICA69 are transcribed in the human thymus throughout fetal life and childhood. There is evidence that variation in the expression of IDDM-related autoantigens in the thymus and peripheral lymphoid organs can affect tolerance to these molecules and in turn influence diabetes risk.

**Fig 3.4 : General hypothesis for the development of self-tolerance to peripheral proteins during thymic selection of T cells** (Pietropaolo and Le Roith, 2001).

**g. Dysfunction and Loss of Regulatory Cells**

Alterations in the number and function of regulatory cells may contribute to the generation of an autoimmune state in type 1 diabetes. Dysfunction or loss of CD1-restricted T cells, T cells with γ/δ receptors, and CD4+/CD25+ T cells may...
all theoretically contribute to disease pathogenesis through inefficient suppression of pathogenic autoreactive T cells. For instance, in monozygotic twins who are discordant for diabetes, levels of CDI-restricted T cells appear to be diminished in the affected twin. The antigens that activate regulatory T cells are unknown, and the mechanisms by which these cells exert their effect on immune responses remain unclear.
3.2 COMPLICATIONS OF DIABETES MELLITUS

Diabetes mellitus is a multifaceted, dynamic expression of pathological disequilibria, resulting in various complications. It has been shown that long-standing hyperglycaemia and poor metabolic control results in increased diabetic complications (Ling et al, 1994). Diabetes can affect nearly every system of the body. The complications of diabetes can be short-term or long-term. Complications are more likely to occur when diabetes is uncontrolled than when it is regulated.

Short Term Complications of Diabetes

A person with uncontrolled diabetes has a diminished resistance to bacterial and fungal infections. Hence, he or she may develop tuberculosis, urinary tract infection, boils, carbuncles or fungal infection of vulva in females and foreskin and glans penis in males. Infection of the outer part of the ear, bleeding gums with pocket of pus between teeth and gums may occur in uncontrolled diabetes. Studies have now clearly demonstrated that diabetes increases the risk of both severe periodontitis and the incidence of periodontal disease progression by approximately 2 to 3 folds. Periodontal disease is a chronic gram-negative infection. Infections increase the secretion of hormones, which oppose the action of insulin, increase blood glucose and if adequate insulin is not available, result in the formation of ketones.

Many conditions can give rise to coma in diabetics. It can be due to abnormal blood glucose level, (either hypoglycaemia or hyperglycaemia). Sweating and trembling are early signals of hypoglycaemia. Some features of hypoglycaemia like blurred vision, confusion, feelings of uncertainty, intense hunger, throbbing headache are due to an impaired function of the central nervous system. Palpitation (awareness of the heartbeats), tingling and numbness of the fingers and lips, trembling, perspiration and a feeling of doom are due to increased release of catecholamines. An inability to carry out
coordinated movement or to concentrate is early features of hypoglycaemia. Features such as unsteady gait, slurred speech, abnormal behaviour can occur. In severe hypoglycaemia, temporary loss of speech or weakness of one side of the body may take place; a person may get fits or may become unconscious. A prolonged and severe hypoglycemia can result in irreversible brain damage, especially in the elderly. Deaths due to hypoglycaemia are uncommon but may occur if the person is living alone or is ignorant of its prevention and treatment. Recurrent severe hypoglycaemia can give rise to cumulative brain damage. Such tragedies are preventable and hence the importance of education of the diabetics and their families. The levels of blood glucose level at which symptoms of hypoglycaemia develop vary from person to person and in the same person from time to time. The faster and the bigger the fall of blood glucose, the higher is the threshold. The threshold is higher in diabetics used to have high blood glucose levels.

Hyperglycaemia can lead to ketoacidotic and nonketotic coma. Ketoacidotic hyperglycaemic coma is a very serious complication of diabetes. Before the discovery of insulin, this was the most common cause of death amongst the diabetics. An omission of insulin in insulin-dependent diabetes is the most important cause of diabetic ketoacidosis and coma. Any infection, like a carbuncle, abscess, pneumonia or vomiting or diarrhoea worsens diabetes and may result in diabetic coma, if not treated properly. An infection may give rise to loss of appetite, nausea or vomiting. A diabetic may not be able to have his usual diet. Often it is assumed that since the usual meal has not been taken, insulin is not necessary and hence is omitted. This mistake is disastrous since infection increases the insulin requirement. Any stress such as an infection or a surgical operation, increases the insulin requirement. The body fat is broken down when insulin is deficient. The breakdown products of fats, called as ketone bodies, accumulate in blood and later appear in urine when diabetes is severe. The ketone bodies are acidic and hence their accumulation in the body causes acidosis. Blood glucose level is high and there is a marked loss of water, sodium,
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Potassium and bicarbonates. Coma develops gradually. Before actual coma or unconsciousness develops the diabetic loses appetite, gets nausea or vomiting and abdominal pain. The tongue becomes dry, face is flushed, breathing becomes deep and rapid and the pulse becomes rapid and feeble. The patient becomes drowsy and unconsciousness.

Nonketotic hyperglycaemic coma occurs more commonly in the elderly. The blood glucose level is very high and there is a marked loss of water from the body. Ketosis is absent since insulin deficiency is not as marked as in the ketoic coma. Coma due to accumulation of lactate in blood may occur in diabetics taking biguanides, especially phenformin, in the presence of impaired kidney, liver and heart function.

Long Term Complications of Diabetes

Long term diabetes can affect all parts of the body. The severity of the damage to various tissues depends on the quality of control of diabetes and the duration of diabetes. Considerable research is going on at present to explain the mechanism of this long-term tissue damage. Individual genetic predisposition is also an important factor. Blood vessels of all sizes are often affected. The affection of large blood vessels is called macroangiopathy or macrovascular complications, while the affection of the small or minute blood vessels is called microangiopathy or microvascular complications. Macrovascular complications include coronary artery disease, peripheral arterial disease, and stroke whereas microvascular complications include diabetic nephropathy, neuropathy, and retinopathy. Moreover, the association between diabetes and cataract formation has been shown in clinical epidemiological and basic research studies.

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Table 3.1 Prevalence of Complications in patients with IDDM (Nathan, 1993)

<table>
<thead>
<tr>
<th>Complications</th>
<th>Cumulative prevalence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Visual impairment</td>
<td>14</td>
</tr>
<tr>
<td>Blindness</td>
<td>16</td>
</tr>
<tr>
<td>Renal failure</td>
<td>22</td>
</tr>
<tr>
<td>Stroke</td>
<td>10</td>
</tr>
<tr>
<td>Amputation</td>
<td>12</td>
</tr>
<tr>
<td>Myocardial infarction</td>
<td>21</td>
</tr>
<tr>
<td>Median survival after diagnosis of IDDM (yr)</td>
<td>36</td>
</tr>
<tr>
<td>Median age at death (yr)</td>
<td>49</td>
</tr>
</tbody>
</table>

Mechanisms for microvascular disease in diabetes include the pathologic effects of AGE accumulation, overproduction of endothelial growth factors, and abnormal stimulation of the PKC and polyol pathways and the RAS. Mechanisms for macrovascular disease in diabetes include the pathologic effects of AGE accumulation, impaired vasodilatory response attributable to NO inhibition, smooth muscle cell dysfunction, overproduction of endothelial growth factors, chronic inflammation, hemodynamic dysregulation, impaired fibrinolytic ability, and enhanced platelet aggregation (clotting) (Todd Cade, 2008).

**Microvascular complications of diabetes**

**Diabetic retinopathy**

Diabetic retinopathy may be the most common microvascular complication of diabetes. It is responsible for ~ 10,000 new cases of blindness every year in the United States alone (Fong et al, 2004). The risk of developing diabetic retinopathy or other microvascular complications of diabetes depends on both the duration and the severity of hyperglycemia (Pirart, 1978; Krolewski et al,
Prospective Diabetes Study (UKPDS), and most patients with type 1 diabetes develop evidence of retinopathy within 20 years of diagnosis (UKPDS, 1998). Retinopathy may begin to develop as early as 7 years before the diagnosis of diabetes in patients with type 2 diabetes (Fong et al, 2004). There are several proposed pathological mechanisms by which diabetes may lead to development of retinopathy.

Aldose reductase may participate in the development of diabetes complications. Aldose reductase is the initial enzyme in the intracellular polyol pathway. This pathway involves the conversion of glucose into glucose alcohol (sorbitol). High glucose levels increase the flux of sugar molecules through the polyol pathway, which causes sorbitol accumulation in cells. Osmotic stress from sorbitol accumulation has been postulated as an underlying mechanism in the development of diabetic microvascular complications, including diabetic retinopathy. In animal models, sugar alcohol accumulation has been linked to microaneurysm formation, thickening of basement membranes, and loss of pericytes. Treatment studies with aldose reductase inhibitors, however, have been disappointing (Gabbay, 1975; Gabbay, 2004; Fong et al, 2004).

Cells are also thought to be injured by glycoproteins. High glucose concentrations can promote the nonenzymatic formation of advanced glycosylated end products (AGEs). In animal models, these substances have also been associated with formation of microaneurysms and pericyte loss. Evaluations of AGE inhibitors are underway (Fong et al, 2004).

Oxidative stress may also play an important role in cellular injury from hyperglycemia. High glucose levels can stimulate free radical production and reactive oxygen species formation. Animal studies have suggested that treatment with antioxidants, such as vitamin E, may attenuate some vascular dysfunction associated with diabetes, but treatment with antioxidants has not yet been shown to alter the development or progression of retinopathy or other microvascular complications of diabetes (Kunisaki et al, 1995; Fong et al, 2004).
Growth factors, including vascular endothelial growth factor (VEGF), growth hormone, and transforming growth factor β, have also been postulated to play important roles in the development of diabetic retinopathy. VEGF production is increased in diabetic retinopathy, possibly in response to hypoxia. In animal models, suppressing VEGF production is associated with less progression of retinopathy (Aiello et al, 1995; Fong et al, 2004; Keenan et al, 2007).

Diabetic retinopathy is generally classified as either background or proliferative. It is important to have a general understanding of the features of each to interpret eye examination reports and advise patients of disease progression and prognosis.

Background retinopathy includes such features as small hemorrhages in the middle layers of the retina. They clinically appear as “dots” and therefore are frequently referred to as “dot hemorrhages.” Hard exudates are caused by lipid deposition that typically occurs at the margins of hemorrhages. Microaneurysms are small vascular dilatations that occur in the retina, often as the first sign of retinopathy. They clinically appear as red dots during retinal examination. Retinal edema may result from microvascular leakage and is indicative of compromise of the blood-retinal barrier. The appearance is one of grayish retinal areas. Retinal edema may require intervention because it is sometimes associated with visual deterioration (Watkins, 2003).

Proliferative retinopathy is characterized by the formation of new blood vessels on the surface of the retina and can lead to vitreous hemorrhage. White areas on the retina (“cotton wool spots”) can be a sign of impending proliferative retinopathy. If proliferation continues, blindness can occur through vitreous hemorrhage and traction retinal detachment. With no intervention, visual loss may occur. Laser photocoagulation can often prevent proliferative retinopathy from progressing to blindness; therefore, close surveillance for the existence or progression of retinopathy in patients with diabetes is crucial (Watkins, 2003).

Patients with diabetes are also at higher risk for other ophthalmic disorders like cataract (Nathan et al, 1986).
Diabetic Nephropathy

Diabetic nephropathy is the leading cause of renal failure in the United States. It is defined by proteinuria > 500 mg in 24 hours in the setting of diabetes, but this is preceded by lower degrees of proteinuria, or “microalbuminuria.” Microalbuminuria is defined as albumin excretion of 30-299 mg/24 hours. Without intervention, diabetic patients with microalbuminuria typically progress to proteinuria and overt diabetic nephropathy. This progression occurs in both type 1 and type 2 diabetes.

As many as 7% of patients with type 2 diabetes may already have microalbuminuria at the time they are diagnosed with diabetes (Gross et al, 2005). In the European Diabetes Prospective Complications Study, the cumulative incidence of microalbuminuria in patients with type 1 diabetes was ~12% during a period of 7 years (Chaturvedi et al, 2001; Gross et al, 2005). In the UKPDS, the incidence of microalbuminuria was 2% per year in patients with type 2 diabetes, and the 10-year prevalence after diagnosis was 25% (Adler et al, 2003; Gross et al, 2005).

The pathological changes to the kidney include increased glomerular basement membrane thickness, microaneurysm formation, mesangial nodule formation (Kimmelsteil-Wilson bodies), and other changes. The underlying mechanism of injury may also involve some or all of the same mechanisms as diabetic retinopathy.

Screening for diabetic nephropathy or microalbuminuria may be accomplished by either a 24-hour urine collection or a spot urine measurement of microalbumin. Measurement of the microalbumin-to-creatinine ratio may help account for concentration or dilution of urine, and spot measurements are more convenient for patients than 24-hour urine collections. It is important to note that falsely elevated urine protein levels may be produced by conditions such as urinary tract infections, exercise, and hematuria.

Initial treatment of diabetic nephropathy, as of other complications of diabetes, is prevention. Like other microvascular complications of diabetes, there
are strong associations between glucose control (as measured by hemoglobin A\(_1c\) [A1C]) and the risk of developing diabetic nephropathy. Patients should be treated to the lowest safe glucose level that can be obtained to prevent or control diabetic nephropathy (DCCT Research Group, 1993; Adler et al, 2003; Gross et al, 2005). Treatment with angiotensin-converting enzyme (ACE) inhibitors has not been shown to prevent the development of microalbuminuria in patients with type 1 diabetes but has been shown to decrease the risk of developing nephropathy and cardiovascular events in patients with type 2 diabetes (HOPE study, 2000; Gross et al, 2005).

In addition to aggressive treatment of elevated blood glucose, patients with diabetic nephropathy benefit from treatment with antihypertensive drugs. Renin-angiotensin system blockade has additional benefits beyond the simple blood pressure-lowering effect in patients with diabetic nephropathy. Several studies have demonstrated renoprotective effects of treatment with ACE inhibitors and antiotensin receptor blockers (ARBs), which appear to be present independent of their blood pressure-lowering effects, possibly because of decreasing intraglomerular pressure. Both ACE inhibitors and ARBs have been shown to decrease the risk of progression to macroalbuminuria in patients with microalbuminuria by as much as 60-70%. These drugs are recommended as the first-line pharmacological treatment of microalbuminuria, even in patients without hypertension (Gross et al, 2005).

Similarly, patients with macroalbuminuria benefit from control of hypertension. Hypertension control in patients with macroalbuminuria from diabetic kidney disease slows decline in glomerular filtration rate (GFR). Treatment with ACE inhibitors or ARBs has been shown to further decrease the risk of progression of kidney disease, also independent of the blood pressure-lowering effect.

Combination treatment with an ACE inhibitor and an ARB has been shown to have additional renoprotective effects. It should be noted that patients treated with these drugs (especially in combination) may experience an initial increase in
creatinine and must be monitored for hyperkalemia. Considerable increase in creatinine after initiation of these agents should prompt an evaluation for renal artery stenosis (Rossing et al, 2003; Gross et al, 2005).

**Diabetic neuropathy**

Diabetic neuropathy is recognized by the American Diabetes Association (ADA) as "the presence of symptoms and/or signs of peripheral nerve dysfunction in people with diabetes after the exclusion of other causes" (ADA, 2007). As with other microvascular complications, risk of developing diabetic neuropathy is proportional to both the magnitude and duration of hyperglycemia, and some individuals may possess genetic attributes that affect their predisposition to developing such complications.

The precise nature of injury to the peripheral nerves from hyperglycemia is not known but likely is related to mechanisms such as polyol accumulation, injury from AGEs, and oxidative stress. Peripheral neuropathy in diabetes may manifest in several different forms, including sensory, focal/multifocal, and autonomic neuropathies. More than 80% of amputations occur after foot ulceration or injury, which can result from diabetic neuropathy (Boulton et al, 2005). Because of the considerable morbidity and mortality that can result from diabetic neuropathy, it is important for clinicians to understand its manifestations, prevention, and treatment.

Chronic sensorimotor distal symmetric polyneuropathy is the most common form of neuropathy in diabetes. Typically, patients experience burning, tingling, and "electrical" pain, but sometimes they may experience simple numbness. In patients who experience pain, it may be worse at night. Patients with simple numbness can present with a painless foot ulceration, so it is important to realize that lack of symptoms does not rule out presence of neuropathy. Physical examination reveals sensory loss to light touch, vibration, and temperature. Abnormalities in more than one test of peripheral sensation are > 87% sensitive in detecting the presence of neuropathy. Patients also typically
experience loss of ankle reflex (Boulton et al, 2005). Patients who have lost 10-g monofilament sensation are at considerably elevated risk for developing foot ulceration (Abbott et al, 2002).

Pure sensory neuropathy is relatively rare and associated with periods of poor glycemic control or considerable fluctuation in diabetes control. It is characterized by isolated sensory findings without signs of motor neuropathy. Symptoms are typically most prominent at night (Boulton et al, 2005).

Mononeuropathies typically have a more sudden onset and involve virtually any nerve, but most commonly the median, ulnar, and radial nerves are affected. Cranial neuropathies have been described but are rare. It should be noted that nerve entrapment occurs frequently in the setting of diabetes. Electrophysiological evaluation in diabetic neuropathy demonstrates decreases in both amplitude of nerve impulse and conduction but may be useful in identifying the location of nerve entrapment. Diabetic amyotrophy may be a manifestation of diabetic mononeuropathy and is characterized by severe pain and muscle weakness and atrophy, usually in large thigh muscles (Boulton et al, 2005).

Several other forms of neuropathy may mimic the findings in diabetic sensory neuropathy and mononeuropathy. Chronic inflammatory polyneuropathy, vitamin B_{12} deficiency, hypothyroidism, and uremia should be ruled out in the process of evaluating diabetic peripheral neuropathy (Boulton et al, 2005).

Diabetic autonomic neuropathy also causes significant morbidity and even mortality in patients with diabetes. Neurological dysfunction may occur in most organ systems and can by manifest by gastroparesis, constipation, diarrhea, anhidrosis, bladder dysfunction, erectile dysfunction, exercise intolerance, resting tachycardia, silent ischemia, and even sudden cardiac death (Boulton et al, 2005). Cardiovascular autonomic dysfunction is associated with increased risk of silent myocardial ischemia and mortality (Maser et al, 2003).

There is no specific treatment of diabetic neuropathy, although many drugs are available to treat its symptoms. The primary goal of therapy is to
Control symptoms and prevent worsening of neuropathy through improved glycemic control. Some studies have suggested that control of hyperglycemia and avoidance of glycemic excursions may improve symptoms of peripheral neuropathy. Amitriptyline, imipramine, paroxetine, citalopram, gabapentin, pregablin, carbamazepine, topiramate, duloxetine, tramadol, and oxycodone have all been used to treat painful symptoms, but only duloxetine and pregablin possess official indications for the treatment of painful peripheral diabetic neuropathy (Boulton et al, 2005). Treatment with some of these medications may be limited by side effects of the medication, and no single drug is universally effective. Treatment of autonomic neuropathy is targeted toward the organ system that is affected, but also includes optimization of glycemic control.

Macrovascular complications of diabetes

The macrovascular complications of diabetes affecting major large arteries include cardiovascular diseases (CVD), cerebrovascular disease and peripheral artery diseases.

Cerebrovascular disease

Stroke is the third leading cause of death in the United States, after CVD and cancer (Centers for disease control and prevention). Diabetes is an independent risk factor across all ages (Abbott et al, 2003) for stroke; the risk in people with diabetes is up to 2- to 4-fold greater, more so in white people and women (Centers for disease control and prevention; Rohr et al, 1996; Folsom et al, 1999; Ohira et al, 2006). Diabetes is also a risk factor for sudden and eventual death from stroke (Haheim et al, 1995; Tuomilehto et al, 1996), and people who have diabetes and who have a stroke have more severe neurological deficits and disability (Parsons et al, 2002; Megherbi et al, 2003; Ribo et al, 2005), a poorer long-term prognosis, (Sprafka et al, 1994) and a higher incidence of stroke recurrence than people without diabetes (Elneihoum et al, 1998).
As in CVD, the presence of diabetes adversely affects the cerebrovascular circulation by increasing the risk of intracranial and extracranial (eg, carotid artery) atherosclerosis (Fabris et al, 1994; Friedlander et al, 2000). People with diabetes have an increased incidence of traditional risk factors for stroke, including hypertension, dyslipidemia, heart failure, and atrial fibrillation (Stegmayr and Asplund, 1995). However, after these factors are controlled for, diabetes remains a strong predictor for stroke, suggesting that the presence of diabetes carries an independent risk for stroke apart from the increased presence of traditional risk factors (Tuomilehto et al, 1996).

As in other diabetes-related complications, hyperglycemia appears to be a significant factor in stroke. Hyperglycemia is a significant predictor of fatal and nonfatal stroke (Niskanen et al, 1998) and death from stroke (Sasaki et al, 1995). Hyperinsulinemia (ie, elevated blood insulin levels) also appears to be a risk factor for stroke (Shinozaki et al, 1996; Zunker et al, 1996), although this relationship is still unclear (Davis et al, 1999). The presence of DR, proteinuria, microalbuminuria, and hyperuricemia (ie, elevated blood uric acid levels) are other diabetes-related factors associated with an increased risk for stroke (Sasaki et al, 1995; Lehto et al, 1998; Guerrero-Romero et al, 1999). Finally, elevated blood levels of chronic inflammatory markers are associated with an increased risk for stroke in people with diabetes (Engstrom et al, 2003).

Peripheral Artery Disease:

Currently in the United States, more than 3.5 million people (African-American > white > Hispanic people) with diabetes have peripheral artery disease (PAD) (ADA, 2003). Peripheral artery disease is characterized by occlusion of the lower-extremity arteries (Kullo et al, 2003), which can cause intermittent claudication and pain, especially upon exercise and activity (Schainfeld, 2001), and which can result in functional impairments (Vogt et al, 1994) and disability (McDermott et al, 2004). Physical therapists frequently encounter people with diabetes-related PAD because of these functional impairments.
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Impairments and because of common events of more severe PAD: foot ulceration and lower-extremity amputation (Adler et al, 1999). Because people with diabetes are 15 times more likely to have lower-extremity amputation than people without diabetes (Dickinson et al, 2002), physical therapists frequently treat people with diabetes-related amputations. As the incidence of diabetes increases, physical therapists will more frequently see, treat, and prescribe exercise for these people. An elevated awareness of PAD is needed among physical therapists because death in people with diabetes and PN is frequently attributed to CVD (Forsblom et al, 1998). Moreover, lower-extremity amputation is more common in people with diabetes and PAD than in people without diabetes but with PAD (Jude et al, 2001); these data suggest that physical therapists should carefully assess lower-extremity blood flow (ie, peripheral pulses) and skin integrity for all patients with diabetes, especially those with known PAD.

Peripheral artery disease, like the aforementioned vascular diseases, is related to the duration and severity of diabetes (Jude et al, 2001, Al-Delaimy et al, 2004). Hyperglycemia, specifically, glycation hemoglobin, has been shown to be an independent risk factor for PAD (Selvin et al, 2004). In addition to diabetes, other risk factors for PAD include hypertension, tobacco use, obesity (ie, waist-to-hip ratio), elevated serum fibrinogen levels, dyslipidemia, a history of CVD, and physical inactivity (American heart association, Wattanakit et al, 2005).

Cardiovascular Diseases

Diabetes increases the risk that an individual will develop cardiovascular disease. CVD is the primary cause of death in people with either type 1 or type 2 diabetes (Laing et al, 2003; Paterson et al, 2007). People with diabetes have a 4-fold-greater risk for having a CVD event than people without diabetes after controlling for traditional risk factors for CVD, such as age, obesity, tobacco use, dyslipidemia, and hypertension (Bonora et al, 2002; Buyken et al, 2007). In fact, CVD accounts for the greatest component of health care expenditures in people...
with diabetes (Hogan et al, 2003; Paterson et al, 2007). Among macrovascular diabetes complications, coronary heart disease has been associated with diabetes in numerous studies beginning with the Framingham study (Kannel and McGee, 1979a). More recent studies have shown that the risk of myocardial infarction (MI) in people with diabetes is equivalent to the risk in nondiabetic patients with a history of previous MI (Haffner et al, 1998). Further, people with diabetes have a poorer long-term prognosis after an MI, including an increased risk for congestive heart failure and death (Malmberg et al, 2000). Recent estimates indicate that 80% of people with diabetes worldwide will die from CVD (Turner et al, 1996). These discoveries have lead to new recommendations by the ADA and American Heart Association that diabetes be considered a coronary artery disease risk equivalent rather than a risk factor (Buse et al, 2007).

People with diabetes (particularly type 2 DM) frequently have many traditional risk factors for CVD, including central obesity, dyslipidemia (ie, elevated serum triglyceride, LDL, and free fatty acid levels and low high-density lipoprotein levels), and hypertension (Reusch and Draznin, 2007). The combination of central adiposity, dyslipidemia, hyperglycemia, and hypertension in the general population is termed “metabolic syndrome” (NCEP, 2001). These factors, along with the independent risk factor of diabetes, can act both independently and cumulatively over time to significantly increase risk for CVD. The combination of hyperglycemia, insulin resistance, dyslipidemia, hypertension, and chronic inflammation can injure the vascular endothelium, leading to macrovasculopathy and CVD in people with type 2 DM (Beckman et al, 2002).

Accumulating data from experimental, pathological, epidemiological, and clinical studies have shown that diabetes mellitus results in functional and structural changes in large arteries (Fang et al, 2004). Structural changes result mainly from glycation of wall components. Functional changes originate in endothelial dysfunction (Nicolaiades and Jones, 2002).
Functional changes in diabetes:

Functional changes do occur in diabetic patients independent of hypertension, CAD, or any other known cardiac disease. These alterations can involve either the diastolic or systolic functions of the heart or both. The inter-relationship between hypertension, diabetes and heart failure is given in Fig. 3.5

Diastolic dysfunction in diabetics

Changes in diastolic function have been widely reported in diabetic patients without evidence of heart disease caused by other factors (Raev 1994; Lind et al, 1996). Dent et al, (2001) suggested that the presence of diastolic dysfunction in diabetic hearts may relate to uncoupling of the contractile apparatus (which drives early relaxation), without concomitant increases in chamber stiffness (which produces more late diastolic changes).
The LV ejection time is often reduced, and the length of the pre-ejection period and the ratio of pre-ejection period to LV ejection time are often increased (Fang et al, 2004). Diastolic inflow patterns are frequently abnormal, reflecting underlying abnormalities in relaxation and/or reduced myocardial compliance. Studies suggest that patients with Type-2 diabetes are more likely to have diastolic dysfunction than Type-1 diabetics. In 20 Type-1 and 10 Type-2 diabetic patients, ventricular filling was impaired more significantly in the latter group of patients (Astori et al, 1997).

**Systolic dysfunction in diabetics**

Animal studies have shown diabetes to also be associated with systolic dysfunction (Hoit et al, 1999, Joffe et al, 1999, Wold et al, 2001). Similar findings were reported in intact animals; heart rate, systolic blood pressure, and fractional shortening were significantly reduced in diabetic animals compared with control animals (Hayashi et al, 2001). Several epidemiological and clinical studies have shown diabetes to be associated with systolic dysfunction. There is a significant association of idiopathic dilated cardiomyopathy with diabetes mellitus (Coughlin et al, 1994). Many diabetics may have normal LV systolic function at rest with abnormal systolic function only during exercise or dobutamine stress. Although many studies have shown that diabetic patients have abnormal diastolic dysfunction but preserved systolic function, this may well be due to techniques used for systolic function evaluation. Techniques utilized for systolic function evaluation are less sensitive than those used for assessment of diastolic dysfunction. Diabetic patients who did not have overt heart disease demonstrated evidences of LV systolic dysfunction and increased myocardial reflectivity. Although the changes observed were similar to those that were caused by LVH, they were independent and incremental to the effects of LVH (Fang et al, 2003).
A number of studies have shown structural changes in diabetic hearts in the absence of hypertension, CAD, and valvular heart diseases. The conspicuous histopathological findings are fibrosis, which may be perivascular, interstitial, or both. As the disease progresses, there is increased myocyte loss and replacement fibrosis. Mizushige et al, (2000) indicated that LV fibrosis is seen in the early stages of type 2 diabetes. In another study using modern stereological techniques to quantify changes in the morphology accompanying streptozotocin-induced diabetes, the results showed that the time to peak tension and relaxation of papillary muscles was prolonged, the heart weight to body weight ratio was increased, and the volume of extracellular components was increased 3-fold in diabetic rats. At the same time, this study also demonstrated that the volume, surface density, and total surface area of capillaries as well as volume fraction of myocyte mitochondria were reduced, and oxygen diffusion distance to myocyte mitochondria was increased in the diabetic animals (Warley et al, 1995). Other studies have identified ultrastructural changes in diabetic hearts (Bhimji et al, 1986, Eto et al, 1987, Seager et al, 1984). More recently, the 2-D Haar wavelet decomposition method has been used as a tool to identify textural changes in diabetic rats, which showed increased texture energy compared with controls. These changes were detected before development of echocardiographic structural changes (Kerut et al, 2000).

In a study by Nunoda et al, (1985), the mean diameter of RV myocardial cell has been shown to be significantly larger and the percentage of interstitial fibrosis in diabetics is significantly greater than controls. Fibrosis in diabetic hearts has been quantified using new techniques such as assessment of ultrasonic backscatter, which is directly related to collagen content. Collagen is a primary determinant of echocardiographic scattering in myocardial tissues and there is a linear relationship between collagen deposition and backscatter magnitude (Picano et al, 1990). Positive associations have been found between heart weight and total fibrosis with the semiquantitative scale in patients with
diabetes alone and with both hypertension and diabetes (van Hoven and Factor, 1990). The increased myocardial tissue reflectivity in diabetics may represent an early marker of diabetic cardiomyopathy (Fang et al, 2004).

**Correlation of structural changes to LV dysfunction**

The functional abnormality in diabetic myocardium is considered to be associated with myocardial structural changes, and indeed, these structural changes might play a role in progressive deterioration of cardiac hemodynamics. Experimental data strongly support the connection between structural changes and heart muscle dysfunction in diabetes. After 2 months of streptozotocin-induced diabetes, *in vitro* study of myocytes showed a 30% increase in time to peak shortening, which corresponded to a significant reduction in resting sarcomere length and a change in the microtubular cytoskeleton (Howarth et al, 2002), suggesting that myocardial structural change may be the basis for cardiac dysfunction. Another study showed that rats with streptozotocin-induced non-insulin-dependent diabetes had prolonged isovolumic relaxation time, elevated LV end-diastolic pressure, and increased chamber stiffness; these functional changes were accompanied with increased LV mass (Joffe et al, 1999). A similar experimental study in animals also showed that functional changes (e.g., reduced LV compliance) after 1 yr of diabetes were associated with increased interstitial connective tissue (Regan et al, 1981). A clear relationship between functional and structural changes is indicated by a study showing that diabetic rats exhibited prolonged deceleration time and low peak velocity of early diastolic transmitral flow, which is associated with extracellular fibrosis in LV myocytes, and higher ratio of collagen area/visual field of LV wall and ratio of collagen content/dry heart weight compared with control rats (Mizushige et al, 2000).

Abnormal systolic and diastolic function is present in many diabetic patients, particularly in the presence of hypertension and myocardial hypertrophy and fibrosis are commonly present in these patients. A correlation between histological and clinical features in a study of endomyocardial biopsies in 16
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diabetics, with myocardial changes more pronounced in the symptomatic group and less so in asymptomatic patients has been reported (Das et al, 1987). This suggests that myocardial dysfunction in diabetics might be secondary to accumulation of glycoprotein within the interstitium together with mild interstitial fibrosis. There is increased myocardial fibrosis in diabetics, particularly in those with cardiomegaly, suggesting that changes in cardiac interstitial collagen might increase myocardial wall stiffness which is usually associated with functional changes (Zoneraich, 1988).

Systolic dysfunction may be more dependent on the degree of myocyte loss and myocyte injury, which may impair the ability of the myocardium to develop force, and thereby resulting into reduced contractility, decreased pump function, and ejection fraction. Abnormal LV systolic function in diabetic patients may be transient, reversible, and related to changes in diabetic control within a certain range and need not indicate structural myocardial disease (Harrower et al, 1986). The development of systolic dysfunction during exercise in some patients may reflect loss of contractile reserve related to limited myocyte loss, insufficient to influence resting function.

Diastolic dysfunction is a result of both accumulation of collagen and myocyte injury in the heart and so there is greater prevalence of diastolic dysfunction in type 2 diabetes. In type 2 diabetes, aging-related increments in cardiac collagen are likely additive, although less satisfactory glycemic control may be an important factor as well. Myocyte injury does affect diastolic function and diabetes mellitus can produce a stiff myocardium before the development of myocardial fibrosis due to formation of advanced glycosylation end products (Norton et al, 1996). At an early stage of diabetes, the alterations in myocardial structure are usually small, and these may be mainly related to myocyte injury, which may be reversible or partially reversible (Fang et al, 2004). As diabetes progresses, accumulation of collagen becomes obvious and may play a major role in the development of diastolic dysfunction. These chronic alterations are
believed to result from repeated acute cardiac responses to suddenly increased glucose levels at the earlier stage of diabetes (Fang et al, 2004).

**Pathophysiology of diabetic cardiomyopathy**

Several factors like metabolic disturbances, myocardial fibrosis, small vessel disease, autonomic dysfunction and insulin resistance have been implicated as putative mechanisms leading to diabetic cardiomyopathy. There are three major stages of diabetic cardiomyopathy (Table 3.2).

**Table 3.2: Three stages of diabetic cardiomyopathy** (Fang et al, 2004)

<table>
<thead>
<tr>
<th>Stages</th>
<th>Characteristics</th>
<th>Functional features</th>
<th>Structural features</th>
<th>Study methods</th>
</tr>
</thead>
<tbody>
<tr>
<td>Early stage</td>
<td>Depletion of GLUT4</td>
<td>No overt functional abnormalities or possible overt diastolic dysfunction but normal ejection fraction</td>
<td>Normal LV size, wall thickness, and mass</td>
<td>Sensitive methods such as strain, strain rate, and myocardial tissue velocity</td>
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<td></td>
<td>Increased FFA</td>
<td></td>
<td></td>
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<td></td>
<td>Carnitine deficiency Ca$^{2+}$ homeostasis</td>
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<td></td>
<td>changes Insulin resistance</td>
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<tr>
<td>Middle stage</td>
<td>Apoptosis and necrosis</td>
<td>Abnormal diastolic dysfunction and normal or slightly decreased ejection fraction</td>
<td>Slightly increased LV mass, wall thickness, or size</td>
<td>Conventional echocardiography or sensitive methods such as strain, strain rate, and myocardial tissue velocity</td>
</tr>
<tr>
<td></td>
<td>Increased AT II</td>
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<tr>
<td></td>
<td>Reduced IGF-I</td>
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<td></td>
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<tr>
<td></td>
<td>Increased TGF-β1 Mild CAN</td>
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<table>
<thead>
<tr>
<th>Late stage</th>
<th>Microvascular changes</th>
<th>Abnormal diastolic dysfunction</th>
<th>Significantly increased LV size, wall thickness, and mass</th>
<th>Conventional echocardiography</th>
</tr>
</thead>
<tbody>
<tr>
<td>Hypertension</td>
<td>CAD Severe</td>
<td>and ejection fraction</td>
<td></td>
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<tr>
<td>CAN</td>
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*Metabolic disturbances*

Normally, the heart utilizes free fatty acids (FFA) as its primary energy source during aerobic perfusion at normal workloads and increasingly relies on glycolysis and pyruvate oxidation during periods of ischaemia or increased work (Mishra and Rath, 2005).

- **Alterations in substrate supply and utilization**

Hyperglycaemia triggers metabolic changes in diabetes directly. Diabetic hearts have primary defect in the stimulation of glycolysis and glucose oxidation (Mokuda et al, 1990). Altered substrate supply and utilisation by cardiac myocytes could be the primary injury in the pathogenesis of diabetic cardiomyopathy (Rodrigues et al, 1998). A major restriction to glucose utilization in the diabetic heart is the slow rate of glucose transport across the sarcolemmal membrane into the myocardium, due to cellular depletion of glucose transporters (GLUTS) 1 and 4 (Russell et al, 1998). Another mechanism of reduced glucose oxidation is via the inhibitory effect of fatty acid oxidation on pyruvate dehydrogenase complex due to high circulating free fatty acid (FFA) (Liedtke et al, 1988) and the net effect is reduced availability of ATP.

- **Free fatty acid (FFA) metabolism**

Elevated FFA levels are believed to be one of the major contributing factors in the pathogenesis of diabetes. Independent of the effects of hyperlipidaemia on coronary artery endothelial function, the increase in, and dependence of diabetic myocardium on fatty acid supply, results in several major
cellular metabolic perturbations (Hayat et al, 2004). FFAs enhance peripheral insulin resistance and trigger cell death. Disturbances of FFA metabolism may be an important contributor to abnormal myocardial function in diabetes. These changes are characterized by elevation of circulating FFAs caused by enhanced adipose tissue lipolysis, as well as high tissue FFAs caused by hydrolysis of augmented myocardial triglyceride stores. Moreover, in addition to the FFA-induced inhibition of glucose oxidation (which may contribute to the above effects by limiting the entry of glucose into the cell), high circulating and cellular FFA levels may result in abnormally high oxygen requirements during FFA metabolism and the intracellular accumulation of potentially toxic intermediates of FFA, all of which lead to impaired myocardial performance and severe morphological changes (Rodrigues et al, 1998; Nakayama et al, 2001). Abnormalities in FFA metabolism have been demonstrated in idiopathic dilated cardiomyopathy in which the rate of FFA uptake by myocardium is inversely proportional to the severity of the myocardial dysfunction (Yazaki et al, 1999). It is possible that similar defects contribute to the development of diabetic cardiomyopathy. There is increased \( \beta \)-oxidation and mitochondrial accumulation of long-chain acyl carnitines, leading to uncoupling of oxidative phosphorylation (Stanley et al, 1997). Enhanced fatty acid oxidation decreases glucose and pyruvate utilization by inhibiting pyruvate dehydrogenase. Pyruvate oxidation is reduced further by pdk4 and activated by PPAR (peroxisome-proliferator-activated receptor). The net result is an excess of glycolytic intermediates and increased synthesis of ceramide leading to apoptosis, which can be prevented by the PPAR-\( \alpha \) and -\( \gamma \) agonist troglitazone (Zhou et al, 2000). Thus impaired glycolysis, pyruvate oxidation, lactate uptake and a greater dependence on fatty acids as a source of acetyl CoA leads to a perturbation of myocardial bioenergetics and contraction/relaxation coupling (Rodrigues et al, 1998). The FFA-induced impairment of glucose oxidation may be a major factor in the development of diabetic cardiomyopathy, and would explain why cardiac function tends to improve upon metabolic improvement. Furthermore, the availability of carnitine,
an essential substance for myocardial FFA metabolism, is usually reduced in diabetes.

- Abnormality in calcium homoeostasis
  
  Oxidative stress caused by toxic molecules may play a critical role in subcellular remodeling and abnormalities of calcium handling that lead to subsequent diabetic cardiomyopathy. A fall in calcium sensitivity, shift in cardiac myosin heavy chain (Takeda et al, 1988), reduction of sarcoplasmic reticulum Ca\(^{2+}\)-ATPase and decreased sarcoplasmic reticulum calcium (SERCA2a) pump protein may all contribute to impaired LV function. The important contributors to abnormal myocardial carbohydrate and lipid metabolism in diabetes are alterations in regulatory proteins and contractile proteins, sarcoplasmic (endoplasmic) reticulum Ca\(^{2+}\)-ATPase and Na\(^{+}\)-Ca\(^{2+}\) exchanger function. These changes likely result from accumulation of toxic molecules such as long-chain acylcarnitines, free radicals, and abnormal membrane lipid content. The consequences of these changes include alterations to the calcium sensitivity of regulatory proteins involved in the regulation of the cardiac actomyosin system, possibly due to phosphorylation of sarcomeric protein troponin I (Malhotra and Sanghi, 1997). Other changes which have been demonstrated to contribute to the development of myofibrillar remodeling in the diabetic heart are alterations in the expression of myosin isoenzymes and regulatory proteins as well as myosin phosphorylation (Dhalla et al, 1998).

- Advanced glycation end products accumulation
  
  The hyperglycaemia leads to non-enzymatic glycation of macromolecules. The sugars linked to macromolecules are condensed into large heterocyclic derivatives by complex reaction and they are labeled as advanced glycation end products (AGEs). These AGEs accumulate in tissues and are implicated in morphological changes that occur in the diabetic heart. Inelasticity of the vessel wall occurs due to accumulation of AGE-modified extra-cellular matrix and this

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results in interference with the myocardial function. It is reported that in diabetics, prolongation of isovolumic relaxation time, as assessed by Doppler echography, correlated with serum levels of AGEs after adjustment for age, diabetes duration, renal function, blood pressure and autonomic function parameters (Berg et al, 1999).

> **Activation of protein kinase C (PKC)**

Increased activation of the DAG (diacylglycerol)-activated PKC signal transduction pathway has been identified in vascular tissues from diabetic animals and in vascular cells exposed to elevated glucose (Way et al, 2001). Hyperglycaemia induced up-regulation of PKC by diacylglycerol (DAG) has been proposed as a mechanism in the development of vascular complications in diabetes (Xia et al, 1994). This has been shown to induce many of the changes in diabetic cardiomyopathy which include a reduction in tissue blood flow, enhanced extracellular matrix deposition, capillary basement membrane thickening and increased vascular permeability with alterations in neovascularization. PKC interferes with the contraction protein Troponin-T, Troponin-I, Troponin-tropomyosin complex (Xia et al, 1994). Increased PKC activity influences nuclear gene transcription by way of the mitogen-activated protein kinase (MAPK) cascade to induce the immediate early gene programme with subsequent stimulation of late genes that increase production of ACE, α-MHC and skeletal α-actin (Koj, 1996). ACE, in particular, may account for development of abnormalities that contribute to the development of diabetic cardiomyopathy (Giles and Sander, 2004).

**Myocardial fibrosis**

Myocardial fibrosis and myocyte hypertrophy are the most frequently proposed mechanisms to explain cardiac changes in diabetic cardiomyopathy. Several studies have shown that in diabetes causes defects in cellular calcium transport (Ganguly et al, 1983), defects in myocardial contractile proteins
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(Giacomelli and Wiener, 1979), and an increase in collagen formation (Regan et al, 1981), which result in anatomic and physiological changes in the myocardium.

- **Myocyte cell death**

  Myocyte cell death may be caused by apoptosis or necrosis or both. Apoptosis is programmed cell death involving genetically controlled process that removes unwanted or damaged cells, whereas myocyte necrosis refers to myocyte cell death due to biochemical damage. Identification of double-strand DNA cleavage with single base or longer 3' overhangs is the measure for evaluation of apoptosis where as myocyte necrosis can be assessed by detection of DNA damage with blunt end fragments (Guerra et al, 1999). Apoptosis does not cause scar formation or significant interstitial collagen accumulation (Haunstetter and Izumo, 1998), with nuclear fragmentation and cell shrinkage being replaced by the surrounding cells (Gerschenson and Rotello, 1992; Anversa and Kajstura, 1998). Conversely, myocyte necrosis results in widening of the extracellular compartments among myocytes and increased deposition of collagen in a diffuse or scattered manner (Anversa et al, 1998; Li et al 1999), resulting from both replacement fibrosis due to myocyte necrosis and connective tissue cell proliferation (Weber and Brilla, 1991). In diabetic heart disease, both apoptosis and necrosis are involved.

- **Process of myocardial fibrosis**

  Hyperglycemia also results in the production of reactive oxygen and nitrogen species, which increases oxidative stress and causes abnormal gene expression, alters signal transduction, and activates the pathways leading to programmed myocardial cell death or apoptosis. This process is associated with the glycosylation of p53 resulting in an increment in angiotensin II synthesis which leads to p53 phosphorylation, increased Bax expression, and also to myocyte apoptosis. Collagen accumulation in the diabetic myocardium may be...
due in part to impaired collagen degradation resulting from glycosylation of the lysine residues on collagen (Fang et al, 2004).

**Small vessel disease**

- **Structural abnormalities of vessels**

  Microangiopathy involving arterioles, capillaries and venules and hyaline arteriosclerosis are the characteristic morphological changes of small vessels seen in diabetic myocardium. There occurs basement membrane thickening, arteriolar thickening, capillary microaneurysms, and reduced capillary density, which may be the results of periarterial fibrosis and focal subendothelial proliferation and fibrosis, possibly due to abnormal permeability of diabetic capillaries. Examination of the myocardium in diabetic animals shows that the volume of extracellular components is increased 3-fold and the volume of capillaries is reduced. The surface density and total surface area of capillaries was reduced, and oxygen diffusion distance to myocyte mitochondria increased (Warley et al, 1995). The combination of cell loss and slowly decreasing contractility resulting from the reactive hypertrophy due to a compensatory response to myocardial necrosis culminates in a cardiomyopathy (Sonnenblick et al, 1985). Moreover, a study by Kawaguchi et al, (1997) suggested that alterations in capillaries due to diabetes may lead to myocardial cell injury and interstitial fibrosis and, ultimately, to diabetic cardiomyopathy. All of these features have been described in diabetic hearts, suggesting a similar disease process in the cardiac microcirculation and the presence of diffuse myocardial small vessel disease in diabetes.

- **Functional abnormalities of vessels**

  In both dilated cardiomyopathy and diabetes mellitus, abnormalities in coronary small vessel function occurs; maximal pharmacological coronary flow reserve is reduced, and endothelium-dependent coronary vasodilation is impaired (Nitenberg et al, 1985; 1993; Treasure et al, 1990; Strauer et al, 1997). Metabolic...
substrates or products such as adenosine play an important role in regulating microvascular tone to maintain constant coronary blood flow for a given level of metabolic demand. There was a reduction in increase of coronary blood flow induced either by pacing or inotropic agents, (to increase myocardial oxygen demand) in spontaneously diabetic rats compared with non-diabetic rats (Durante et al, 1989). Reduced coronary flow reserve may lower the threshold for myocardial ischemia, particularly when coronary stenoses are present. Repeated episodes of myocardial ischemia, resulting from both structural and functional abnormalities in small vessels during increased myocardial demand or from microvascular spasm due to changes in calcium distribution, results in diabetic cardiomyopathy. Such a process would lead to focal cell loss due to microvascular spasm and reperfusion injury, with the subsequent development of focal fibrosis and reactive hypertrophy in response to the myocardial necrosis.

In type 1 young adult diabetic patients with no or minimal microvascular complications and without any evidence of coronary heart disease, 29% reduction of myocardial blood flow and significant increase in total coronary resistance during hyperemia and consequent impairment of coronary flow reserve have been reported (Pitkanen et al, 1998). Another study confirmed reduction of flow reserve, which was ascribed to a significantly higher resting myocardial blood flow (Meyer and Schwaiger, 1997). A number of other studies report that there is association of diabetic cardiomyopathy with stenosis of small coronary arteries (Sutherland et al, 1989).

Endothelial dysfunction

Commonly, in coronary vasculature of the diabetic patients, endothelial dysfunction occurs leading to abnormal control of blood flow and there is impairment in endothelium-dependent responses of both small and large vessels in diabetic rats (Mayhan et al, 1991; Taylor et al, 1995). Diabetic patients with an otherwise low likelihood of atherosclerosis also have impaired endothelium-dependent dilatation in the epicardial coronary arteries (Nitenberg et al, 1993).
and in forearm arteries (Johnstone et al, 1993). Several mechanisms have been implicated in the abnormal endothelium-dependent vasodilation in diabetes. There is an increase in protein kinase C activity in hyperglycemia and may also play a role in development of endothelial dysfunction in diabetes (Tesfamariam et al, 1991). Protein kinase C activation is associated with abnormal retinal and renal hemodynamics in diabetic animals, and overexpression of the β-isofrom in myocardium is associated with cardiac hypertrophy and failure (Koya and King, 1998), implying that this may play a role in the development of diabetic cardiomyopathy by affecting vascular cells. The nitric oxide activity is reduced partly due to accumulation of glycosylation end products (Bucala et al, 1991) and partly due to increased oxidative stress (Hattori et al, 1991; Pieper et al, 1993; Rosen et al, 1995; Joffe et al, 1999). Tesfamariam et al, (1989) have reported that synthesis of vasoconstrictor prostanoids by the endothelium increases, and hence the vasoconstriction is enhanced in diabetic subjects.

Cardiac autonomic neuropathy (CAN)

Diabetic cardiomyopathy is also associated with CAN. There is change in sympathetic innervation in the diabetic heart leading to alterations of catecholamine levels and adrenergic receptors in the myocardium. There is an significantly increased cardiac norepinephrine content and β-adrenergic receptor density in short-term diabetics and these changes precede both the development of cardiac hypertrophy and the enhanced adenylyl cyclase activity. However, as the diabetic state develops, cardiac norepinephrine content, β-adrenergic receptor density, and adenylyl cyclase activity returned to control levels (Uekita et al, 1997). The reason for the increased norepinephrine in the early stages of diabetes can attributed to increased bradykinin-induced release of norepinephrine, which has been shown to be four times greater in diabetic than in normal preparations (Pietrzyk et al, 2000), as well as the acute effects of high glucose levels on sympathetic activity (Manzella et al, 2001). The alterations in cardiac sympathetic activity during the early stage of diabetes enhance the cardiac β-adrenergic system, which may induce toxic effects on the heart.
3.3 DIABETES MELLITUS AND OXIDATIVE STRESS

Glucose in chronic excess causes toxic effects on structure and function of organs, including the pancreatic islet. Multiple biochemical pathways and mechanisms of action for glucose toxicity have been suggested. These include glucose autoxidation, protein kinase C activation, methylglyoxal formation and glycation, hexosamine metabolism, sorbitol formation, and oxidative phosphorylation. There are many potential mechanisms whereby excess glucose metabolites traveling along these pathways might cause beta cell damage. However, all these pathways have in common the formation of reactive oxygen species that, in excess and over time, cause chronic oxidative stress, which in turn causes defective insulin gene expression and insulin secretion as well as increased apoptosis. Diabetes mellitus is a disease characterized by hyperglycemia and is caused by absolute or relative insulin deficiency, sometimes associated with insulin resistance. It has multiple etiologies and segregates into two major forms. Type 1 diabetes is an autoimmune disease in which the patient's own immune system reacts against islet antigens and destroys the beta cell. Type 2 diabetes is a polygenic syndrome with multiple etiologies rather than a single specific disease. As the hyperglycemia of diabetes becomes chronic, the sugar that normally serves as substrate, fuel, and signal takes on the darker role of toxin. Chronic hyperglycemia is the proximate cause of retinopathy, kidney failure, neuropathies and macrovascular disease in diabetes. The beta cell in type 2 diabetes is also adversely affected by chronic hyperglycemia and in this sense, is also a target for secondary complications. As hyperglycemia worsens, the beta cell steadily undergoes deterioration, secretes less and less insulin, and becomes a participant in a downward spiral of loss of function. This relentless deterioration in cell function caused by constant exposure to supraphysiologic concentrations of glucose is termed glucose toxicity.
Mechanisms of Hyperglycemia-induced Oxidative Stress

In physiologic concentrations, endogenous reactive oxygen species (ROS) help to maintain homeostasis. However, when ROS accumulate in excess for prolonged periods of time, they cause chronic oxidative stress and adverse effects. This is particularly relevant and dangerous for the islet, which is among those tissues that have the lowest levels of intrinsic antioxidant defenses. Multiple biochemical pathways and mechanisms of action have been implicated in the deleterious effects of chronic hyperglycemia and oxidative stress on the function of vascular, retinal and renal tissues. Considerably less work has been performed using islet tissue. At least six pathways are emphasized in the literature as being major contributors of ROS.

![Diagram of glucose metabolism](image-url)

**Fig 3.6:** Under physiologic conditions, glucose primarily undergoes glycolysis and oxidative phosphorylation (6). Under pathologic conditions of hyperglycemia, excessive glucose levels can swamp the glycolytic process and inhibit glyceraldehyde catabolism which cause glucose, fructose-1:6-bis-P, and glyceraldehyde-3-P to be shunted to other pathways: (1) enolization and α-ketoaldehyde formation; (2) PKC activation; (3) dicarbonyl formation and glycation; (4) sorbitol metabolism; and (5) hexosamine metabolism (Robertson, 2004).
Glyceraldehyde Autoxidation

Glyceraldehyde 3-phosphate is a phosphorylation product formed from glucose during anaerobic glycolysis. The partner product, dihydroxyacetone phosphate, also contributes to intracellular glyceraldehyde concentrations via enzymatic conversion by triose-phosphate isomerase. Thereafter, glyceraldehyde 3-phosphate is oxidized by glyceraldehyde-phosphate dehydrogenase (GAPDH). Continuance of glycolysis yields pyruvate, which enters the mitochondria where it is oxidized to acetyl-CoA and the processes of the tricarboxylic acid cycle and oxidative phosphorylation begin. One alternative to this classic pathway of glucose metabolism is the less familiar route of α-glyceraldehyde autoxidation. The potential relevance of this pathway to diabetes mellitus was pointed out by Wolff and Dean (Wolff and Dean, 1987), who emphasized that autoxidation of α-hydroxyaldehydes generates hydrogen peroxide (H₂O₂) and ketoaldehydes. In the presence of redox active metals, H₂O₂ can form the highly toxic hydroxyl radical. This pathway, therefore, forms two potentially toxic substances, α-ketoaldehydes, which contribute to glycosylation-related protein chromophore development and the hydroxyl radical, a reactive oxygen species that can cause mutagenic alterations in DNA. Although glyceraldehyde is characteristically thought of as an insulin secretagogue, when present in excess it may also inhibit insulin secretion (Hellman et al, 1974). Long term exposure to high glucose concentrations decreases GAPDH activity in islets (Sakai et al, 2003), which favours excess glyceraldehyde accumulation. Exposure of endothelial cells to 30 mM glucose caused GAPDH inhibition (Du et al, 2003) through the mechanism of ROS-activated poly (ADP-ribosylation) of GAPDH by poly-(ADP-ribose) polymerase. This in turn was associated with intracellular advanced glycation end product (AGE) formation and activation of PKC, the hexosamine pathway, and NF-κB.

PKC Activation

Dihydroxyacetone can undergo reduction to glycerol 3-phosphate and acylation and thereby increase de novo synthesis of diacylglycerol, which
activates protein kinase C, of which there at least 11 isoforms (Fig. 3.6, pathway 2). Activation of PKC has many biochemical consequences that relate to microvascular disease in diabetes. PKC activation is associated with increases in TGF-β1, vascular endothelial growth factor, endothelin-1, NAD (P)H oxidase, NF-κB and ROS (Ishii et al, 1996; Brownlee, 2001).

Methylglyoxal, Glycation and Sorbitol pathway

Three reactive intracellular dicarbonyls (glyoxal, methylglyoxal, and 3-deoxyglucosone) form AGEs by reacting with amino groups on intracellular and extra cellular proteins (Thornalley et al, 1999) (Fig. 3.6, pathways 3 and 4). AGEs play important roles in the pathogenesis of secondary complications of diabetes, especially with regard to microvascular disease in the retina, nerves, and kidney and likely islets. When GAPDH-mediated catabolism of glyceraldehyde-3-P is impaired, such as in the presence of high glucose concentrations, accumulation of glyceraldehyde-3-P and dihydroxyacetone favors formation of methylglyoxal. Additionally, increased flux along the polyol pathway as a result of hyperglycemia results in aldose reductase-mediated NADPH-dependent reduction of glucose to form sorbitol. Oxidation of sorbitol by NAD⁺ increases the cytosolic NADH: NAD⁺ ratio, which tends to inhibit GAPDH activity. This can lead to increased levels of triose phosphates, methylglyoxal, and diacylglycerol. This chain of events is also associated with consumption of NAD⁺ by activated poly (ADPribose) polymerase, which can be activated by hyperglycemia via increased production of ROS and DNA strand breaks (Du et al, 2003). Above and beyond the damage that reactive dicarbonyls can cause through enhancement of glycation and the formation of AGEs, the Maillard reaction between carbohydrates and proteins also generates ROS (Wells-Knecht et al, 1995). Thus, hyperglycemia simultaneously enhances both glycative and oxidative stress, which together synergistically contributes to the development of diabetic complications.
Hexosamine Pathway

In states of excess intracellular glucose, fructose 6-phosphate via glutamine:fructose-6-phosphate aminotransferase (GFAT) can form glucosamine 6-phosphate and then UDP-N-acetylglucosamine, which supports proteoglycan synthesis and the formation of (O)-linked glycoproteins (Fig. 3.6, pathway 5). This pathway has been shown to be related to increases in transcription of TGF-β, TGF-β1, and PAI-1 and has been implicated in insulin resistance (Brownlee, 2001; Marshall et al, 1991). Glucosamine infusions in rodents and in humans have been associated with interference with glucose sensing by the beta cell and with insulin sensitivity (Monauni et al, 2000). Adenovirus-mediated over expression of GFAT was reported to impair glucose-stimulated insulin secretion and to reduce expression levels of the insulin, GLUT2, and glucokinase genes (Kaneto et al, 2001). The DNA binding activity of PDX-1, a critical transcription factor for these genes, was also markedly reduced. In these experiments glucosamine was found to increase hydrogen peroxide levels, and the antioxidant n-acetylcysteine prevented the adverse effects of GFAT over expression.

Oxidative Phosphorylation

High glucose concentrations increase the mitochondrial proton gradient as a result of overproduction of electron donors by the tricarboxylic acid cycle, which in turn increase production of mitochondrial superoxide (Du et al, 2000) (Fig. 3.6, pathway 6). In these experiments, inhibition by Mn-SOD or UCP-1 of hyperglycemia-induced overproduction of mitochondrial superoxide prevented the increases in polyol pathway flux, intracellular AGE formation, PKC activation, and hexosamine pathway activity in endothelial cells. High glucose concentrations were shown to increase mitochondrial superoxide production, proton leak, lower ATP levels, and impaired glucose-induced insulin secretion in islets from wild type but not from UCP-2-knock-out animals (Krauss et al, 2003), suggesting that superoxide-mediated activation of UCP-2 could play a role in
type 2 diabetes. It has also been reported that a 2 mM glyceraldehyde concentration in 24-h islet incubations increased ROS levels and inhibited insulin secretion, effects that were abrogated by n-acetylcysteine (Takahashi et al, 2004). However, in these studies neither inhibitors of mitochondrial oxidative phosphorylation nor adenovirus overexpression of Mn-SOD prevented the ability of glyceraldehyde to increase islet reactive oxygen species levels. 2 mM glyceraldehyde has been reported to increase intraislet glyceraldehyde concentrations to a level similar to that achieved with 20 mM glucose (Taniguchi et al, 2000). Thus, when the glycolytic pathway is swamped by glucose, it seems likely that both mitochondrial and non-mitochondrial pathways contribute ROS to the glucotoxic process that impairs beta cell function.

**Therapeutic targets for oxidative stress in diabetes**

Oxidative stress is increased in diabetes mellitus and may play an important role in the pathogenesis of the typical long-term complications of human diabetes, like neuropathy and microangiopathy. Protein glycation and glucose autoxidation can generate free radicals that can catalyze lipid peroxidation. Other potential mechanisms of oxidative stress include the reduction of anti-oxidant defense. There are several members of the antioxidant system. Use of anti-oxidants may improve glucose metabolism and protein glycation and can prevent the development of diabetic complications.
Preventive antioxidants (sequestration of transition metal ions that catalyze free radical reactions)
- Transferrin
- Ceruloplasm
- Albumin
- Ferritin

Enzymatic anti-oxidants (catalyze the reduction of oxidants)
- Superoxide dismutase
- Glutathione peroxidase
- Catalase
- Paraoxonase

Scavenging antioxidants (electron donors to radicals in which the anti-oxidant is sacrificed)
- Ascorbic acid
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- α-Tocopherol
- Thiols (glutathione)
- β-Carotene
- Uric acid
- Flavonoids
- Coenzyme Q (ubiquinone)

Synthetic anti-oxidants
- N-acetylcysteine
- Penicillamine
- Xantine oxidase inhibitors (e.g. allopurinol)
- Probucol
- Deferroxamine
- Butylated hydroxytoluene (a food additive)
3.4 DIABETES MELLITUS AND CATARACT

Cataract is considered a major cause of visual impairment in diabetic patients as the incidence and progression of cataract is elevated in patients with diabetes mellitus (Kahn et al, 1977; Harding et al, 1993). Several clinical studies have shown that cataract development occurs more frequently and at an earlier age in diabetic compared to non-diabetic patients (Pollreisz and Schmidt-Erfurth, 2010). It is one of the earliest secondary complications of diabetes mellitus. Since extracellular glucose diffuses into the lens uncontrolled by the hormone insulin, the lens is one of the body parts most affected in diabetes mellitus. The proteins of the lens are extremely long-lived, and there is virtually no protein turnover that provides great opportunities for posttranslational modification to occur (Kyselova et al, 2004).

The association between diabetes and cataract formation has been shown in clinical epidemiological and basic research studies. Due to increasing numbers of type 1 and type 2 diabetics worldwide, the incidence of diabetic cataracts steadily rises. Even though cataract surgery, the most common surgical ophthalmic procedure worldwide, is an effective cure, the elucidation of pathomechanisms to delay or prevent the development of cataract in diabetic patients remains a challenge. Furthermore, patients with diabetes mellitus have higher complication rates from cataract surgery (Stanga et al, 1999). Some studies have reported that cataract surgery may have adverse effects, including progression of retinopathy, vitreous hemorrhage, iris neovascularization and decrease or loss of vision (Aiello et al, 1983; Poliner et al, 1985; Jaffe et al, 1992).

Pathogenesis of Cataract

Three molecular mechanisms seem to be involved in the development of diabetic cataracts: non-enzymatic glycation of lens proteins, oxidative stress and activated polyol pathway (Javadi and Zarei-Ghanavati, 2008).
1. Non-enzymatic glycation of lens proteins

Under hyperglycemic conditions, part of the excess glucose reacts nonenzymatically with proteins or other tissue or blood constituents, thus increasing the physiological rate of nonenzymatic glycation (Fig. 3.8) (Brownlee, 1996). Chronic, irreversible abnormalities unaffected by normalization of blood glucose levels primarily involve long-lived molecules including extracellular matrix, eye lens crystallins, and chromosomal DNA. Due to their characteristic chemical properties, advanced products of nonenzymatic glycation play a critical role in the evolution of diabetic complications.

The formation of advanced glycation end products (AGEs) begins with the attachment of glucose carbonyl group to a free amino group of proteins or amino acids to form a labile Schiff base adduct as the first step of the complex Maillard process. Levels of the unstable Schiff base increase rapidly, and equilibrium is reached after several hours. Once formed, Schiff base adducts undergo a slow chemical rearrangement over a period of weeks to form a more stable, but still chemically reversible, Amadori product (Monnier et al, 1992). Finally, AGEs are formed as a rather heterogeneous mixture of protein-bound, nitrogen-and/or oxygen-containing heterocyclic compounds through a complex cascade of dehydration, condensation, fragmentation, oxidation, and cyclization reactions of the intermediate Amadori ketoamine. The AGEs are frequently pigmented or fluorescent, and — most importantly for diabetic complications — they participate in glucose-derived cross-link formation (Brownlee, 1996; Schinzel et al, 2001; Westwood & Thornalley, 1997).

Specific chemical characterization of AGE proteins has been difficult, as Amadori products can theoretically undergo a large number of potential rearrangements. Immunological and chemical evidence indicates that progressive accumulation of AGEs in diabetic eye lens contributes to accelerated cataractogenesis in hyperglycemic experimental animals and diabetic humans (Lyons et al, 1991; Nagaraj et al, 1991; Araki et al, 1992; Duhaiman, 1995; Shamsi et al, 2000).
Fig 3.8 Non-enzymatic reactions of excessive glucose in diabetic state

2. Oxidative stress

Diabetes mellitus was found to be inextricably connected with increased oxidative stress both in diabetic humans and hyperglycemic animals (Baynes, 1991; Cameron et al, 1995; Dai and McNeill, 1995; Kowluru and Kennedy, 2001). Among the number of mechanisms proposed as a pathogenic link between hyperglycemia and diabetic complications, oxidative stress is an equally tenable hypothesis as the Maillard advanced glycation hypothesis or the AR-mediated osmotic hypothesis. Irreversible AGEs were shown to be formed via a sequence of glycation and oxidation reactions (Kowluru et al, 1996; Wohaieb & Godin, 1987). Under physiological conditions, glucose, like other alpha-hydroxyaldehydes, can enolize and thereby reduce molecular oxygen, catalyzed by transition metals yielding reactive alpha-ketoaldehydes and oxidizing free radical intermediates. The ketoamine Amadori product undergoes autooxidation as well, contributing to the oxidative damage of proteins exposed to hyperglycemia (Baynes, 1991; Baynes and Thorpe, 1999).
Hyperglycemia would not only generate more reactive oxygen species (ROS) but also attenuate endogenous antioxidative mechanisms through glycation of scavenging enzymes and depletion of low molecular antioxidants, for example, glutathione. Shifts in redox balances due to derangement in energy metabolism of carbohydrates and lipids also contribute to the overt oxidative stress in diabetic individuals.

Reactive dicarbonyls, products of carbohydrate autooxidation, contribute to covalent attachment of monosaccharide to protein with high cross-linking potential. Indeed, glycation and oxidation are closely connected and the complex process if often referred to as glycooxidation (Baynes, 1991).

A hypothesis parallel to the aforementioned glucose autooxidation theory has been recently proposed, suggesting that the initial event leading to the oxidative stress in hyperglycemia would be the enhanced generation of ROS occurring at the mitochondrial level as a consequence of the increased intracellular glucose metabolism (Mario and Pugliese, 2001; Nishikawa et al, 2000a). Under these conditions, the increased proton gradient produced by the accelerated electron flow through the respiratory chain associated with excess glucose disposal is capable of generating ROS. The blockade of ROS production by manganese superoxide dismutase and by an inhibitor of pyruvate transport (Nishikawa et al, 2000b) indicated the role of superoxide anion radical ($O_2^-$) and pyruvate, the substrate of the tricarboxylic or Kreb's cycle (Nishikawa et al, 2000a). The mechanism by which the polyol pathway activity is increased at elevated $O_2^-$ concentrations under hyperglycemia may be linked to its ability to quench nitric oxide which inhibits aldose reductase (AR) by S-thiolation of a cysteine-298 residue located at the active site (Dixit et al, 2001; Chandra et al, 2002). However, at this posttranslational level, AR could equally well be activated via nitrosation of the sensitive cysteine-298 residue, depending on the nature of the NO donor (Dixit et al, 2001). In addition, nitric oxide and, more generally, oxidative stress, can also affect the transcription of the AR gene resulting in the up-regulation of the rate-limiting AR enzyme (Spycher et al, 1997; Seo et al,
2000), which is coupled with depletion of reduced glutathione leading to further enhancement of the oxidative stress (Giugliano et al, 1996). Superoxide anion radical is responsible for inhibition of glyceraldehyde-3-phosphate dehydrogenase (GAPDH; Buchanan and Armstrong, 1978; Marin et al, 1995; Rivera-Nieves et al, 1999; Salvemini and Cuzzocrea, 2002). Inhibition of GAPDH would be responsible for an increased formation of the AGE-forming compound methylglyoxal (Baynes and Thorpe, 1999; Nishikawa et al, 2000a; Thornalley, 1996), for an elevated production of the activator of endogenous protein kinase C (PKC) diacylglycerol (Koya and King, 1998), and the activation of the hexosamine pathway (conversion of fructose-6-phosphate into glucosamine-6-phosphate by the glucosamine-fructose-amidotransferase; (Schleicher and Weigert, 2000). As recently shown by Chang et al (2002), methylglyoxal is also responsible for substrate-induced upregulation of AR, which may further facilitate development of diabetic complications.

It is now widely accepted that oxidative free-radical damage is an initiating or very early event in the overall sequence that leads to cataract (Sarma et al, 1994). Oxidative stress may cause direct modification of the inner lens proteins, such as cross-linking, aggregation, and precipitation (Reddy et al, 1988; Young, 1991). Toxic aldehydes generated by peroxidation of lens epithelium and by oxidative damage of the vulnerable retina may contribute to the final damage of lens proteins yielding opacity (Altomare et al, 1997).

3. Polyol Pathway

Under physiological conditions, the bulk of glucose is metabolized through the glycolytic pathway and the pentose shunt. When hyperglycemia occurs, glucose disposal through these pathways tends to increase (Pugliese et al, 1991). In addition, an increased amount of glucose is converted into sorbitol by the enzyme AR via the polyol pathway, normally operating for converting aldehydes into alcohols at physiological glucose concentrations (Williamson et al, 1993). The glucose conversion into sorbitol by utilizing NADPH results in the
reduction of the NADPH/NADP+ ratio. This reaction uses NADPH as a hydrogen donor. Moreover, sorbitol oxidation to fructose by sorbitol dehydrogenase (SD) using NAD+ as a cofactor is associated with an increased NADH/NAD+ ratio (Fig. 3.9). Sorbitol does not easily cross cell membranes, and it can accumulate in cells and cause damage by disturbing osmotic homeostasis. Intralenticular accumulation of polyols produced in hyperglycemic conditions has long been suggested to be a major factor in acute models of sugar cataract.

**Fig 3.9: Polyol pathway in hyperglycemic and normoglycemic state**

![Polyol pathway diagram]

The fact that AR is responsible for initiating the cataractous process provides an explanation for the difference in the rate of cataract progression observed between diabetic and galactosemic rats. First, galactose is a better substrate than glucose for AR, so that more polyol is formed per unit time from galactose than from glucose. Second, galactitol formed in the AR reaction is not further metabolized by SD, as is sorbitol in the diabetic state. Since fructose can be further metabolized and can leak from the lens, the sorbitol pathway

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intermediates in the diabetic state never accumulate to the level of polyol found in the galactosemic lens. Therefore, there is a greater osmotic change in the lens of galactosemic rats, and, consequently, the rate of cataract development is more rapid (Kinoshita et al., 1981; Kinoshita and Nishimura, 1988; Kinoshita, 1990; Sato et al., 1998; Ohta et al., 1999; Ohta et al., 2000).

As the lens begins to swell in response to the hyperosmotic effects of polyol accumulation, membrane permeability changes result in an increase of lenticular sodium and in a decrease of lenticular potassium, reduced glutathione, myoinositol, ATP, and free amino acids. Eventually, as the lenticular levels of sodium exceed those of potassium, a shutdown of protein synthesis with loss of dry weight occurs. These biochemical changes are accompanied by morphological changes (Fig 3.10), which include an initial swelling of the lens epithelial cells and those in the central lens region increase in height and display aberrant vacuoles and dilution of cell contents followed by swelling of the superficial cortical fibers, which eventually rupture to form visible vacuoles (Robison et al., 1990; Kador et al., 2000). As lens fiber degeneration progresses, the entire cortex becomes opaque, and, eventually, nuclear opacity formation occurs along with liquefaction of the cortical regions.

Increased flux of glucose via polyol pathway has also consequences for the overall antioxidant status of the lens leading to depletion of GSH as a result of competition between AR and glutathione reductase for NADPH (Cheng and Chylack, 1985; Varma and Kinoshita, 1990). The NADPH depletion, combined with leakage of GSH and compounds essential for its synthesis, such as amino acids and ATP, results in a significant fall in lenticular GSH levels, an important intralenticular antioxidant (Gonzalez et al., 1983; Lee and Chung, 1999).
4. Other mechanisms:

Lens signal transduction mechanisms are severely disrupted by diabetes, with upregulated phosphorylation of MAPK and inhibition of phosphatidylinositol-3 kinase (Zatechka et al, 2003). Early diabetes has also been reported to cause activation of phospholipase C-γ1 with concomitant elevation of diacylglycerol concentration, increase in protein kinase C-γ activity, phosphorylation of Cx43 on Ser368, and inhibition of lens gap junction dye transfer activity (Lin et al, 2007). Contribution of all these changes to diabetes-associated cataractogenesis is unclear. MAPK activation plays an important role in other diabetic complications, and a p38 MAPK inhibitor corrected neurovascular dysfunction and nerve conduction deficits in experimental diabetic neuropathy (Tomlinson and Gardiner, 2008). Clearly, MAPK inhibitors should be tested for their ability to inhibit diabetic cataract formation. Recently, induction of endoplasmic reticulum stress and subsequent activation of the unfolded protein response have been implicated in lens epithelial cell apoptosis and cataract formation in galactose-fed rats (Mulhern et al, 2006). Furthermore, cellular osmolytes such as 4-phenylbutyric acid, trimethylamine N-oxide, and tauroursodeoxycholic acid suppressed endoplasmic reticulum and oxidative stresses in lens epithelial cells and attenuated galactose-induced cataract formation (Mulhern et al, 2007).
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results have been obtained in diabetic rats (Obrosova, 2009). Two structurally diverse PARP inhibitors counteracted diabetic cataract formation in mature STZ-diabetic rats, a model that clearly manifests accumulation of poly(ADP-ribosyl)ated proteins in the lens early after the induction of diabetes (Drel et al, 2009). The latter is in line with other studies suggesting an important role for this mechanism in the pathogenesis of diabetic neuropathy, nephropathy, and retinopathy (Jagtap and Szabó, 2005; Obrosova and Julius, 2005). Transition metal imbalance is another mechanism that deserves a thorough evaluation. Copper concentrations are increased in cataractous lenses of diabetic subjects, compared with non-diabetic controls (Lin, 1997). The copper chelator trientine counteracted oxidative stress and nerve dysfunction associated with peripheral diabetic neuropathy (Nakamura et al, 2002); furthermore, this agent has been reported to effectively counteract oxidative and carbonyl stress in diabetic lens (Hamada et al, 2005). A common manifestation of several diabetic complications including neuropathy is accumulation of cytosolic Ca++, a phenomenon closely linked to oxidative stress, PARP activation, and premature apoptosis. Elevated Ca++ concentrations are present in the diabetic lens (Cekic et al, 1998; Levy, 1999), and Ca++ antagonist verapamil displayed anticataract properties in the STZ-diabetic rat model (Ettl et al, 2004). Elevation of Ca++ is essentially required for activation calpains, intracellular cysteine proteases, implicated in unregulated proteolysis of lens crystallins, and lens opacification (Biswas et al, 2004). A number of calpains have been identified in the lens, including calpain 2, calpain 10, and two isozymes of calpain 3: Lp82 and Lp85 (Biswas et al, 2004). Evidence for the important role of calpains in both neuropathy and cataract formation in diabetic animal models is emerging (Kilic and Trevithick, 1998; Sakamoto-Mizutani et al, 2002). Calpain 2 is the strongest candidate of the calpains for a role in human types of cataractogenesis (Biswas et al, 2004). Evidence for the important role of Na+/H+-exchanger-1, a ubiquitously distributed isoform of NHE, in diabetic complications is emerging. Our recent findings (Obrosova, 2009)

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suggest that the Na\(^+\)/H\(^+\)-exchanger-1 inhibitor cariporide delays although does not prevent diabetic cataract formation

**Prevention and management of cataract**

At present, no definitive pharmacological therapy is available, and, thus, the only solution for the patient with advanced cataract is surgery, with all its disadvantages. Nevertheless, there are certain measures and treatment modalities based on possible molecular mechanisms of cataractogenesis, which can improve the visual outcome of this disabling eye disease.

As the first possibility of delaying cataract, protection of critical amino groups of long-lived proteins is offered. An efficient inhibitor of nonenzymatic glycation should inhibit glucose-derived AGE generation and cross-link formation. Such glycation inhibitors may include aminoguanidine (Swamy et al, 1996; Harding, 2001), aspirin (Blakytny and Harding, 1992; Harding, 2001), ibuprofen (Blakytny and Harding, 1992; Harding, 2001), paracetamol (Blakytny and Harding, 1992; Harding, 2001), pyruvate (Varma et al, 1995; Zhao et al, 1998, 2000) and \(\alpha\)-lipoic acid (LPA) (Obrosova et al, 1998; Packer, 1998).

The second way to prevent cataractogenesis is to reduce the oxidative stress by antioxidants. Antioxidants may generally act at different levels, for example, by preventing the formation of ROS, by eliminating already created ROS by scavenging, trapping, and quenching them, or by binding metal ions into inactive chelates. The lens may defend itself against oxidative stress by means of endogenous antioxidants like vitamin C, vitamin E, carotenoids, and antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), and Selenium dependent GSH peroxidase (Se-GPx) (Sarma et al, 1994; van der Pols, 1999). Various antioxidants have been studied for their effectiveness in preventing the development of cataract like vitamin C (Jacques et al, 1997; Leske et al, 1998; Harding, 2001), vitamin E (Knekt et al, 1992; Leske et al, 1998; Ohta et al, 2000), LPA (Kilic et al, 1995; Borenshtein et al, 2001), pyruvate (Varma et al, 1995; Zhao et al, 2000; Harding, 2001), carotenoids (Bates et al,
1996; Chasan-Taber et al, 1999), trolox (Ansari et al, 1994), butylated hydroxytoluene (Linklater et al, 1986; Srivastava and Ansari, 1988), venoruton (Kilic et al, 1996), and quercetin (Sanderson et al, 1999)

The third approach is the development of potential new agents to interfere with cataract formation through the inhibition of accumulation of polyols in eye lens cells. A wide variety of molecules were synthesized to inhibit AR (ARIs), which themselves may be acting in ways other than lowering the sorbitol pathway. Progress has been made in this area with reports of delaying cataract formation by zenarestat (FR74366) (Ao et al, 1991), BALARI8 (Gervasi et al, 1991), zopolrestat (Beyer-Mears et al, 1996), and imirestat (AL-1576) (Kador et al, 1998), all of which are classified as ARIs.

Fig 3.11: Pharmacological possibilities of prevention of cataract formation

Currently the only therapy available for the cataract is surgical extraction of the lens and replacement with an artificial intraocular lens. Complications from cataract surgery can arise at the time of surgery or days, weeks, months or even years later. Early complications include corneal oedema, raised intraocular pressure, corneal abrasion, wound leak, suture complications, iris prolapse, incarcerated vitreous, severe anterior uveitis, and displacement of the intraocular lens. Later complications include cystoid macular oedema, endophthalmitis, retinal detachment, posterior capsule opacification, and unsatisfactory refractive
error (Obrosova et al, 2010). Thus, supplementation with a potential anticataract agent is envisaged as an adjunct therapy to help preserve vision in diabetic patients and future clinical trials are needed to assess the benefits of pharmacological interventions in lowering the risk of cataract development. Significant improvement of the quality of life and reduction of the cost of caring for diabetic patients may be expected (Kyselova et al, 2004).

**Age-related cataractogenesis**

Several factors are postulated to be of importance in the generation of lens opacities in the older individual. These have been summarized by Taylor (1999) as the five 'D's: daylight, diet, diabetes, dehydration and don't know. The latter catch-all category probably predominantly involves genetic influences in nuclear and cortical opacification (Congdon et al, 2004; 2005). But the final common pathway by which these different factors exert their influence is predominantly through oxidation of lens proteins (Davies and Truscott, 2001) and peroxidation of lipids (Harding, 2002). In addition, the deleterious effects of glucose metabolism in the lens and associated changes in lens epithelial cell redox potential should not be overlooked, given their exacerbating effect on these oxidative changes (Hegde and Varma, 2005). Age-related cataract is thus not the result of one metabolic reaction in the lens but rather a final common pathway of many cataractogenic effects. A complete assessment of factors in age-related cataractogenesis sees photo-oxidation as the key event and thus considers light on the one hand and antioxidants on the other as being central to the problem and its solution with dehydration as a critical third factor.

The lens is designed to focus light onto the retina throughout the individual's life, but a necessary consequence of this is photo-oxidation of lens structures. The lens might appear a relatively inert structure, but has ATP levels as high as those found in muscle, a much more active tissue (Greiner et al, 1985). Oxidative metabolism is clearly important in maintaining the lens in a transparent state. However, this means that, as well as a continuous bathing in
light, the lens is also 'bathed' in oxygen that can lead to generation of superoxide radicals. Oxidative stress associated with increased reactive oxygen species is known to accelerate cataract formation in laboratory rodent models (Bhuyan et al, 1997). Superoxide is converted in most tissues of the body, including the lens, to hydrogen peroxide by superoxide dismutases but even hydrogen peroxide can become highly toxic because it produces the hydroxyl radical OH. This toxicity is prevented by catalase and glutathione peroxidase. These enzymes protect the lens by a system of antioxidant molecules, the lynch-pin of which is glutathione, the role of which is expertly reviewed by Lou (2003). The oxidation-reduction cycle of this tripeptide links dietary antioxidants such as ascorbate, riboflavin, carotenoids and tocopherols with the prevention of photo-oxidation.

One key link between photo-oxidation and cataract is that photo-oxidation of thiol groups on lens crystallins produces disulfide bridges between molecules (Ozaki et al, 1987) and, given time, the build-up of these will lead to protein aggregation and hence cataract. As Harding notes (2002), these aggregative changes are not confined to the lens – they occur in other age-related tissue degenerations such as central nervous tissue in Alzheimer's disease, but are perhaps particularly evident in a tissue through which light is continually passing. The lens contains UV filters that reduce the effects of the electromagnetic spectrum on lens proteins, but with age, these are deleteriously reduced (Bova et al, 2001; Truscott, 2003).

Lipid peroxidation is another key event in cataractogenesis according to some authorities, although the relative importance of protein oxidation and lipid peroxidation is unclear. Again this is unlikely to be an either/or situation but one in which both events may lead to lens opacification. In both cases antioxidants are a key prophylactic agent in preventing oxidation-related cataractogenesis and the dietary route is vital in providing these molecules (Williams, 2006).
3.5 TRADITIONAL SYSTEM OF MEDICINE

From the time immemorial, man has depended on plants as medicine. From a historical perspective, it is evident that the fascination with plants is as old as mankind itself. Herbs have provided us some of the very important life saving drugs used in the armamentarium of modern medicine. The plant kingdom represents a rich storehouse of organic compounds, many of which have been used for medicinal and other purpose. There exists a plethora of knowledge and information and benefits of herbal drugs in our ancient literature of Ayurvedic and Unani medicine. Ethnomedicines are replete with descriptions of plant medicines and the grandmothers pouch that has been called for years of medical wisdom is excellent proof of efficacy of these experimental medicines.

Ayurveda

Ayurveda is one of the major traditional medicinal systems from India. It is presumed that the knowledge of Ayurveda is given by Gods of different world. It is accepted as the oldest written medical system. The word “Ayurveda” means “science of life”. It is the ancient Indian system of health care and longevity. Ayurveda takes a holistic view of man, his health and illness. It aims at a positive health, which has been defined as a well balanced metabolism coupled with a healthy state of being. Disease, according to Ayurveda, can arise from body and/or mind due to external factors or intrinsic causes. Ayurvedic treatment consists of salubrious use of drugs, diets and certain practices. The basic concept of diagnosis and drug development in Ayurveda is based on Tridosha (three major components of disorders) theory, which includes Vayu, Pitta and Kapha. Vayu (Vata) it explains the entire biological phenomenon, which are controlled by the functions of central and autonomous nervous system. Pitta: It is manifestation of energy (Tejas) in the living organisms that helps digestion, assimilation, tissue building, heat production, blood pigmentation, activities of endocrine glands and so on. Kapha: It implies the function of thermotaxis or heat.
regulation and also the formation of various preservative fluids e.g. mucus, sinovial fluid etc.

Ayurveda has a vast literature in Sanskrit and in various Indian languages, covering all aspects of diseases, therapeutics and pharmacy. Pharmaceutics occupies an important place in Ayurveda. Medicinal preparations are invariably complex mixtures, derived from plant and animal products as also minerals and metals forming a dominant part. Earliest references to such plants are found in two holy books Rigveda and Atharva Veda, dating back to second millennium BC. The Ayurveda is said to be an Upaveda (part of Atharva Veda) whereas, the Charaka Samhita (1900 BC) is the first recorded treatise fully devoted to concept and practice of Ayurveda. This describes 341 plants and plant products for use in medicine. The next landmark of the Ayurvedic literature was the Sushruta Samhita (600 BC), which has special emphasis on surgery. It has six sections covering 186 chapters, and describes 395 medicinal plants, 57 drugs of animal origin and 64 minerals and metals as therapeutic agents. With the introduction of Western scientific methods in India, many Ayurvedic drugs and other Indian plants with curative properties soon came under some sort of scrutiny. Such investigations have continued to the present day and are being reviewed continuously.

**Siddha System of Medicine**

The term Siddha comes from 'Siddhi' means attainment of perfection. This system is almost akin to Ayurveda. This system describes 96 principal constituents of human beings, which include physical, physiological, moral and intellectual components of individuals. When there is any imbalance or slight deviation with these 96 units, diseases occurs. The Siddha medicine consists of psychosomatic system where attention is given to minerals and metals rather than plant constituents. The use of metals and minerals form an integral part of Siddha system of therapy to cure diseases.
There are similarities between Siddha and Ayurveda in their basic principles based on the theory of *panchamahabhuta* meaning that everything in the world and the universe around it are made up of five basic elements – earth, water, fire, air and space. Detoxification is a common phenomenon for any given drug to increase their therapeutic potency thereby minimizing the toxicity, known as *Suddhi Seithal*.

**Unani System of Medicine**

Unani medicine owes its origin to Greece. In this system, diseases are considered as a natural process and its symptoms are the reaction of the body to the diseases. Unani system based on humoral theory, there are several humors in the body like Dam (Blood), Bhalgham (Phlegm), Safra (Yellow bile), and Souda (Black bile). Unani system believes that every person has a unique humoral constitution, which represents his healthy state. Any change in his state affects his health. There is a power of self-preservation or adjustment called 'medicatrix naturae' or defense mechanism, which strive to restore disturbances. If this power weakens, imbalance in humoral composition occurs and causes diseases. The medicines help the body to regain this power to an optimum level and thereby restore humoral balance and thus retaining health.

Various types of treatments are prescribed in Unani system of medicine. There are:
(a) Regimental therapy, includes Deaphoresis, Diuresis, Turkish bath, massage, emesis, purging etc.
(b) Dietotherapy, aims at treating certain ailments by administration of specific diets or by regulating the quantity and quality of food.
(c) Pharmacotherapy deals with the use of naturally occurring drugs mostly herbal.
Homeopathic Remedies

Homeopathy is based on the idea that "like cures like"; that is, substances that cause certain symptoms in a health person can also cure those same symptoms in someone who is sick. This so-called law of similar gives homeopathy its name.

Linking of various systems of medicine

The above mentioned traditional systems of medicine have their uniqueness, no doubt, but there has to be a common thread running through these systems in their fundamental principles and practices. Further, we also believe that there has to be a common link with the principles of modern medicine with these systems of the medicine. Use of the herbs is the common thread for various traditional systems. In many countries it is traditional to use medicinal plants, either a single herb or a polyherbal formulation, to control diabetes. The antihyperglycaemic effect of several plants extracts or herbal formulations that are used as antidiabetic remedies has been confirmed (Modak et al, 2007). A database of natural hypoglycemics collected by researchers in Mexico lists almost 800 plants (Lozoya, 1994). Researchers in India have documented the use of 150 plants in families with reported hypoglycemic activity (Handa and Chawla, 1989). A recent cross-cultural compendium cites 1,200 medicinal plants used for diabetes (Marles and Farnsworth, 1995). Hundreds of products are marketed in India as "natural" agents for lowering blood sugar and decreasing long term complications. These include antibiotic, Alphabetic, Diabets, DB-7, Diabetica, Diabetiks, Dia-Comp, DiaVite, GlucoCare, Glucotize, GlycoNase, SugarMax, and Sugar Loss. These formulations are typically the combination products containing the individual components presented here along with others. However, in order to link them with the principles of modern medicine we must understand the molecular, cellular and molecular mechanism involved in the anti-diabetic activity of these drugs. Under these circumstances, one must...
consider that herbal drugs may not contain directly the insulin and even if there is protein substance, it can not be absorbed in total from gastrointestinal tract. Herbal drugs have to be considered as the sensitizers of insulin or possessing insulin like substances. We should not forget that the most important drugs biguanides like phenformin, metformin were discovered from a plant Galega officinalis or goat’s rue. An attempt has been to first classify the herbal anti-diabetics based on the molecular mechanism investigated by various researchers.

**Plants and treatment of diabetes mellitus**

Traditional medicines for the treatment of diabetes mellitus are probably based mainly on treatment of its obvious symptoms of pronounced thirst and polyuria. Even glucosuria was recognized as a symptom of diabetes in ancient Ayurvedic medical texts such as the Sushruta Samhita and Charaka Samhita (Nagarajan et al, 1982). The Greek physician aretaeus recommended treatment of diabetes by treatment of profound thirst. For this, he recommended starting with a purgative to strengthen the stomach, followed by consuming water boiled with autumn fruit a good source of soluble fibre and complex carbohydrates like pectin, milk, gruels of a variety of whole grains (an excellent source of soluble and insoluble fibre and glycans) and astringent wines (Hengesh and Holcomb 1981; Lomeo et al, 1988). He also recommended a crude drug of animal origins: venom of the “dipsas” viper, which in bite victims causes a severe thirst. Aretaeus suggested it could be used as a mithridate, i.e., a poison which is deliberately administered in small, gradually increasing doses in order to develop immunity to the effect of the poison (Adams, 1978). In fact, the venom of the Middle Eastern viper Piscivorus piscivorus (crotalidae) was found to be hypoglycemic when administered i.v. at a dose of 10 μg/kg in normal rats and rabbits, but was inactive against alloxan induced hyperglycemia in rats (Taha, 1982).
More than 1200 species of organisms have been used ethnopharmacologically or experimentally to treat symptoms of diabetes mellitus. They represent more than 725 genera in 183 families, extending phylogenetically all the way from marine algae and fungi to advanced plants such as the composites. The most frequently cited families are shown in Table 3.3. They are very large and widely distributed families, so the large number of species reported to have been used traditionally or experimentally for the treatment of diabetes may be coincidental. The phylogenetic distance between even these select groups of families is a strong indication of the varied nature of the active constituents. Thus, chemotaxonomic studies are often useful in the discovery of new plants with biologically active constituent. It will be necessary to learn more about particular groups of hypoglycemic natural products and their mechanism action before this method of drug discovery can be successfully employed.

Table 3.3: Plants family more often cited for antidiabetic activity (Throne et al, 1981).

<table>
<thead>
<tr>
<th>Family</th>
<th>Species cited for antidiabetic activity</th>
<th>Total species</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fabaceae</td>
<td>127</td>
<td>18,000</td>
</tr>
<tr>
<td>Asteraceae</td>
<td>98</td>
<td>21,000</td>
</tr>
<tr>
<td>Lamtaceae</td>
<td>36</td>
<td>3,500</td>
</tr>
<tr>
<td>Liliaceae</td>
<td>35</td>
<td>6,460</td>
</tr>
<tr>
<td>Poaceae</td>
<td>30</td>
<td>11,000</td>
</tr>
<tr>
<td>Euphorbiaceae</td>
<td>30</td>
<td>7,000</td>
</tr>
</tbody>
</table>

Half of the species found in our literature review have been used in traditional medicine to treat the symptoms of diabetes. Half of these traditional medicines have some experimental testing for hypoglycemic activity, e.g., in normal, glucose-loaded, alloxan or streptozotocin induced diabetic or naturally

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diabetic subjects. Distinctions of the experimental model used are clearly important for gaining and understanding of the mechanism of action of the botanical drugs.

If the same or closely related plants are used traditionally for the same purpose in more than one country, it suggests either cultural contact among the countries or independent discovery. In either case, the conservation of that traditional use indicates a higher probability that the traditional practitioners found the remedy to be effective. Table 3.4 give the list of most widely used traditional antidiabetic plants.

**Table 3.4: Indian medicinal plants with antidiabetic and related beneficial properties** (Modak et al, 2007)

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Ayurvedic/common name/herbal formulation</th>
<th>Antidiabetic and other beneficial effects in traditional medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Annona squamosa</em></td>
<td>Sugar apple</td>
<td>Hypoglycemic and antihyperglycemic activities of ethanolic leaf-extract, Increased plasma insulin level</td>
</tr>
<tr>
<td><em>Artemisia pallens</em></td>
<td>Davana</td>
<td>Hypoglycemic, increases peripheral glucose utilization or inhibits glucose reabsorption</td>
</tr>
<tr>
<td><em>Areca catechu</em></td>
<td>Supari</td>
<td>Hypoglycemic</td>
</tr>
<tr>
<td><em>Beta vulgaris</em></td>
<td>Chukkander</td>
<td>Increases glucose tolerance in OGTT</td>
</tr>
<tr>
<td><em>Boerhavia diffusa</em></td>
<td>Punarnava</td>
<td>Increase in hexokinase activity, decrease in glucose-6-phosphatase and fructose bisphosphatase activity, increase plasma insulin level, antioxidant</td>
</tr>
<tr>
<td><em>Bombax ceiba</em></td>
<td>Semul</td>
<td>Hypoglycemic</td>
</tr>
<tr>
<td><em>Butea monosperma</em></td>
<td>Palasa</td>
<td>Antihyperglycemic</td>
</tr>
<tr>
<td><em>Camellia sinensis</em></td>
<td>Tea</td>
<td>Anti-hyperglycemic activity, antioxidant</td>
</tr>
<tr>
<td>Plant Name</td>
<td>Ayurvedic/common name/herbal formulation</td>
<td>Antidiabetic and other beneficial effects in traditional medicine</td>
</tr>
<tr>
<td>------------------------</td>
<td>-----------------------------------------</td>
<td>---------------------------------------------------------------</td>
</tr>
<tr>
<td>Capparis decidua</td>
<td>Karir or Pinju</td>
<td>Hypoglycemic, antioxidant, hypolipidaemic</td>
</tr>
<tr>
<td>Caesalpinia bonducella</td>
<td>Sagarghota, Fevernut</td>
<td>Hypoglycemic, insulin secretagogue, hypolipidemic</td>
</tr>
<tr>
<td>Coccinia indica</td>
<td>Bimb or Kanturi</td>
<td>Hypoglycemic</td>
</tr>
<tr>
<td>Emblica officinalis</td>
<td>Amla, Dhatriphala, a constituent of herbal formulation, &quot;Triphala&quot;</td>
<td>Decreases lipid peroxidation, antioxidant, hypoglycemic</td>
</tr>
<tr>
<td>Eugenia uniflora</td>
<td>Pitanga</td>
<td>Hypoglycemic, inhibits lipase activity</td>
</tr>
<tr>
<td>Enicostema littorale</td>
<td>krimihrita</td>
<td>Increase hexokinase activity, Decrease glucose 6-phosphatase and fructose 1,6 bisphosphatase activity. Dose dependent hypoglycemic activity</td>
</tr>
<tr>
<td>Ficus bengalensis</td>
<td>Bur</td>
<td>Hypoglycemic, antioxidant</td>
</tr>
<tr>
<td>Gymnema sylvestre</td>
<td>Gudmar or Merasingi</td>
<td>Anti-hyperglycemic effect, hypolipidemic</td>
</tr>
<tr>
<td>Hemidesmus indicus</td>
<td>Anantamul</td>
<td>Anti snake venom activity, anti-inflammatory</td>
</tr>
<tr>
<td>Hibiscus rosa-sinensis</td>
<td>Gudhal or Jasson</td>
<td>Initiates insulin release from pancreatic beta cells</td>
</tr>
<tr>
<td>Ipomoea batatas</td>
<td>Sakkargand</td>
<td>Reduces insulin resistance</td>
</tr>
<tr>
<td>Momordica cymbalaria</td>
<td>Kadavanchi</td>
<td>Hypoglycemic, hypolipidemic</td>
</tr>
<tr>
<td>Murraya koenigii</td>
<td>Curry patta</td>
<td>Hypoglycemic, increases glycogenesis and decreases gluconeogenesis and glycogenolysis</td>
</tr>
<tr>
<td>Musa</td>
<td>Banana</td>
<td>Anti-hyperglycemic, antioxidant</td>
</tr>
</tbody>
</table>
## Review of Literature

<table>
<thead>
<tr>
<th>Plant Name</th>
<th>Ayurvedic/common name/herbal formulation</th>
<th>Antidiabetic and other beneficial effects in traditional medicine</th>
</tr>
</thead>
<tbody>
<tr>
<td>sapientum</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phaseolus vulgaris</td>
<td>Hulga, white kidney bean</td>
<td>Hypoglycemic, hypolipidemic, inhibit alpha amylase activity, antioxidant, Altered level of insulin receptor and GLUT-4 mRNA in skeletal muscle</td>
</tr>
<tr>
<td>Punica granatum</td>
<td>Anar</td>
<td>Antioxidant, anti-hyperglycemic effect</td>
</tr>
<tr>
<td>Salacia reticulata</td>
<td>Vairi</td>
<td>Inhibitory activity against sucrase, α-glucosidase inhibitor</td>
</tr>
<tr>
<td>Scoparia dulcis</td>
<td>Sweet broomweed</td>
<td>Insulin-secretagogue activity, antihyperlipidemic, hypoglycemic, antioxidant</td>
</tr>
<tr>
<td>Swertia chirayita</td>
<td>Chirata</td>
<td>Stimulates insulin release from islets</td>
</tr>
<tr>
<td>Syzygium alternifolium</td>
<td>Shahajire</td>
<td>Hypoglycemic and antihyperglycemic</td>
</tr>
<tr>
<td>Terminalia belerica</td>
<td>Behada, a constituent of &quot;Triphala&quot;</td>
<td>Antibacterial, hypoglycemic</td>
</tr>
<tr>
<td>Terminalia chebula</td>
<td>Hirda</td>
<td>Antibacterial, hypoglycemic</td>
</tr>
<tr>
<td>Tinospora crispa</td>
<td></td>
<td>Anti-hyperglycemic, stimulates insulin release from islets</td>
</tr>
<tr>
<td>Vinca rosea</td>
<td>Sadabahar</td>
<td>Anti-hyperglycemic</td>
</tr>
<tr>
<td>Withania somnifera</td>
<td>Ashvagandha, winter cherry</td>
<td>Hypoglycemic, diuretic and hypcholesterolemic</td>
</tr>
</tbody>
</table>

The Indian subcontinent has an extensive indigenous pharmacopoeia, including the Ayurvedic, Unani, and Folkloric medical systems, which has already supplied the world with such useful drugs as reserpine, from *Rauvolfia serpentina*, which is used as an antihypertensive and tranquilizer (Tyler et al, 1981). Reserpine is also reported to be hypoglycemic in normal animals and

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animals made hyperglycemic by pretreatment with epinephrine (Ricci and Ricordati, 1955). Indian traditional medicines may very well supply the world with some new antidiabetic drugs. Several reviews of plants with known antidiabetic activity or traditional use as antidiabetic remedies have been published (Farnsworth and Segelman 1971; Ajgaonakar 1979; Oliver-Bever and Zahnd 1979; Oliver-Bever 1980; Nagarajan et al, 1982; Mossa 1985; Oliver-Bever 1986; Day and Bailey 1988; Bailey and Day 1989; Handa and Sharma, 1989, Rahman and Zaman 1989; Ivorra et al, 1989; Winkelman 1989; Modak et al, 2007; Khan et al, 2012). Table 3.5 gives the classification of some herbal anti-diabetics based on the mechanisms of action.

Table 3.5: Some herbal anti-diabetics based on the mechanisms of action
(Goyal et al, 2007).

<table>
<thead>
<tr>
<th>Mechanism of action</th>
<th>Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Drugs acting like Insulin</td>
<td><em>Momordica charantia.</em></td>
</tr>
<tr>
<td>Drugs that increase insulin secretion from beta-cells</td>
<td><em>Panax ginseng</em> (release of insulin from cells)</td>
</tr>
<tr>
<td></td>
<td><em>Pterocarpus marsupium, Gymnema sylvestre,</em></td>
</tr>
<tr>
<td></td>
<td><em>Morus bombysis</em> (regeneration of beta-cells)</td>
</tr>
<tr>
<td></td>
<td><em>Lythrum salicaria, Trigonella foenum-graecum,</em></td>
</tr>
<tr>
<td></td>
<td><em>Cassia tamala &amp; Swertia chirayata</em> (increase circulating insulin levels).</td>
</tr>
<tr>
<td>Drugs inhibiting glucagon secretion</td>
<td><em>Ke-Tang-Ling</em></td>
</tr>
<tr>
<td>Drugs that reduce absorption of glucose from gut</td>
<td><em>Cyamopsis tetragonolobus, Cuminum nigrum,</em></td>
</tr>
<tr>
<td></td>
<td><em>Andrographis paniculata, Pterocarpus marsupium,</em></td>
</tr>
<tr>
<td></td>
<td><em>Ocimum sanctum, Saccharum officinarum</em></td>
</tr>
<tr>
<td>Drugs that increase uptake of glucose by muscle</td>
<td><em>Swertia chirayata</em></td>
</tr>
<tr>
<td>Drugs inhibiting aldose-reductase activity</td>
<td><em>Paeonia latiflora, Glycyrrhiza glabra, Aralia elata, Atractyloides lanata, Phellodendron amurense, Acer ginnula, Cinnamum cortex, Illicium religiosum, Comus macrophylla</em></td>
</tr>
<tr>
<td>----------------------------------------------------------------</td>
<td>----------------------------------------------------------------------------------------------------------------------------------</td>
</tr>
<tr>
<td>Drugs that increase glucose utilization/glycogen formation</td>
<td><em>Gymnema sylvestris, Nelumbo nucifera, Bryonia alba, Lythrum salicaria, Aralia elata, Allium sativum, Panax ginseng, Hygrophila longifolia, Coccinia indica, Swertia chirayta.</em></td>
</tr>
<tr>
<td>Drugs that increase glucose uptake by lipocytes</td>
<td><em>Ocimum sanctum, Swertia japonica</em></td>
</tr>
<tr>
<td>Drugs that increase uptake of GLUT-4 in skeletal muscles</td>
<td><em>Sangbackptang, a preparation containing Morus bombycis, Panax ginseng, Liriope muscari Pueraria thunbergiana, Poria cocos, Dioscorea batas, Cinnamomum cassia Glycyrrhiza uralensis.</em></td>
</tr>
<tr>
<td>Drugs that inhibit gluconeogenesis</td>
<td><em>Bauhinia megalandra, Trigonella foenumgraecum, Syzygium cumini.</em></td>
</tr>
</tbody>
</table>
3.6 PHYTOPHARMACOLOGY OF TEPHROSIA PURPUREA

Botanical Name: *Tephrosia purpurea* Linn.
Family: Leguminosae
Sub-family: Papilionaceae

**Classification of *T. purpurea***

<table>
<thead>
<tr>
<th>Kingdom</th>
<th>Plantae - Plants</th>
</tr>
</thead>
<tbody>
<tr>
<td>Subkingdom</td>
<td>Tracheobionta – Vascular plants</td>
</tr>
<tr>
<td>Superdivision</td>
<td>Spermatophyta – Seed plants</td>
</tr>
<tr>
<td>Division</td>
<td>Magnoliophyta – Flowering plants</td>
</tr>
<tr>
<td>Class</td>
<td>Magnoliopsida – Dicotyledons</td>
</tr>
<tr>
<td>Subclass</td>
<td>Rosidae</td>
</tr>
<tr>
<td>Order</td>
<td>Fabales</td>
</tr>
<tr>
<td>Family</td>
<td>Fabaceae – Pea family</td>
</tr>
<tr>
<td>Genus</td>
<td><em>Tephrosia Pers</em> – hoarypea</td>
</tr>
<tr>
<td>Species</td>
<td><em>Tephrosia purpurea</em> (L.) Pers.</td>
</tr>
</tbody>
</table>

**Other vernacular names:**

<table>
<thead>
<tr>
<th>Eng.</th>
<th>Purple <em>Tephrosia</em></th>
</tr>
</thead>
<tbody>
<tr>
<td>Guj.</td>
<td>Thila</td>
</tr>
<tr>
<td>Hindi</td>
<td>Sarponkha</td>
</tr>
<tr>
<td>Sans.</td>
<td>Sharapankha</td>
</tr>
<tr>
<td>Tam.</td>
<td>Kolluk-kay-velai</td>
</tr>
</tbody>
</table>
Botanical description:

An erect or spreading annual or short-lived perennial herb, sometimes bushy, 40-80 cm tall, rarely up to 1.5 m; indumentum sericeous, strigose or velutinous.

Stem: slender, erect or decumbent at base.

Leaves imparipinnate; stipules narrowly triangular, 1.5-9 mm x 0.1-1.5 mm; rachis up to 14.5 cm long, including the petiole of up to 1 cm; petiolule 1-3 mm long; leaflets 5-25, obovate to narrowly elliptical, terminal leaflet 7-28 mm x 2-11 mm, lateral leaflets 5-30 mm x 2-11 mm, acute at base, apex rounded to emarginate, venation usually distinct on both surfaces.

Inflorescence: an axillary or leaf-opposed pseudo-raceme, (1.5-)10-15(-25) cm long, sometimes with basal leaf-like bracts; flowers in fascicles of 4-6; bracts to
fascicles and to flowers small, bracteoles usually absent; pedicel 2-6 mm long; flower 4-8.5 mm long, purplish to white; calyx campanulate, persistent, cup 1.4-2.3 mm x 1.5-3.2 mm, unequally 4-toothed, teeth pubescent inside; standard broadly ovate, 3.5-7.3 mm x 5-10 mm, clawed; wings 2.5-6 mm x 1.5-3.8 mm, auricled on vexillary side, clawed; keel 2.2-4.5 mm x 2-3 mm, auricled on vexillary side, clawed; stamens 10, staminal tube 4-6 mm long, filaments alternately longer and shorter, free part up to 3.5 mm long, vexillary filament free at base, connate halfway, 5-8 mm long; style up to 4.5 mm long, upper half glabrous, stigma penicillate at base.

Pod: flat, linear, 2-4.5 cm x 3-5 mm, somewhat up-curved towards the end, convex around the seeds, flattened between, margins thickened, dehiscent with twisted valves, 2-8(-10)-seeded.

Seed: rectangular to transversely ellipsoid, 2.5-5 mm x 1.8-3 mm, light to dark brown to black, sometimes mottled.

Natural habitat

*T. purpurea* occurs naturally in grassy fields, waste places and thickets, on ridges, and along roadsides. *T. purpurea* is native to tropical Asia, and is found from India and Sri Lanka to southern China, and through South-East Asia to tropical Australia and the Polynesian Islands. It is now naturalized and cultivated pantropically.

Altitude: Up to 400 m altitude, it generally grows at low altitudes, but may be found to 1300 m altitude.

Soil types: It prefers dry, gravelly or rocky and sandy soils

Traditional Uses:

According to Ayurveda, plant is digestible, anthelmintic, alexiteric, antipyretic, alternative, cures diseases of liver, spleen, heart, blood, tumours, ulcers, leprosy, asthma, poisoning etc. According to Unani system of medicine, root is diuretic, allays thirst, enriches blood, cures diarrhea, useful in bronchitis,
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asthma, liver, spleen diseases, inflammations, boils and pimples; Leaves are tonic to intestines and a promising appetizer. Good in piles, syphilis and gonorrhoea

Chemical constituents and phytochemical investigations

Phytochemical investigations on *T. purpurea* have revealed the presence of glycosides, retenoids, isoflavones, flavonones, chalcones, flavanonols and sterols.

Glycosides:

Several workers have reported the presence of glycoside rutin in the *T. purpurea* (Clarke 1910; Clarke 1941; Basu 1977a). Later Basu (1977b) and Basu (1981) quantitatively determined rutin in the leaves of *T. purpurea*. Saxena and Choubey (1997), have isolated a novel neoflavanoid glycoside, serration 7-O [β-D-glucopyranosyl-(1 to 4)-O-β-D-galactopyranoside} from the chloroform-soluble fraction of the ethanolic extract of the stem. Quercetin glycoside was isolated from the tissues and tissue cultures of *T. purpurea*, the content being higher in the leaves (0.29%) as compared to stem (0.03%) and root (0.15%) (Khanna et al, 1977). Clarke and Banerjee (1910), have reported the occurrence of a glucoside identical to osyritin from the leaves of *T.purpurea*. A novel oleanen type triterpenoid glycoside has been isolated from the butanolic extract of the seeds of *Tephrosia purpurea*. Its structure was elucidated as 3-O-β-D-glucopyranosyl-(1→6)- [α-L-rhamnopyranosyl- (1→2)]-β-D-glucopyranosyl- (1→4)- [β-D-glucopyranosyl-(1→2)] -β-D-xylopyranosyl} -2,16-dihydroxy-23,29-dihydroxymethylolean-11,13(18)-dien-28-oic acid on the basis of spectral evidences (Khan, 2011).

Rotenoids:

Sharma and Khanna (1975) analyzed the root, stem and leaves as well as the unorganized static cultures of *T. purpurea* for their rotenoid content. Rotenoid content was higher in leaves as compared to the roots and stem. Presence of...
degulin, elliptone, rotenone and tephrosin was confirmed. Kamal and Jain (1978) reported the presence of 12α-hydroxyrotenone in *T. purpurea*.

**Flavanoids:**

Rao and Raju (1979), isolated (-)-isolonchocarpin, a flavanone, from the petrol soluble fraction of the hot chloroform extract of the roots of *T. purpurea*. This was the first report of isolation of active isolonchocarpin from a natural source. Isolonchocarpin was later isolated from the petrol extract of seeds and roots of the plant (Gupta et al, 1980; Pelter et al, 1981). Along with this, Rao and Raju (1979) isolated 3 other crystalline compounds from the same fractions which were identified as lanceolatin A, lanceolatin B, and pongamol. Lanceolatin B and pongamol were also isolated from the seeds (Gupta et al, 1980; Sinha et al, 1982). Lanceolatin and o-methylprongamol were isolated from the roots of the plant (Pelter et al, 1981). Parmar et al (1989) reported the occurrence of pongamol in its pure enol form in the whole plant of the *T. purpurea*. Its structure was analysed as β-hydroxybenzofuranchalcone, the enol form of pongamol. Gupta et al (1980) isolated a another flavanone, (-)-purpurin from the seeds of *T. purpurea*. The structure of purpurin was found to be 2, 3 dihydrosemiglabrin. Later, Rao and Raju (1984) isolated (+)-purpurin, a diastereoisomer of (-)-purpurin from the petrol soluble fraction of the chloroform extract of roots of *T. purpurea*. Along with purpurin, they isolated a β-hydroxychalcone-purpurenone, (-)-dehydroisoderricin, (-)-maackiai, (-)-semiglabrin and pseudoglabrin from the same fraction. Karajin was isolated on column chromatography of the petrol extract and the ethanolic extract of the seeds of *T. purpurea* (Sinha et al, 1982). Two prenylated flavanoids, purpuritenin and purpureamethide have been characterized from the seeds of the *T. purpurea* (Sinha et al, 1982). Prenylated flavanoids are well known in literature but, purpuritenin is a rare example of a methylated chalcone, which may perhaps be a secondary fungal metabolite of seeds. Three other novel flavonoids (+)-tephrorins A and B and (+)-tephrosone were also isolated from *T. purpurea* by Chang et al, (2000). Tephorins were flavanones containing an unusual tetrahydrofuran moiety. Moreover, Hegazy et
al, (2009) reported rare prenylated flavonoids, tephropurpulin A and isoglabratephrin, in addition to a previously identified flavonoid, glabratephrin. Investigations of the methylenechloride/methanol (1:1) extract of the aerial parts of *Tephrosia purpurea* yielded a prenylated flavonoid which was identified as apollinine (Khalafalah et al, 2010).

An isoflavone, 7,4-dihydroxy-3,5-dimethoxyisoflavone and chalcone, (+)-tephropurpurin along with (+)-purpurin, pongamolm lanceolatin-B and pterocarps (-)-maackiain, and (-)-3-hydroxy-4methoxy-8,9-methylene dioxypterocarpan were obtained as active compounds from *T. purpurea*. Additionally, three known compounds, 3-methoxydaidezin, desmethocyphyllin B and 3, 9-dihydroy-8-methoxycoumestan were isolated (Chang et al, 1997). Anthocyanin A and B, anthocyanidin A and B and delphindin chloride were extract from the purple floweres of *T. purpurea* and identified. Cyanidin chloride was also isolated (Basu and Sircar 1978). Tephrosin, pongaglanol and semiglabrin were isolated from the aerial parts of *T. purpurea* (Ahmed et al, 1999). Pelter et al, (1981) isolated and characterized unusual and closely related flavanoids from the roots of *T. purpurea*, three of them being new natural products. Apollinine, a 8-substituted 7-methoxyflavone, was isolated from the benzene-soluble fraction of the chloroform extract of the roots of the plant (Pelter et al, 1981). It represented the first example of a natural product containing a butenolide unit attached to a flavone. Tephroglabrin, which had not been previously reported as a natural product but had been prepared by treating semiglabrinite with methyl iodide and dry K₂CO₃, was isolated from the roots. Other flavanoids isolated from the roots of the plant were semiglabrin, semiglabrinol and tepurindiol.

**Steroids:**

β-sitosterol and lupeol was isolated from the petroleum ether extract of the leaves of *T.purpurea*. It also yielded sitosterol. β-sitosterol and spinasterol-α were isolated (Parmar et al, 1989). The steroid constituents of chloroform extracts of *T.purpurea* have been identified as stigmasterol, β-sitosterol,
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campesterol, stigmasta-4, 22-dien-3-one. Khanna et al, (1977) isolated β-sitosterol form the tissue cultures of *T. purpurea*, the content being higher in leaves as compared to root and stem. β-sitosterol, cholesterol and stigmasterol were also isolated from the tissue cultures of *T. purpurea* with the total crude sterol content of 1.7% (Kamal, 1978).

**Miscellaneous:**

Sinha et al, (1982) isolated a new aliphatic ketone, tephrone, in combination with n-tricontanol from the pods of *T.purpurea*. Basu, (1977) reported the presence of an alkaloid in the basic fraction of the ethanolic extract of *T.purpurea* leaves. A phenolic acid resembling in its chromatographic behaviour, melting point, ultraviolet and infrared absorption and also biological properties with caffeic acid has been isolated from the seeds of *T.purpurea* (Sinha et al, 1982). This substance showed both stimulation and germination of seed depending on the concentration. Petroleum ether extract of seeds of *T. purpurea* yielded a yellowish brown oil, consisting of palmitic, palmitoleic, stearic, oleic, linoleic and linolenic acids. 18 amino acids were identified by chromatography (Joshi et al, 1979). The leaves have been reported to contain high amounts of nitrogen and potassium, which are valuable of green manuring. Also, investigations on methylenechloride/methanol (1:1) extract of the aerial parts of *T.purpurea* reported an aromatic ester identified as 2-propenoic acid, 3-(4-(acetyloxy)-3-methoxyphenyl)-3(4-actyloxy)-3-methoxyphenyl)-2-propenyl ester and a sesquiterpene which was found to be of rotundane skeleton 4-isopropyl-1,8-dimethyl-decahydro-azulene-5,8,9-triol (Khalafalah et al, 2010) which was previously isolated from *Ferula sinaica* (Ahmed, 1990).

**Pharmacological activity**

**Hepatoprotective activity**

*Tephrosia purpurea* (aerial parts) are incorporated frequently in a large number of commercial herbal hepatoprotective formulations like Livfit, Amlycure,
Livomyn, Tefroli, Livin, Lvikin, Livospin, Himoliv, Neoliv-100, Rohitak Arisht, Rohitakghritam, Amlycure, and Livarin. Joshi et al, (1979) showed significant hepatoprotective activity. In experimental carbon tetrachloride induced acute and chronic liver damage in rats, pretreatment with a composite herbal drug containing *T. purpurea* was effective in inhibiting triglyceride accumulation in hepatic cells and in protecting liver against increased hydroxyproline content (Chauhan et al, 1992). Administration of *T.purpurea* (aerial parts) along with the hepatotoxins offered a protective action in both acute (D-galactosamine) and chronic (CC14) hepatotoxic models (Murthy and Srinivasan, 1993). Treatment with HD-30, a polyherbal preparation led to significant amelioration of toxin-induced changes in the biochemical parameters. HD-30 (a polyherbal product in which *T. purpurea* is one of the component) was also investigated for its anticholestatic activity in thioacetamide induced experimental cholestasis in anesthetized guinea pigs (Mitra et al, 1998). Pretreatment with HD-30 at a dose of 750mg/kg body weight, orally for 15 days, significantly prevented thioacetamide induced changes in bile flow, bile acids and bile salts secretion (Mitra et al, 1999). Lucas and Rajasekhar (2000) studied antihepatotoxic activity of *T. purpurea* used in Ayurveda. From the results, it was evident that plant exhibited anthepatotoxic effect by stimulating cell regeneration. Shankar et al, (2005) studied the hepatoprotective activity of a new benzopyrone derivative (TP) isolated from the alcoholic extract of aerial parts of *T.purpurea*. Hepatoprotective activity of TP and the alcoholic extract was evaluated using carbon tetrachloride, paracetamol and rifampican as toxicants. Results suggest that alcoholic extract (200 mg/kg) and benzopyrone derivative (100 mg/kg) caused significant fall of the serum enzyme levels (SGOT, SGPT) of the animals. Alcoholic extract of *T. purpurea* and TP have shown significant hepatoprotective activity in rats against all the toxicants that were studied. Nair (2006) showed protective effect of Tefroli tonic (a polyherbal mixture containing *T. purpurea*) against cadmium induced hepatotoxicity in experimental rats. Subcutaneous injection of cadmium chloride to rats caused liver damage and was observed by analysis of serum bilirubin and
assay of marker enzymes such as transaminase and phosphates of serum and liver. The administration of Tefroli tonic has maximum protective effect against cadmium chloride induced hepatotoxicity in rats. Investigations on the ethanolic extracts of the leaves and the flavonoid fraction isolated from the leaves of *T. purpurea* showed significant hepatoprotective activity against CC14 induced liver damage in rats. It was also found that hepatoprotective activity was more with ethanolic extract rather than the flavonoid fraction isolated from the leaves (Jain et al, 2006a). Khatri et al (2009) showed potential hepatoprotective activity of the aerial parts of *T. purpurea* at a dose of 500mg/kg/day and attenuates the hepatotoxic effects of TAA by membrane stabilizing effect and acting as an antioxidant. The ethanolic extract of root of *T. purpurea* was evaluated for hepatoprotective activity which showed significant protection of liver against CC14 induced oxidative damage probably by increasing antioxidative defense activities (Sangeetha and Krishnakumari, 2010). The dried herb extract of *T. purpurea* showed significant results in restoring the induced biochemical and histological ultra structural changes in liver induced by CC14 which may be due to its free radical scavenging and antioxidant properties (Johri et al, 2010). Gunjegaonkar et al, (2010) also showed that *T. purpurea* whole plant aqueous extract has potent hepatoprotective action against CC14 induced hepatic damage in rats. Kashaw et al, (2011) were studied the dried ethanolic extract of *T. purpurea* for its efficacy in both acute (D-galactosamine) and chronic models (CC14) of experimentally induced hepatotoxicity. Results revealed the mechanism of hepatoprotection by *T. purpurea* mainly involves membrane stabilization of liver cells as indicated by decrease in levels of SGOT, SGPT and bilirubin levels, wherein it prevents cellular leakage and loss of functional integrity of liver cell membranes caused by various hepatotoxic agents. *T. purpurea* also leads to increase in hepatic regeneration, which again contributes to its hepatoprotective efficacy. Ethyl acetate fraction of ethanol extract of roots of *T. purpurea* also demonstrated the hepatoprotective activity of roots against CC14-induced hepatotoxicity (Shah et al, 2011). The root callus extract of *T. purpurea* offered better antihepatotoxic action
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as compared to the active principles present in natural root extract against in-vitro CCl4 hepatic damage (Mujeeb et al, 2012).

Hypoglycemic activity

The hypoglycaemic effects of fresh extract of the roots of *T. purpurea* and its chemical principle rutin have been investigated (Swift et al, 1949). Fresh extract could produce a transient hypoglycaemic effect. The oral administration of the aqueous extract of seeds of *T. purpurea* led to a marked lowering of blood glucose levels in normal and alloxan-induced diabetic rabbits (Rahman et al, 1985). The hypoglycaemic effect of the extract was comparable to that of tolbutamime in normal rabbits. In diabetic rabbits, the extract exerted 60-70% hypoglycaemic effect as compared to tolbutamime (Rahman et al, 1985). *T.purpurea* leaf extract has not only shown antihyperglycemic effect but also resulted in elevated plasma insulin concentration, probably by stimulating insulin secretion from remnant pancreatic β-cells, which might have corrected other metabolic alterations. The plant extract has also revealed antihyperlipidemic effect in a manner similar to that of the reference drug glibenclamide (Pavana et al, 2007a) The ethanolic seed extract of *T.purpurea* at a dose of 300mg/kg body weight showed potent antihyperglycemic and antilipidperoxidative effects in streptozotocin induced diabetic rats. It was also observed that the antihyperglycemic effect of the plant drug was comparable to that of the reference drug glibenclamide (Pavana et al, 2007b). The effect of ethanolic seed extract on glycoprotein components in streptozotocin diabetic rats was studied which indicated potent role in modifying altered glycoprotein components in the diabetic rats. The protective effects of the seed extract may be due to its inhibitory role in abnormal glycoprotein synthesis or on the activity of the enzymes involved in glycoprotein metabolism (Pavana et al, 2008). Repeated dose administration of alcoholic and hydro-alcoholic extract of roots of *T.purpurea* produced significant hypoglycemic activity in normal healthy wistar rats as well as produced significant anti-diabetic activity in alloxan induced
diabetic rats (Joshi et al, 2008). Pavana et al (2009) evaluated the effects of aqueous seed extract of *T.purpurea* on hyperglycemia associated with an altered hexokinase and glucose-6-phosphatase activities, elevated lipid peroxidation, disturbed enzymatic and non enzymatic antioxidant status in streptozotocin induced diabetic rats. Oral administration of the aqueous seed extract of *T.purpurea* at a dose of 600mg/kg body weight showed significant improvement in blood glucose levels as well as the antioxidant status (Pavana et al, 2009).

**Antihyperlipidemic activity**

Balakrishnan et al (2007) investigated the lipid lowering properties of methanolic extract of *Tephrosia purpurea* leaves on experimentally triton induced rats. Extract on oral administration at doses of 300 and 600 mg/kg in triton induced rats showed significant reduction in total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), very low density lipoprotein cholesterol (VLDL-C) and triglycerides (TG) levels, while high density lipoprotein cholesterol (HDL-C) level was significantly increased when compared to control group. The results suggested that methanolic plant extract can reduce lipid levels. The ethanolic extract of leaves of *T.purpurea* can reduce the lipid levels like total cholesterol, LDL-C, VLDL-C as well as triglycerides significantly in dexamethasone induced rats (Akhtar et al, 2011). The ethanolic extract of plant of *T.purpurea* possess significant antihyperlipidemic activity in experimentally dexamethasone induced hyperlipidemic wistar rats. The antihyperlipidemic activity of ethanolic extract at dose of 400 mg/kg b.w. and 800 mg/kg b.w. was found to be significant as indicated by decrease in total cholesterol level of rats when compared to hyperlipidemic control (Mustak, 2012).

**Antimicrobial, antifungal and anthelmintic activity**

Cold and hot water extracts of the whole plant of *T. purpurea* were inactive as antibacterial when tested against *M. Pyogenes var, aureus, E. coli, B. dysenteriae, B. typhousu* and *V. Cholera* by the agar cupplate method (Godbole Shraddha Shah 101
and Pendse, 1960). The ethanolic and methanolic extracts of *T. purpurea* possessed potential antibacterial activity (Mahajan et al, 1999). The flavanoids were found to have antimicrobial activity (Rao and Raju, 1978). Fungicidal activity of extract of *T. purpurea* was tested on 3 sclerotia forming pathogens, *Sclerotium rolfsii*, *Rhizoctonia solani* and *Sclerotinia sclerotiorum* (Chaudhuri and Sen, 1982). The pollen suspension of *T. purpurea* was tested for antifungal activity, which showed a marked stimulatory action on fungal spore germination (Tripathi et al, 1982). Sharma et al, (2003) reported that n-butanol fraction of *T.purpurea* extract at dose of 50 mg/kg for 5 days treatment exhibited significant antileishmanial activity against *Leishmania donovani* infection in hamsters. Thetwa et al (2006) tested the seed extracts of the plant *T.purpurea* for their antimicrobial and antifungal properties against some human, animal and plant pathogenic organisms. The study revealed that the seed extract showed a good inhibition effect against all the tested microorganisms. Kumar et al (2007) evaluated antimicrobial activity of ethanolic extract of *T.purpurea* roots by disc diffusion and broth dilution methods against acne inducing bacteria. The results generated from the study revealed the significant antimicrobial activity of test extract. Moreover, the ethanolic root extract of *T.purpurea* shows significant activity against *Pseudomonas aeruginosa*, two other *Pseudomonas* strains and two coliform strains but the ethanolic leaf extracts and water extracts of *T.purpurea* shows no activity against any of the isolates. The MIC of ethanolic root extracts of *T. purpurea* was found to be 128mg/L (Rangama et al, 2009). Surve and Patil (2009) were evaluated pet ether, ethanol and aqueous extracts of seeds of *T.purpurea* for anthelmintic activity. Ethanol and aqueous seeds extracts exhibited anthelmintic activity in dose-dependent manner giving shortest time of paralysis and death of *Pheretima posthuma* at 100 mg/ml concentration. Ethanolic extract was found to be most potent among the all extracts and showed the maximum anthelmintic activity due to the presence of different constituents such as alkaloids, glycosides, steroids and sterols, anthraquinones, flavonoids, triterpenoids and fixed oil. The *T.purpurea* seeds extract shows MIC values
below 50µg/mL, therefore representing a good activity against the selected bacteria *Streptococcus pneumoniae*. However, the MIC value was maximum for *B.subtilis* and *K.pneumoniae*- therefore the extract was least effective against these bacteria. Among fungi, complete inhibition on the growth of fungus - *Alternaria alternata* was observed at a concentration of 200µg/mL (Khan, 2011). The petroleum ether, ethanol and aqueous extracts of seeds of *T.purpurea* was evaluated for antimicrobial activity. Among all extracts, pet ether extract was found to be potent while aqueous extract showed the least activity. Seed extracts was effective against both i.e gram positive and gram negative bacteria and fungi while pet ether extract showed the highest zone of inhibition for gram negative *Escherichia coli* and fungi *Candida albicans* (Venkatraman et al, 2011). Devprakash et al (2011) investigated *T. purpurea* for in-vitro antimicrobial activity against pathogens namely *Staph. aureus*, *E. coli*, *P. aeruginosa*, and *B. subtilis* by disc diffusion method compared with standard antibiotic. The ethanolic extract of plant showed better antibacterial activity than aqueous extract against all organisms. The observed antibacterial activity of the plant extracts was linked to the presence of tannins in the test extracts. Juma et al (2011) found that *T.purpurea* stem extract showed antiplasmodial activity against the chloroquine sensitive and chloroquine-resistant strains of *Plasmodium falciparum*. The new compound, terpurinflavone, showed the highest antiplasmodial activity (Juma et al, 2011). Anti microbial activity of 50% alcoholic extract showed significant antibacterial activity against *Escherichia coli*, *Serratia marcescens* and *Staphylococcus* but did not show positive antifungal activity against *Aspergillus fumigates*, *Aspergillus niger*, *Ganoderma lucida* and *Candida albicans* (Nivedithadevi et al, 2012). The same study also showed potent antihistaminic activity of the 50% alcoholic extract of *T.purpurea* by inhibiting the contraction of isolated guinea pig ileum.
Wound healing potential

The wound healing potential of ethanolic extract of *T. purpurea* (aerial parts) in the form of simple ointment using three types of wound models in rats as incision wound, excision wound and dead space wound was evaluated. The results were comparable to standard drug Fluticasone propionate ointment, in terms of wound contraction, tensile strength, histopathological and biochemical parameters such as hydroxyproline content, protein level (Lodhi et al, 2006). The petroleum ether, ethyl acetate and methanolic extracts of the roots of *T. purpurea* was evaluated by excision, incision and dead space wound models in rats. The results of study showed that the methanolic extract of *Tephrosia purpurea* effectively stimulates wound contraction (excision wound); petroleum ether, ethyl acetate and methanol shows significant increase in tensile strength of incision and dead space wounds as compared to control group. These finding could justify the inclusion of this plant in the management of wound healing (Chaudhari et al, 2010). The flavonoid rich fraction of *T. purpurea* L. aerial parts was screened for its burn wound repairing efficacy in partial thickness and full thickness burn wound models. It was observed that, wound contraction and tensile strength of skin tissues were significantly greater in partial thickness burn in the case of flavonoid rich fraction when compared to the control group. In the full thickness burn model, protein and hydroxyproline contents were found to be significantly higher. Presence of matured collagen fibers and fibroblast cells with better angiogenesis was observed as a result of histopathological studies, which was visibly improved in case of treated groups compared to control group (Lodhi et al, 2010).

Anti-ulcer activity

The aqueous extract of roots of *T. purpurea* possess significant anti-ulcer activity in different models of gastric (oral administration of ethanol, 0.6M HCl, indomethacin or pylorus ligation) and duodenal (oral administration of cysteamine HCl) ulcers. However, the anti-ulcer activity was less than that of omeprazole. It
was suggested that the anti-ulcer property could be either due to cytoprotective action of the drug or by strengthening of the gastric and duodenal mucosa and thus enhancing mucosal defence (Deshpande et al, 2003). In vitro anti-
*Helicobacter pylori* activity profile of the plant extract and its fractions was evaluated by Chinniah et al, (2009). The methanolic extract showed promising activity against clinical isolates and standard strains of *Helicobacter pylori*, including metronidazole-resistant strains. Fractionation of the extract revealed the *n*-hexane and chloroform fractions to possess marked activity. The extract and the less polar fractions exhibited consistent bacteriostatic activity during repeated exposure, and demonstrated synergism, complete or partial, even with antibiotic-resistant strains (Chinniah et al, 2009).

**Anticancer activity**

The petroleum ether- and ethyl acetate-soluble extracts of *T.* purpurea were found to significantly induce quinone reductase (QR) activity which is a major mechanism of protection against tumor initiation with cultured Hepa 1c1c7 mouse hepatoma cells (Chang et al, 1997). Saleem et al (2001) assessed the effect of *T.* purpurea on 12-O-tetradecanoyl phorbol-13-acetate (TPA; phorbol ester) induced cutaneous oxidative stress and toxicity in murine skin. The study shows that topical application of *T.* purpurea prior to TPA and croton oil treatment resulted in significant inhibition of TPA-induced cutaneous ODC activity, [3H]thymidine incorporation and croton oil-promoted skin tumorigenesis, respectively, in a dose-dependent manner. The present study also suggests a delay in onset of tumor formation with the animals pre-treated with *T.* purpurea in DMBA-initiated and croton oil-promoted mice skin, which further suggests the antitumor promoting potential of *T.* purpurea. In addition, *T.* purpurea reversed TPA-mediated inhibition of the activities of antioxidant enzymes such as glutathione S-transferase, glutathione reductase, catalase and cutaneous glutathione. The ethanolic root extract of *T.* purpurea has found to possess potent chemopreventive efficacy on 7, 12- dimethylbenz(a)anthracene (DMBA)- induced
hamster buccal pouch carcinoma at a dose of 300 mg/kg, body weight (Kavitha and Manoharan, 2006). Gulecha and Sivakuma (2011) investigated the anticancer activity of different fractions of *T. purpurea* using human breast cancer cell lines MCF-7 by trypan blue exclusion method. They showed that the fractions obtained from the plant were effective in attenuating the viable tumor cell count which may be attributed to the flavonoids and phenolic constituents of the fractions. Hydroalcoholic, chloroform and ethyl acetate extracts of the aerial parts of *T. purpurea* was evaluated for cell survival on human cancerous cell line (HeLa) using MTT assay. The ethyl acetate extract showed a comparable cytotoxic effect to taxol against HeLa cell (15 µg/ml) which may be related to the presence of lipophilic glycosides as non-polar compounds extracted by ethylacetate (Shanmugapriya et al, 2011). The chemopreventive potential of *T. purpurea* extract on N-nitrosodiethylamine (NDEA)-induced hepatocellular carcinoma in Wistar rats was assessed by Hussain et al (2012). They showed that *T. purpurea* prevented lipid peroxidation, suppressed the tumour burden, and promoted enzymatic and nonenzymatic antioxidant defence systems during NDEA-induced hepatocarcinogenesis which might be due to modulating the antioxidant defence status, which contributed to its anticarcinogenic potential (Hussain et al, 2012).

**Neproprotective activity**

Prophylactic treatment of rats with *T. purpurea* at doses of 5 mg/kg body weight and 10 mg/kg body weight prevented N-diethylnitrosamine-initiated and potassium bromate promoted renal oxidative stress and toxicity (Khan et al, 2001). They investigated chemopreventive efficacy of *T. purpurea* against Ndiethylnitrosamine-initiated and potassium bromate-mediated oxidative stress and toxicity in rat kidney. The data indicate that *T. purpurea* is a potent chemopreventive agent against renal oxidative stress and carcinogenesis induced by N-diethylnitrosamine and KBrO3 by reducing lipid peroxidation and xanthine oxidase activities and enhancing antioxidant enzymes activity.
Prashanth kumar et al (2001) demonstrated significant hydroxyl radical scavenging activity in vitro. Moreover, using a trypan blue exclusion assay, they found that the extract markedly increased the percentage viability of the isolated rat kidney cortical cells in gentamicin-induced cell damage. Swathi et al, (2008) evaluated aqueous extract of *T. purpurea* roots for its antilithiatic activity in two models of urolithiasis. The aqueous extract of *T. purpurea* was found to be effective in reducing the formation of and dissolving existing calcium oxalate (Gentamicin and 5% ammonium oxalate) and magnesium ammonium phosphate stones(zinc discs). Jain and Singhai, (2009) shown *T. purpurea* leaves possesses marked nephroprotective and curative activities without any toxicity. The proposed mechanisms of activities are antioxidant activity and inhibition of overproduction of NO and COX-2 expression and it may be attributed to phenolic and flavonoidal compounds like quercetin.

**Antioxidant activity**

*T.purpurea* ameliorates benzoyl peroxide-induced oxidative stress in murine skin (Saleem et al, 1999). Soni et al, (2003) observed that *T.purpurea* showed significant free radical scavenging and antilipid peroxidation properties but did not scavenge the hydroxyl radical generated using ascorbic acid iron EDTA model. The ethanol extract of *T.purpurea* was found to significantly inhibit the carbon tetrachloride-induced lipid peroxidation and superoxide generation in vivo. The ethyl acetate fraction of the same extract also showed significant potential in vitro for free radical scavenging and antilipid peroxidation activity (Soni et al, 2006). Jain et al, (2006b) have taken up the investigation of in-vitro antioxidant activity of *Tephrosia purpurea* leaves in DPPH free radical scavenging, and nitric oxide scavenging methods. The ethanol extract showed good antioxidant activity in these above methods. This activity may be due to the presence of flavonoids. Bhaskar Rao et al, (2007) demonstrated that *T.purpurea* possess significant levels of enzymatic antioxidants, non-enzymatic antioxidants and also exhibits antioxidant capacity. Patel et al, (2010) showed that the
ethanolic extract of the leaves of *T. purpurea* possesses the antioxidant substance which may be potentially responsible for the treatment of jaundice and other oxidative stress related diseases. The aqueous and the ethanolic root extracts of the plant showed potential in vitro antioxidant activity by DPPH assay and nitric oxide scavenging assay (Sonawane et al, 2010). The hydroalcoholic root extract of *T. purpurea* have marked amount of total phenols which could be responsible for the marked antioxidant activity in DPPH assay, superoxide free radical activity and nitric oxide scavenging activity (Shah et al, 2010). *T. purpurea* root extract exhibited significant anti-oxidant activity in various *in vitro* assays like ABTS, DPPH, FRAP and ORAC assays. The root extract also showed potent xanthine oxidase inhibition activity which may be useful in the treatment of hyperuricemia associated with gout and kidney diseases (Nile and Khobragade, 2011).

**Anti-inflammatory and analgesic activity**

The ethanolic extract of *T. purpurea* showed significant anti inflammatory activity in subacute inflammation using cotton peller granuloma model but did not show any effect in acute inflammation (carrageenan induced rat paw edema) (Shenoy et al, 2010). Gopalakrishnan et al, (2010) also demonstrated significant anti inflammatory and analgesic activity of the ethanolic extracts of aerial and root parts of *T. purpurea*. Five different fractions of *T. purpurea* was evaluated for analgesic activity using acetic acid induced writhing in mice and tail flick test in rats and for anti inflammatory activity using carrageenan induced rat paw edema and cotton pellet granuloma formation in rats out of which two fractions showed potent activity (Gulecha et al, 2011). The ethanol, ethyl acetate, chloroform and petroleum ether extracts of *T. purpurea* root demonstrated potent antipyretic activity in 20% Brewer's yeast induced pyrexia model and anti-inflammatory activity in 1% carrageenan induced paw edema in albino rats. Ethanol extract of 500 mg/kg possessed higher antipyretic activity as that of standard paracetamol.
and also higher anti-inflammatory activity than diclofenac sodium (Valli et al, 2011a).

**Membrane stabilizing potency**

Gokhale et al (2000) reported the ethanolic extract of *T.purpurea* for its *in-vitro* effect on rat mast cell degranulation and erythrocyte membrane integrity *in-vitro*. The extract in concentration of 25-200 μg/ml showed a dose-dependant inhibition of rat mast cell degranulation induced by compound 48/80 and egg albumin. *T.purpurea* extract was found to inhibit haemolysis of erythrocytes induced by hypotonic solution but accelerated haemolysis induced by heat at a concentration of 100 μg/ml. The studies reveal that the ethanolic extract of *T. purpurea* may inhibit degranulation of mast cells by a mechanism other than membrane stabilization. The chloroform, ethyl acetate and methanolic extracts of the root of *T.purpurea* was evaluated for *in vitro* anti inflammatory activity by means of HRBC membrane stabilizing method. The result showed significant HRBC membrane stabilization with regard to standard hydrocortisone (Sandhya et al, 2010).

**Antiallergic Activity:**

The ethanolic extract of the aerial parts of *T. purpurea* showed potential antiallergic activity. The extract at doses of 25, 50 and 100 μg/ml showed dose-dependent inhibition of rat mast cell degranulation *in vitro* induced by compound 48/80 and egg albumin. At doses of 50, 100 and 200mg/kg, it significantly inhibited systemic anaphylaxis and active paw anaphylaxis in mice and passive pay anaphylaxis in rats. The extract significantly inhibited histamine release during passive peritoneal anaphylaxis in rats. The extract at doe of 200mg/kg significantly protected guinea pigs against histamine induced bronchoconstriction. The extract showed significant inhibition of enzyme soyabean lipoxidase at dose of 200μg/ml (Gokhale and Saraf 2000).
Review of Literature

Immunomodulatory activity

Oral administration of flavonoid fraction of *T.purpurea* (10–40 mg/kg) significantly inhibited sheep red blood cells-induced delayed type hypersensitivity reactions. It also produced a significant, dose-related decrease in sheep erythrocyte-specific haemagglutination antibody titre. However, the fraction failed to show a significant change in the macrophage phagocytic activity. These results suggest the ability of the flavonoidal fraction of *T. purpurea* to modulate both the cell-mediated and the humoral components of the immune system (Damre et al, 2003). Moreover, the methanolic extract of aerial parts of *T.purpurea* also showed significant immunomodulatory activity evaluated by carbon clearance and WBC count method (Jain et al, 2010).

Anti-epileptic activity

Asuntha et al (2010) studied the anti-epileptic activity of *T.purpurea* in *Status epilepticus* induced in rats by administration of pilocarpine after lithium chloride. The results of lithium-pilocarpine induced *status epilepticus* model demonstrated that the ethanolic extract of *Tephrosia purpurea* has significant ability in reducing the severity of *status epilepticus* and also possess both *in vitro* and *in vivo* antioxidant activity.

Anxiolytic activity

Sathish Kumar et al, (2011) studied the anxiolytic activity of a hydroalcoholic extract of *Tephrosia purpurea* in mice using the elevated plus-maze, elevated zero-maze, Y-maze and hole-board models. The results indicate that hydroalcoholic extract of *T.purpurea* having anxiolytic activity and phytochemical screening revealed the presence of saponins and flavonoids. It may possible that the mechanism of anxiolytic action of plant could be due to the binding of any of these phytoconstituents to the GABA-BZD complex.
Datta et al (1997) examined "Yakrifit" (a polyherbal product in which *T. purpurea* is a part), for its appetite stimulant activity. All the animals recovered in 3 to 7 days, regained appetite for food and water and their general condition had improved.

Root exudates from *T.purpurea* contained a rock-iron [Fe(OH)$_3$] - solubilizing compound. The lyophilized purified fraction of it showed maximum Fe(OH)$_3$-solubilizing activity at 50°C under acidic pH (Agg rawal et al, 1999).

Soni et al (2004) have investigated the spasmolytic activity of ethanol extract of *Tephrosia purpurea* on guinea pigs trachea. The results of experiments clearly showed the spasmolytic activity of the drug. The preliminary phytochemical investigation, however shows the presence of glycosides and saponins may be responsible for this activity.

The flavonoids isolated from *T.purpurea* showed more than 90 mortality within 24 hr. period to *Callosobruchus maculatus* grubs, which feeds upon *Phaseolus mungo* indicating potent insecticidal property (Diwan and Saxena, 2010).

The ethanol, ethyl acetate, chloroform and petroleum ether extracts of *T.purpurea* root showed significant CNS depressant activity evaluated using photoactometer (Valli et al, 2011b).

**Formulations containing *T.purpurea***

*T.purpurea* containing formulations are prescribed in ayurveda mainly as liver correctives and restoratives. They contain aqueous or alcoholic extracts of *T.purpurea*. They are found to be effective in treating various disorders (Handa et al, 1989; Sharma et al, 1991; Doreswamy and Sharma, 1995) like

1. Alcoholic liver cirrhosis
2. Viral hepatitis
3. Pre-cirrhotic conditions
4. Protein energy malnutrition
5. Radiation and chemotherapy induced liver damage
6. As an adjuvant with hepatotoxic drugs like antitubercular drugs
7. Urinary tract anti-infective
8. Antibacterial in acne vulgaris and acts as a blood purifier

Though *Tephrosia* is just one of the many ingredients of these formulations, it supports the effects of other herbs and produces a synergistic effect that potentiates the effect of the final product.

### Table 3.6: Some marketed formulations containing *T.purpurea* (Mathews et al, 2012)

<table>
<thead>
<tr>
<th>Product</th>
<th>Manufactured by</th>
<th>Form and content of <em>T.purpurea</em> extract</th>
<th>Uses</th>
</tr>
</thead>
<tbody>
<tr>
<td>G-LIV-D.S. syrup</td>
<td>Morpheme Remedies</td>
<td>200mg/200 ml syrup</td>
<td>Liver corrective and restorative</td>
</tr>
<tr>
<td>Stimuliv</td>
<td>FrancolIndia Ltd.</td>
<td>100mg/sugar coated tablet</td>
<td>For supportive treatment in viral hepatitis, drug induced and alcoholic hepatitis</td>
</tr>
<tr>
<td>Dilapsin</td>
<td>Solumiks</td>
<td>100mg/tablet, 100mg/ml syrup</td>
<td>Digestive, improves appetite, relieves flatulence</td>
</tr>
<tr>
<td>Safi</td>
<td>Hamdard Laboratories</td>
<td>18.06mg/5 ml syrup</td>
<td>Skin diseases like acne vulgaris, skin rashes and blemishes, boils.</td>
</tr>
<tr>
<td>Vimliv Fortified syrup</td>
<td>Solumiks herbaceutical products</td>
<td>25 mg/5ml syrup</td>
<td>Comprehensive liver tonic</td>
</tr>
<tr>
<td>Medicine</td>
<td>Company</td>
<td>Dosage</td>
<td>Uses</td>
</tr>
<tr>
<td>-------------------------</td>
<td>--------------------------------</td>
<td>-------------------------------</td>
<td>----------------------------------------------------------------------</td>
</tr>
<tr>
<td>Vasuliv syrup</td>
<td>Vasu pharmaceuticals</td>
<td>12mg/10ml syrup, 360mg/tablet</td>
<td>Liver corrective and Protective</td>
</tr>
<tr>
<td>Hibril oil</td>
<td>Vital care Pvt Ltd</td>
<td>1% Hair oil</td>
<td>Relieves stress and provides cooling effect. Induces sleep</td>
</tr>
<tr>
<td>Janduna capsules</td>
<td>Ajmera Pharmaceuticals Pvt Ltd</td>
<td>50mg/capsule</td>
<td>UT infection, expels urinary stones. Relieves burning micturition.</td>
</tr>
<tr>
<td>Livina syrup</td>
<td>Deys Medical Stores Mfg,Ltd</td>
<td>50mg/tablet, 100mg/5ml syrup</td>
<td>Viral hepatitis, Jaundice</td>
</tr>
<tr>
<td>Stomyne capsules</td>
<td>Eisen Pharmaceutical Co Pvt Ltd</td>
<td>50mg/capsule</td>
<td>UT infection</td>
</tr>
<tr>
<td>Tefroliv</td>
<td>TTK Healthcare Ltd</td>
<td>60mg/5ml syrup, 120mg/tablet</td>
<td>Acute and chronic hepatitis, liver cirrhosis.</td>
</tr>
<tr>
<td>NewLivfit</td>
<td>NLF</td>
<td>Syrup</td>
<td>Management of hepatitis B in end stage renal disease</td>
</tr>
<tr>
<td>Livex</td>
<td>Ban</td>
<td>Drops, syrup, tablet, capsule</td>
<td>Liver corrective, protective and regenerative</td>
</tr>
<tr>
<td>Hepjaun</td>
<td>S.G Phytopharma Pvt Ltd</td>
<td>Syrup, Capsules</td>
<td>Hepatitis and jaundice</td>
</tr>
</tbody>
</table>