Chapter-1

Introduction

1.1. Diabetes Mellitus

Diabetes mellitus is actually a group of metabolic diseases characterized by hyperglycemia resulting from defects in insulin secretion, insulin action or both. The World Health Organization (WHO) estimated 135 million diabetics in 1995 and this number would increase to 300 million by the year 2025. Much of this increase will occur in developing countries and will be due to population growth, ageing, unhealthy diets, obesity and sedentary lifestyles. Over 20 million people are affected by diabetes in India. These numbers are expected to increase to 57 million by 2025. In the 1970s, the prevalence of diabetes among urban Indians was reported to be 2.1 per cent and this has now risen to 12.1 per cent (Pradeepa and Mohan, 2002). People with diabetes are 25 times more likely to develop blindness, 17 times more likely to develop kidney disease, 30-40 times more likely to undergo amputation, two to four times more likely to develop myocardial infarction and twice more likely to suffer a stroke than non-diabetics (Pradeepa et al., 2002).

1.2. Classification

The first widely accepted classification of diabetes mellitus was published by WHO in 1980 (World Health Organization, 1980), and, in modified form, in 1985 (World Health Organization, 1985). The 1985 classification was widely accepted and used internationally which includes both staging of diabetes mellitus based on clinical descriptive criteria and a complimentary etiological classification. Diabetes mellitus is typically classified into two main subtypes: type-1 or insulin-dependent diabetes (IDDM), and type-2 or non-insulin-dependent diabetes (NIDDM).

Type 1 diabetes (formerly known as insulin-dependent) in which pancreas fails to produce insulin primarily due to pancreatic islet beta cell destruction and it is more prone to ketoacidosis for which neither etiology nor pathogenesis is known (idiopathic). This form develops most frequently in children and

adolescents, but is being increasingly noted later in life and is attributable to an autoimmune process.

Type 2 diabetes (formerly named non-insulin-dependent) which results from defect(s) in insulin secretion almost always with a major contribution from insulin resistance, the body's inability to respond properly to the action of insulin. Type 2 diabetes is much more common and accounts for around 90% of all diabetes cases worldwide. It occurs most frequently in adults, but is being noted increasingly in adolescents as well.

Certain genetic markers have been shown to increase the risk of developing Type 1 diabetes. Type 2 diabetes is strongly familial, but it is only recently that some genes have been consistently associated with increased risk for Type 2 diabetes in certain populations. Both types of diabetes are complex diseases caused by mutations in more than one gene, as well as by environmental factors.

People with Type 1 diabetes are usually totally dependent on insulin injections for survival. Such people require daily administration of insulin. The majority of people suffering from diabetes have the Type 2 form. Although they do not depend on insulin for survival, about one third of sufferers need insulin for reducing their blood glucose levels.

Other forms of diabetes include:

- Gestational diabetes.
- Genetic defects of β cell function.
- Genetic defects in insulin action.
- Diseases of the exocrine pancreas.
- Endocrinopathies.
- Drug or chemical induced.
- Infections.
- Uncommon forms of immune mediated diabetes.
- Other genetic syndromes sometimes accompanied with diabetes.

Diabetes in pregnancy may give rise to several adverse outcomes, including congenital malformations, increased birth weight and an elevated risk of perinatal mortality. Strict metabolic control may reduce these risks to the level of those of non-diabetic expectant mothers.

1.3. Diagnostic criteria for diabetes mellitus

Fasting plasma sugar (FPS), postprandial plasma sugar (PP_2PS) and oral glucose tolerance test (OGTT) are the golden criteria for the diagnosis of diabetes mellitus. Generally FPS after overnight fasting and PP_2PS, two hour after a meal is routinely checked for the diagnosis. OGTT is recommended in case where person is not frank diabetic.

Recently new classification and diagnostic criteria for diabetes were proposed by the American Diabetes Association (ADA), WHO and Japan Diabetes Society (JDS) between 1997 and 1999. Diabetes is classified in to four etiological categories; type 1, type 2, diabetes due to other specific mechanism or conditions and gestational diabetes. Following plasma glucose levels [fasting plasma glucose (FPS) 2-h plasma glucose in the 75g oral glucose tolerance test (2-hPG)] been suggested for the diagnosis of diabetes:

Normal type - FPS < 6.1 mmol/L (110 mg/dl) and 2-hBG < 7.7 mmol/L (140 mg/dl)

Borderline type - FPS > 6.1 mmol/L (110 mg/dl) or = < 7.0 mmol/L (126 mg/dl)
PP_2PS>7.7 mmol/L (140mg/dl) or = <11.1 mmol/L (200 mg/dl)

Diabetic type - FPS > 7.0 mmol/L (126 mg/dl) and PP_2PS > 11.1 mmol/L (200 mg/dl)

Borderline corresponds to the sum of impaired fasting glycemia (IFG) and impaired glucose tolerance (IGT) based on ADA and WHO criteria.

1.3.1 Prognostic criteria - Prognosis of the disease is monitored by conducting following tests

Decreased blood pH - < 7.4 is the indicators of acidosis.

ketone body in serum > 1mEq/L can lead to acetone breath and ketoacidosis.

Microalbuminuria and proteinuria are the indicators of diabetic nephropathy.

Diabetic retinopathy is detected clinically by presence of visible opthalmoscopic retinal microvascular lesions and estimation of Advanced Glycated End products (AGEs).

1.4. Diabetes mellitus – consequence of insulin deficiency/resistance

The defect in insulin deficiency or insulin resistance leads to hyperglycemia the hallmark of the X syndrome i.e. diabetes mellitus. As β cell destruction progresses in case of IDDM, plasma insulin levels fall even during the fasted state, hepatic glucose production increases, and the patient requires insulin therapy (Eisenbarth et al., 1987). With more severe insulin deficiency, plasma FFA levels increase in response to enhanced lipolysis and plasma triglyceride levels may increase because of a decrease in lipoprotein lipase activity (Ong and Kern, 1989). Deficiency of insulin and/or increase in counter-insulin hormones is sufficiently severe to increase glycogen, protein and lipid catabolism; leading to elevated plasma FFA and ketone body levels. Whereas an earlier abnormality in NIDDM is hyperinsulinemia associated with insulin resistance (Warram et al., 1990). Patients with type 2 diabetes and obesity have been shown to have a significant defect in glucose uptake in skeletal muscle (DeFronzo, 1992), a decrease in muscle glycogen synthesis (DeFronzo et al., 1985), and an increase in lactate production (Bogardus et.al., 1984). Such inhibition of glycogen synthesis causes an increase in glucose-6-phosphate, suggesting a defect in glucose uptake or hexokinase step. Decrease in pyruvate dehydrogenase activity is also seen contributing to the decrease in glucose oxidation and increase in muscle lactate release (Mandarino et al., 1986). Hyperglycemia may worsen insulin resistance leading to "glucose toxicity" (Rossetti et al., 1990). Insulin has a profound effect on protein turnover through its dual role as a stimulator of protein synthesis and

an inhibitor of protein degradation. In conditions where insulin is lacking, total body protein is lost, particularly evident as wasting of muscle. Hence any failure in insulin function leads to impaired carbohydrate, fat and protein metabolism. As a result, elevated levels of glucose in the plasma, free fatty acids, triglycerides, cholesterol, VLDL, ketone bodies, etc. are encountered. All these consequences lead to oxidative stress and the accompanied diabetic complications.

1.5. Prevalence of diabetes

The prevalence of diabetes is rapidly rising all over the globe at an alarming rate (Huizinga and Rothman, 2006). Over the past 30 years, the status of diabetes has changed from being considered as a mild disorder of the elderly to one of the major causes of morbidity and mortality affecting the youth and middle aged people. It is important to note that the rise in prevalence is seen in all six inhabited continents of the globe (Wild et al., 2004). Although there is an increase in the prevalence of type 1 diabetes also, the major driver of the epidemic is the diabetes, which accounts for more than 90 percent of all diabetes cases. Nowhere is the diabetes epidemic more pronounced than in India as the World Health Organization (WHO) reports show that 32 million people had diabetes in the year 2000 (Wild et al., 2004). The International Diabetes Federation (IDF) estimates the total number of diabetic subjects to be around 40.9 million in India and this is further set to rise to 69.9 million by the year 2025 (Sicree et al., 2006) (Fig. 1.1).

1.6. Evolution of the diabetes epidemic in India

The first national study on the prevalence of type 2 diabetes in India was done between 1972 and 1975 by the Indian Council Medical Research (ICMR, New Delhi) (Ahuja, 1979). Screening was done in about 35,000 individuals above 14 yr of age, using 50 g glucose load. Capillary blood glucose level >170 mg/dl was used to diagnose diabetes. The prevalence was 2.1 per cent in urban population and 1.5 per cent in the rural population while in those above 40 yr of age, the prevalence was 5 per cent in urban and 2.8 per cent in rural areas.

Subsequent studies showed a rising trend in the prevalence of diabetes across different parts of India.

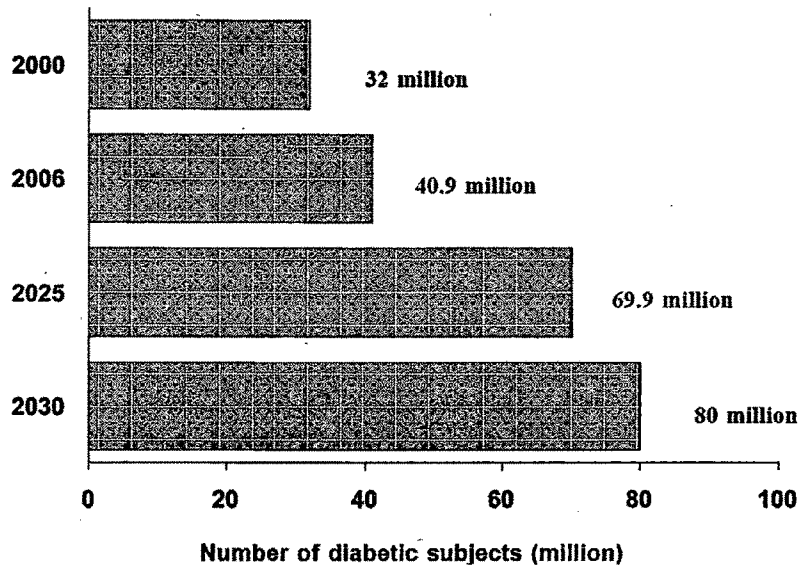


Figure 1.1: Estimated number of diabetic subjects in India.

Source: Ref. - Wild et al., 2004; Sicree et al., 2006

In 1988, a study done in a small township in south India reported a prevalence of 5 per cent (Ramachandran et al., 1988). The prevalence of impaired glucose tolerance in the same study was 2 per cent. A national rural diabetes survey was done between 1989 and 1991 in different parts of the country in selected rural populations (Sridhar et al., 2002). This study which used the 1985 WHO criteria to diagnose diabetes, reported a crude prevalence of 2.8 per cent (Sridhar et al., 2002). The Eluru survey which looked at the prevalence of known diabetes in four villages in Andhra Pradesh showed a prevalence of 1.5 per cent. The prevalence of known diabetes was 6.1 per cent in individuals aged above 40 yr which was unexpectedly high at that time for a rural area with low socio-economic status and decreased health awareness (Rao et al., 1989). A study done in 1988 in Chennai reported a prevalence of 8.2 per cent in the urban and 2.4 per cent in the rural areas (Ramachandran et al., 1998). A subsequent study in the same urban area done after five years showed an age standardized prevalence of

11.6 per cent indicating a rising trend in prevalence of diabetes (Ramachandran et al., 1997). A very high prevalence of 16.3 per cent was reported in Thiruvanathapuram in Kerala State in the year 1999 (Raman et al., 1999). In the same year, a prevalence of 8.2 per cent was reported from Guwahati (Shah et al., 1999). A cross-sectional population survey was done in the Kashmir valley in 2000 and the prevalence of 'known diabetes' among adults aged >40 yr was found to be 1.9 per cent (Zargar et al., 2000).

The National Urban Diabetes Survey (NUDS), a population based study was conducted in six metropolitan cities across India and recruited 11,216 subjects aged 20 yr and above representative of all socio-economic strata (Ramachandran et al., 2001). An oral glucose tolerance test was done using capillary glucose and diabetes was defined using the WHO criteria (Alberti et al., 1998). The study reported that the age standardized prevalence of type 2 diabetes was 12.1 per cent. This study also revealed that the prevalence in the southern part of India to be higher-13.5 per cent in Chennai, 12.4 per cent, in Bangalore, and 16.6 per cent Hyderabad; compared to eastern India (Kolkatta), 11.7 per cent; northern India (New Delhi), 11.6 per cent; and western India (Mumbai), 9.3 per cent. The study also suggested that there was a large pool of subjects with impaired glucose tolerance (IGT), 14 per cent with a high risk of conversion to diabetes. The study done in western India showed, age standardized prevalence of 8.6 per cent in urban population (Gupta et al., 2003). A more recent study reported a high prevalence (9.3%) in rural Maharashtra (Deo et al., 2006). The Amrita Diabetes and Endocrine Population Survey (ADEPS) (Menon et al., 2006), a community based cross-sectional survey done in urban areas of Ernakulam district in Kerala has revealed a very high prevalence of 19.5 per cent (Fig. 1.2).

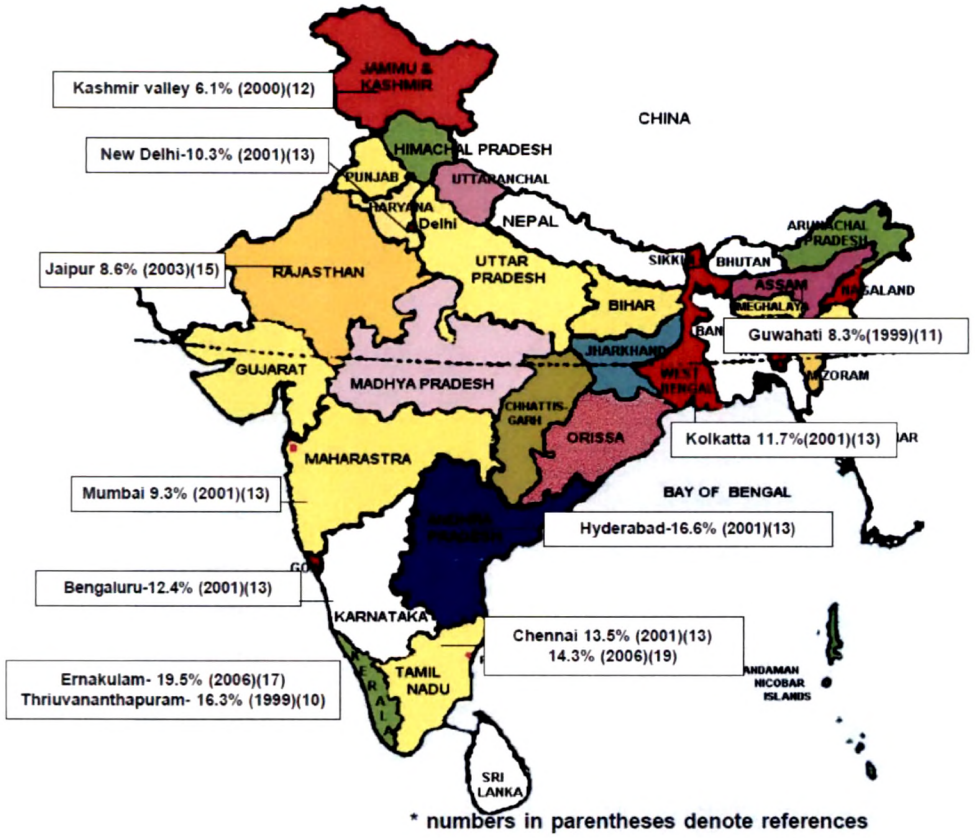


Figure 1.2: Recent population based studies showings the prevalence of type 2 diabetes in different parts of India.

Source: Raman et al., 1999; Shah et al., 1999; Zargar et al., 2000; Ramachandran et al., 2001; Gupta et al., 2003; Menon et al., 2006; Mohan et al., 2006.

1.6.1 Undiagnosed diabetes - the hidden danger

It is important to note that the studies that have shown an increase in prevalence of diabetes have also reported a very high prevalence of undiagnosed diabetes in the community. While in Chennai Urban Rural Epidemiology Study (CURES), the prevalence of known diabetes was 6.1 per cent that of undiagnosed diabetes was 9.1 per cent (Mohan et al., 2006). Similarly, in the Diabetes and Endocrine Population Survey (ADEPS), the prevalence of known and undiagnosed diabetes was 9.0 and 10.5 per cent respectively (Menon et al., 2006). The Kashmir valley study showed that the prevalence of undiagnosed diabetes

was 4.25 per cent, which was more than double to that of the known diabetes (1.9%), (Zargar et al., 2002). The individuals who are unaware of their disease status are left untreated and are thus more prone to microvascular as well as macrovascular complications. Hence, it is necessary to detect the large pool of undiagnosed diabetic subjects in India and offer early therapy to these individuals.

1.7. Urban-rural differences in diabetes prevalence

Urban rural differences in the prevalence of diabetes have been consistently reported from India. The ICMR study reported that the prevalence was 2.1 per cent in urban and 1.5 per cent in rural areas (Ahuja et al., 1979), a later study showed that the prevalence was three times higher among the urban (8.2%), compared to the rural population (2.4%), (Ramachandran et al., 1998). A study done in southern Kerala looked at the variations in the prevalence of type 2 diabetes among different geographic divisions within a region (Kutty et al., 2000). The prevalence of diabetes was the highest in the urban (12.4%) areas, followed by the midland (8.1%), highland (5.8%) and coastal division (2.5%).

1.8. Burden of diabetes related complications in India

Both macrovascular and microvascular complications cause significant morbidity and mortality among diabetic subjects (Zargar et al., 1999). The Chennai Urban Population Study (CUPS) and CURES provided valuable data from India on the complications related to diabetes. The prevalence of coronary artery disease (CAD) was 21.4 per cent among diabetic subjects compared to 9.1 per cent in subjects with normal glucose tolerance (Mohan et al., 2001). The prevalence of CAD in IGT subjects were 14.9 per cent in the same study. It was also seen that the diabetic subjects had increased subclinical atherosclerosis as measured by intimal medial thickness (IMT) at every age point compared to subjects with normal glucose tolerance (Mohan et al., 2000). A recent study showed that carotid intima medial thickness increased with worsening grades of

glucose tolerance as well as with increase in the number of components of metabolic syndrome (Mohan et al., 2006).

The prevalence of peripheral vascular disease (PVD) was 6.3 per cent among diabetic subjects compared to 2.7 per cent in non-diabetic subjects (Premalatha et al., 2000), and these figures are lower than the prevalence reported in western populations (Melton et al., 1980). This is probably due to lower age at onset for diagnosis of type 2 diabetes in India. It is well known that PVD is more common in older individuals. The CURES Eye study is the largest population based data on the prevalence of diabetic retinopathy done in India. This study showed that the overall prevalence was 17.6 per cent, which was lower when compared to the reports from the West (Rema et al., 2005). A recent population based study reported that the prevalence of overt nephropathy was 2.2 per cent in Indians while microalbuminuria was present in 26.9 per cent. Glycated haemoglobin, duration of diabetes and systolic blood pressure were independently associated with diabetic nephropathy. Overall, Asian Indians appear to have a greater redilection for cardiovascular complications whereas the prevalence of microvascular complications appears to be lower than in Europeans. A recent follow up of the original CUPS cohort showed that the overall mortality rates were nearly three-fold higher (18.9 per 1000 person-years) in people with diabetes compared to non diabetic subjects (5.3 per 1000 person-years, P=0.004) (Mohan et al., 2006). The hazard ratio (HR) for all cause mortality for diabetes was found to be 3.6 compared to non diabetic subjects. The study also showed that mortality due to cardiovascular (diabetic subjects: 52.9% vs. non diabetic subjects 24.2%, P=0.042) and renal (diabetic subjects 23.5% vs. non diabetic subjects 6.1%, P=0.072) causes was higher among diabetic subjects (Fig.1.3).

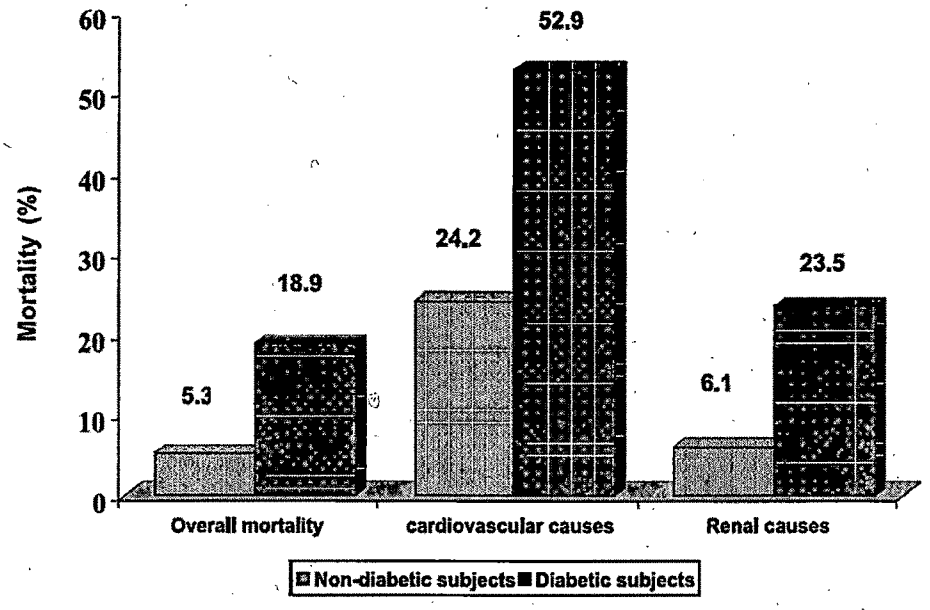


Figure 1.3: Differences in mortality rates among diabetic and non-diabetic individuals, the Chennai urban population study (CUPS).

Source: Mohan et al., 2006.

1.9. The pathobiology of diabetic complications

The general features of hyperglycemia-induced tissue damage are shown schematically in Fig. 1.4. The DCCT (Diabetes Control and Complications Trial) and the UKPDS (U.K. Prospective Diabetes Study) established that hyperglycemia, shown on the far left of the figure, is the initiating cause of the diabetic tissue damage that we see clinically, shown on the far right (The Diabetes Control and Complications Trial Research Group, 1993; UK Prospective Diabetes Study (UKPDS) Group, 1998). Although this process is modified by both genetic determinants of individual susceptibility, shown in the top box, and by independent accelerating factors such as hypertension, shown in the bottom box (Fig. 1.4). The mechanisms that mediate the tissue-damaging effects of hyperglycemia refers to a particular subset of cell types: capillary endothelial cells in the retina, mesangial cells in the renal glomerulus, and neurons and Schwann cells in peripheral nerves. These cells are more vulnerable to hyperglycemia and are involved in diabetic complications. Most of the cells are

able to reduce the transport of glucose inside the cell when they are exposed to hyperglycemia, so that their internal glucose concentration stays constant. In contrast, the cells damaged by hyperglycemia are those that cannot do this efficiently (Kaiser et al., 1993; Heilig et al., 1995). Thus, diabetes selectively damages cells, like endothelial cells and mesangial cells, whose glucose transport rate does not decline rapidly as a result of hyperglycemia, leading to high glucose inside the cell. Thus complications must involve mechanisms going on inside these cells, rather than outside. The first such mechanism that was discovered was the polyol pathway and increased polyol pathway flux, described in peripheral nerve (Gabbay et al., 1966). Then, 10 years later, in the late 1970s, a second piece of the puzzle emerged: increased formation of Advanced Glycation End products (AGEs). In the late 1980s and early 1990s, it was discovered: hyperglycemia-induced activation of protein kinase C (PKC) isoforms, and in the late 1990s, a fourth metabolic alteration was discovered: increased hexosamine pathway flux and consequent overmodification of proteins by *N*-acetylglucosamine.

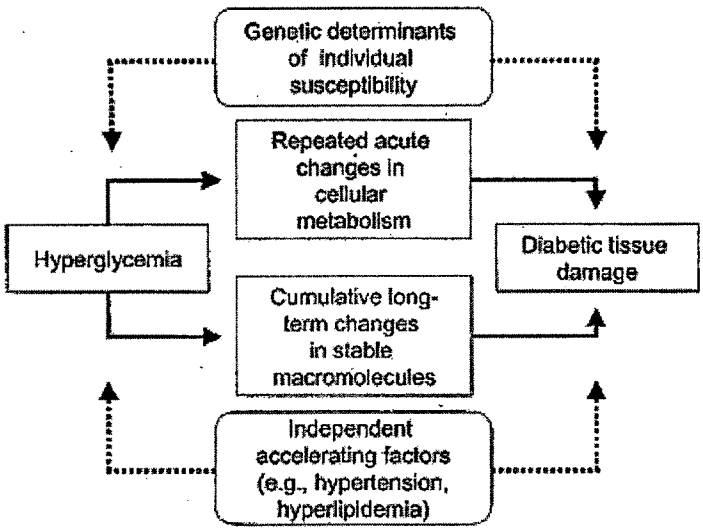


Figure 1.4: General features of hyperglycemia-induced tissue damage.

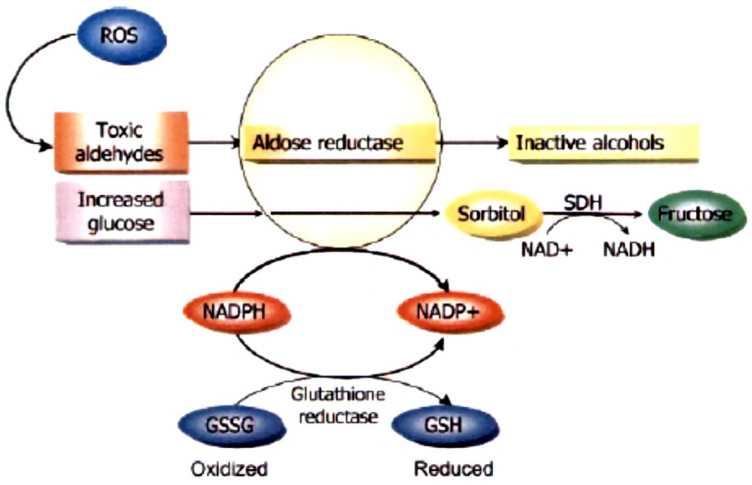


Figure 1.5: Hyperglycemia increases flux through the polyol pathway.

Source: Brownlee M: Biochemistry and cell biology of diabetic complications. Nature 414:813-820, 2001.

1.10. Increased flux through the polyol pathway.

The polyol pathway, shown schematically in Fig. 1.6, focuses on the enzyme aldose reductase. Aldose reductase normally has the function of reducing toxic aldehydes in the cell to inactive alcohols, but when the glucose concentration in the cell becomes too high, aldose reductase also reduces that glucose to sorbitol, which is later oxidized to fructose. In the process of reducing high intracellular glucose to sorbitol, the aldose reductase consumes the cofactor NADPH. Shown in Fig. 1.5, NADPH, which is also the essential cofactor for regenerating a critical intracellular antioxidant, reduced glutathione. By reducing the amount of reduced glutathione, the polyol pathway increases susceptibility to intracellular oxidative stress. Studies conducted by Ron Engerman and Tim Kern (Engerman et al., 1994), in which diabetic dogs were treated for 5 years with an aldose reductase inhibitor. Nerve conduction velocity in the diabetic dogs decreased over time as it does in patients. In contrast, in diabetic dogs treated with an aldose reductase inhibitor, the diabetes-induced defect in nerve conduction velocity was prevented.

1.11. Intracellular production of AGE precursors.

The second discovery listed is the intracellular production of AGE precursors. As shown schematically in Fig. 1.6, these appear to damage cells by three mechanisms. The first mechanism, shown at the top of the endothelial cell, is the modification of intracellular proteins including, most importantly, proteins involved in the regulation of gene transcription (Giardino et al., 1994; Shinohara et al., 1998). The second mechanism, shown on the left, is that these AGE precursors can diffuse out of the cell and modify extracellular matrix molecules (McLellan et al. 1994), which changes signaling between the matrix and the cell and causes cellular dysfunction (Charonis et al., 1990). The third mechanism, shown on the right of 1.6, is that these AGE precursors diffuse out of the cell and modify circulating proteins in the blood such as albumin. These modified circulating proteins can then bind to AGE receptors and activate them, thereby causing the production of inflammatory cytokines and growth factors, which in turn cause vascular pathology (Li et al., 1996; Neeper et al., 1992; Smedsrod et al., 1997; Vlassara et al., 1995; Abordo et al., 1997; Doi et al., 1992; Kirstein et al., 1992; Schmidt et al., 1995; Skolnik et al., 1991; Vlassara et al., 1988). Animal studies done by Hans-Peter Hammes (Hammes et al., 1991), demonstrating that pharmacologic inhibition of AGEs prevents late structural changes of experimental diabetic retinopathy.

1.12. PKC activation.

The third mechanism was the PKC pathway. In this pathway, shown schematically in Fig. 1.7, hyperglycemia inside the cell increases the synthesis of a molecule called diacylglycerol, which is a critical activating cofactor for the classic isoforms of protein kinase-C, β, δ and α (Koya et al., 1998; DeRubertis et al., 1994; Xia et al., 1994). When PKC is activated by intracellular hyperglycemia, it has a variety of effects on gene expression, examples of which are shown in the row of open boxes in Fig. 1.7. In each case, the changes that are good for normal function are decreased and the changes that are bad are increased. For example, Fig. 1.7, the vasodilator producing endothelial nitric oxide (NO) synthase (eNOS)

is decreased, while the vasoconstrictor endothelin-1 is increased. Transforming growth factor β and plasminogen activator inhibitor-1 are also increased. At the bottom of the figure, the row of black boxes lists the pathological effects that may result from the abnormalities in the open boxes (Fig. 1.7), (Koya et al., 1997; Ishii et al., 1996; Kuboki et al., 2000; Studer et al., 1993; Feener et al., 1996). Several animal studies published by George King, showed that inhibition of PKC prevented early changes in the diabetic retina and kidney (Ishii et al., 1996; Bishara et al., 2002; Koya et al., 2000).

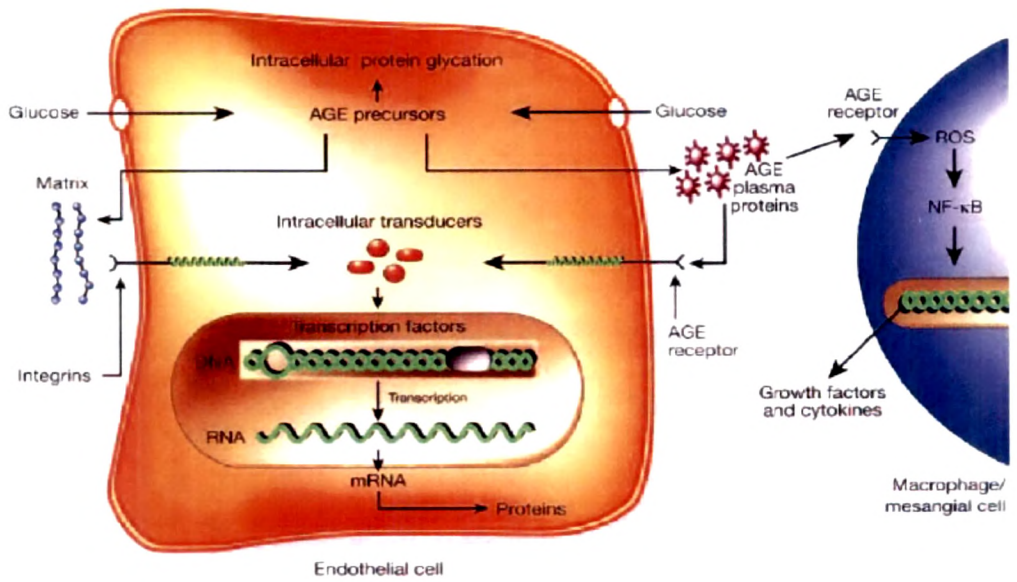


Figure 1.6: Increased production of AGE precursors and its pathologic consequences.

Source: Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001.

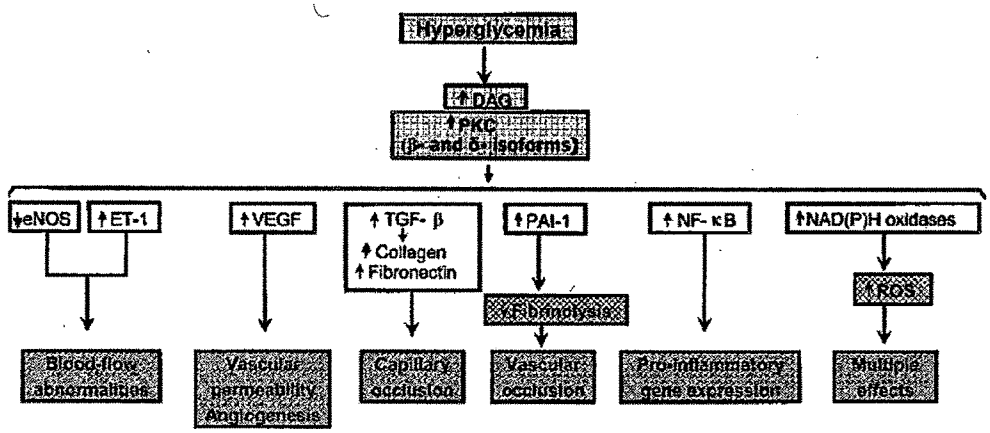


Figure 1.7: Consequences of hyperglycemia-induced activation of PKC.

1.13. Increased hexosamine pathway activity.

The last mechanism was increased flux through the hexosamine pathway. As shown schematically in Fig. 1.8, when glucose is high inside a cell, most of that glucose is metabolized through glycolysis, going first to glucose-6 phosphate, then fructose-6 phosphate, and then rest of the glycolytic pathway. However, some of fructose-6-phosphate gets diverted into a signaling pathway in which an enzyme called GFAT (glutamine:fructose-6 phosphate amidotransferase) converts the fructose-6 phosphate to glucosamine-6 phosphate and finally to UDP (uridine diphosphate) *N*-acetyl glucosamine. *N*-acetyl glucosamine then affect serine and threonine residues of transcription factors, just like the more familiar process of phosphorylation. Overmodification by this glucosamine often results in pathologic changes in gene expression (Kolm-Litty et al., 1998; Sayeski et al., 1996; Wells et al., 2003). For example, (Fig. 1.8), increased modification of the transcription factor Sp1 results in increased expression of transforming growth factor-β and plasminogen activator inhibitor-1, both of which are bad for diabetic blood vessels (Du et al., 2000). This pathway has been shown to play a role both in hyperglycemia-induced abnormalities of glomerular cell gene expression (Kolm-Litty et al., 1998) and in hyperglycemia-induced cardiomyocyte dysfunction in cell culture (Clark et al., 2003). In carotid artery plaques from

type 2 diabetic subjects, modification of endothelial cell proteins by the hexosamine pathway is also significantly increased (Federici et al., 2002).

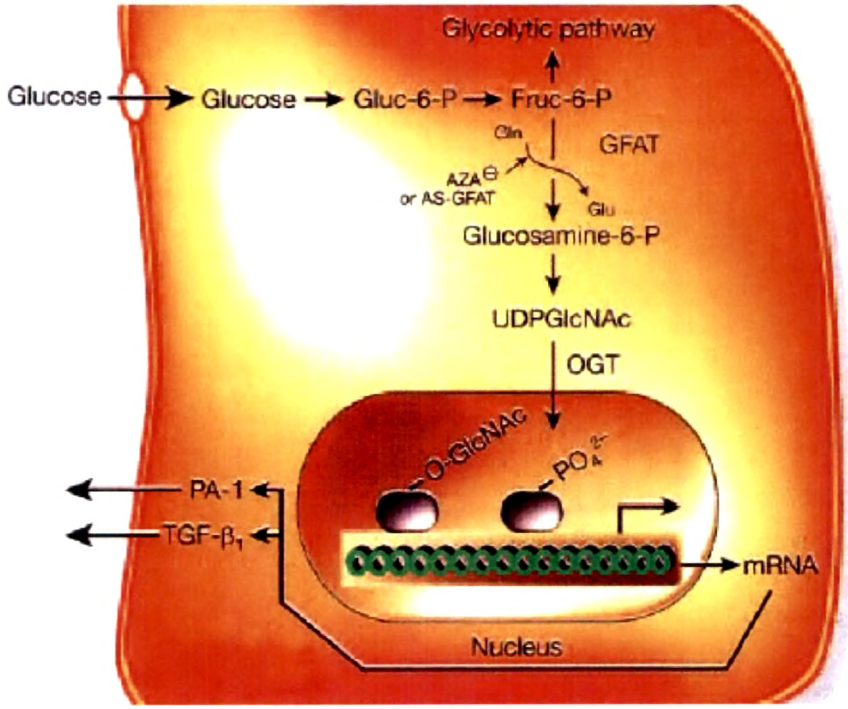


Figure 1.8: Hyperglycemia increases flux through the hexosamine pathway.

Source: Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001.

1.14. A unified mechanism

All of these different pathogenic mechanisms do reflect a single hyperglycemia-induced process and that this single unifying process is the overproduction of superoxide by the mitochondrial electron transport chain. People discovered that a consistent differentiating feature common to all cell types that are damaged by hyperglycemia is an increased production of reactive oxygen species (ROS), (Du et al., 2000, Nishikawa et al., 2000). Although hyperglycemia had been associated with oxidative stress in the early 1960s (Giugliano et al., 1996), neither the underlying mechanism that produced it nor its consequences for pathways of hyperglycemic damage were known.

1.13 How does hyperglycemia increase superoxide production by the mitochondria?

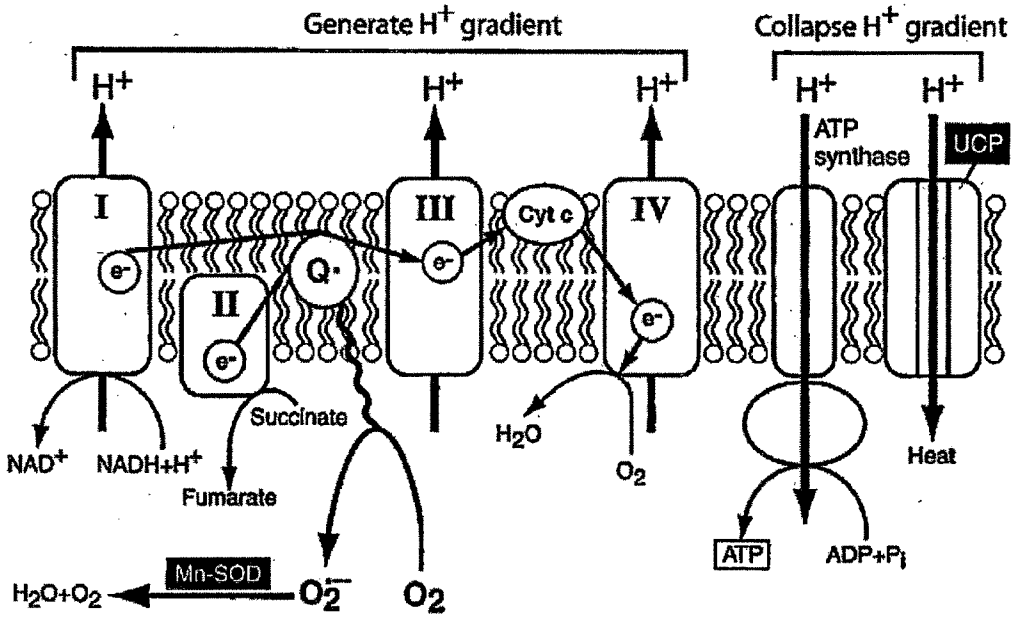


Figure 1.9: Hyperglycemia-induced production of superoxide by the mitochondrial electron transport chain.

There are four protein complexes in the mitochondrial electron transport chain, called complex I, II, III, and IV (Fig. 1.9). In normal cell when glucose is metabolized through the tricarboxylic acid (TCA) cycle, it generates electron donors. The main electron donor is NADH, which gives electrons to complex I. The other electron donor generated by the TCA cycle is $FADH_2$, formed by succinate dehydrogenase, which donates electrons to complex II. Electrons from both these complexes are passed to coenzyme Q, and then from coenzyme Q they are transferred to complex III, cytochrome-C, complex IV, and finally to molecular oxygen, which they reduce to water. The electron transport system is organized in this way so that the level of ATP can be precisely regulated. As electrons are transported from left to right in Fig. 1.9, some of the energy of those electrons is used to pump protons across the membrane at complexes I, III, and

IV. This generates what is in effect a voltage across the mitochondrial membrane. The energy from this voltage gradient drives the synthesis of ATP by ATP synthase (Wallace et al., 1992; Trumpower et al., 1990).

Alternatively, uncoupling proteins (UCPs; Fig. 1.9) can bleed down the voltage gradient to generate heat as a way of keeping the rate of ATP generation constant. In contrast, in diabetic cells with high glucose inside, there is more glucose being oxidized in the TCA cycle, which in effect pushes more electron donors (NADH and $FADH_2$) into the electron transport chain. As a result of this, the voltage gradient across the mitochondrial membrane increases until a critical threshold is reached. At this point, electron transfer inside complex III is blocked (Korshunov et al., 1997), causing the electrons to back up to coenzyme Q, which donates the electrons one at a time to molecular oxygen, thereby generating superoxide (Fig. 1.9). The mitochondrial isoform of the enzyme superoxide dismutase degrades this oxygen free radical to hydrogen peroxide, which is then converted to H_2O and O_2 by other enzymes. This was experimentally proved in cells with a dye that changes color with increasing voltage of the mitochondrial membrane and found that intracellular hyperglycemia indeed increase the voltage across the mitochondrial membrane above the critical threshold necessary to increase superoxide formation (Du et al., 2001). In order to prove further the electron transport chain indeed produces superoxide by the mechanism scientist examined the effect of overexpressing either UCP-1 or manganese superoxide dismutase (MnSOD). Hyperglycemia caused a big increase in production of ROS. In contrast, an identical level of hyperglycemia does not increase ROS at all when the mitochondrial voltage gradient collapsed by overexpressing UCP (Nishikawa et al., 2000). Similarly, hyperglycemia does not increase ROS at all when by overexpressing the enzyme MnSOD. Thus, UCP effect shows that the mitochondrial electron transport chain is the source of the hyperglycemia-induced superoxide. The MnSOD effect shows that the initial ROS formed is indeed superoxide.

In another independent experimental approach, mitochondrial DNA was depleted from normal endothelial cells, which lack a functional mitochondrial electron transport chain. When the mitochondrial electron transport chain is removed, the effect of hyperglycemia on ROS production is completely lost. Similarly, hyperglycemia completely fails to activate the polyol pathway, AGE formation, PKC, or the hexosamine pathway. Hyperglycemia did not activate any of the pathways when either the voltage gradient across the mitochondrial membrane was collapsed by UCP-1 or when the superoxide produced was degraded by MnSOD (Nishikawa et al., 2000). When wild-type animals are made diabetic, all four of the pathways are activated in tissues where diabetic complications occur. In contrast, when MnSOD transgenic mice are made diabetic, there is no activation of any of the four pathways. In endothelial cells, PKC also activates nuclear factor κB (NFκB), a transcription factor that itself activates many proinflammatory genes in the vasculature. As expected, hyperglycemia-induced PKC activation is prevented by either UCP-1 or MnSOD, both in cells and in animals. Importantly, inhibition of hyperglycemia-induced superoxide overproduction using a transgenic approach (superoxide dismutase [SOD]) also prevents long-term experimental diabetic nephropathy in *db/db* diabetic mouse (DeRubertis et al., 2004).

1.15. Hyperglycemia-induced mitochondrial superoxide production activates the four damaging pathways by inhibiting GAPDH.

Figure 10 shows the scheme based on a critical observation on diabetic animals, patients and hyperglycemia in cells, all decrease the activity of the key glycolytic enzyme glyceraldehyde- 3 phosphate dehydrogenase (GAPDH). Inhibition of GAPDH activity by hyperglycemia does not occur when mitochondrial overproduction of superoxide is prevented by either UCP-1 or MnSOD (Du et al., 2000). Inhibition of GAPDH activity increases the level of all the glycolytic intermediates that are upstream of GAPDH. Increased levels of the upstream glycolytic metabolite glyceraldehyde-3-phosphate activate the AGE

pathway because the major intracellular AGE precursor methylglyoxal is formed from glyceraldehyde-3 phosphate. It also activates the classic PKC pathway.

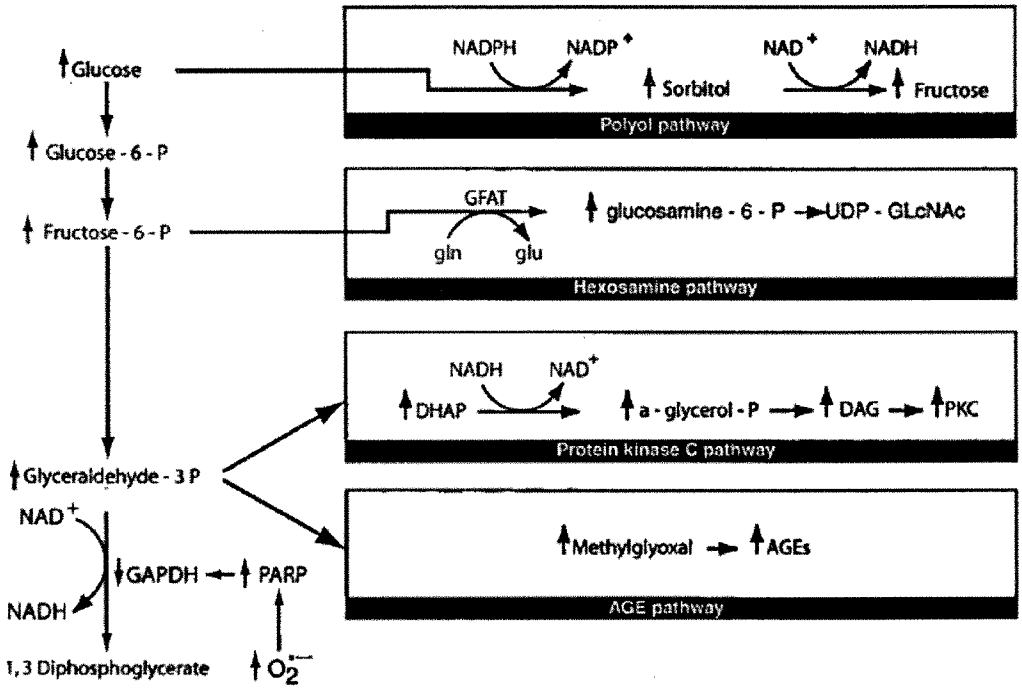


Figure 1.10: Mitochondrial overproduction of superoxide activates four major pathways of hyperglycemia damage by inhibiting GAPDH.

Source: Brownlee M: Biochemistry and molecular cell biology of diabetic complications. *Nature* 414:813–820, 2001.

Further upstream, increased levels of the glycolytic metabolite fructose-6 phosphate; increases flux through the hexosamine pathway. Finally, inhibition of GAPDH increases intracellular levels of the first glycolytic metabolite, glucose. This increases flux through the polyol pathway, where the enzyme aldose reductase reduces it, consuming NADPH in the process. This changes demonstrated by inhibition of GAPDH activity using antisense DNA (Du et al., 2003).

1.16. Hyperglycemia-induced mitochondrial superoxide production inhibits GAPDH by activating poly (ADP-ribose) polymerase.

Hyperglycemia activates the four major pathways of hyperglycemic damage through the overproduction of superoxide by the mitochondria, which then decreases GAPDH activity. In a test tube, superoxide itself directly inactivates GAPDH, but only at concentrations that far exceed levels found in hyperglycemic cells. Hyperglycemia-induced superoxide inhibits GAPDH activity in vivo by modifying the enzyme with polymers of ADP-ribose (Du et al., 2003), (Fig. 1.11). Most importantly, both modification of GAPDH by ADP-ribose and reduction of its activity by hyperglycemia were also prevented by a specific inhibitor of poly(ADP-ribose) polymerase (PARP), the enzyme that makes these polymers of ADP-ribose. This established a cause-and-effect relationship between PARP activation and the changes in GAPDH activity. When increased intracellular glucose generates increased ROS in the mitochondria, these free radicals induce DNA strand breaks, thereby activating PARP. Once activated, PARP splits the NAD molecule into its two component parts: nicotinic acid and ADP-ribose. PARP then proceeds to make polymers of ADP-ribose, which accumulate on GAPDH and other nuclear proteins. Although GAPDH is commonly thought to reside exclusively in the cytosol, in fact it normally shuttles in and out of the nucleus, where it plays a critical role in DNA repair (Sawa et al., 1997; Schmidtz et al., 2001). A schematic summary showing the elements of the unified mechanism of hyperglycemia-induced cellular damage is shown in Fig.1.12. When intracellular hyperglycemia develops in target cells of diabetic complications, it causes increased mitochondrial production of ROS. The ROS cause strand breaks in nuclear DNA, which activates PARP. PARP then modifies GAPDH, thereby reducing its activity. Finally, decreased GAPDH activity activates the polyol pathway, increases intracellular AGE formation, activates PKC and subsequently NF-κB, and activates hexosamine pathway flux.

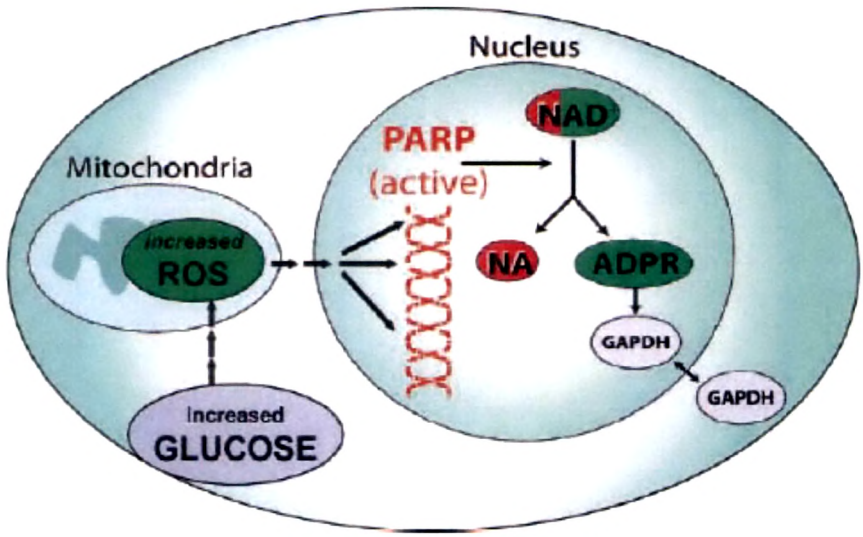


Figure 1.11: ROS-induced DNA damage activates PARP and modified GAPDH.

1.17. How does the unifying mechanism explain diabetic macrovascular disease?

In contrast to diabetic microvascular disease, data from the UKPDS have shown that hyperglycemia is not the major determinant of diabetic macrovascular disease. For microvascular disease end points, there is a nearly 10-fold increase in risk as HbA1c increases from 5.5 to 9.5%. In contrast, over the same HbA1c range, macrovascular risk increases only about twofold (UK Prospective Diabetes Study (UKPDS) Group, 1998). If hyperglycemia is not the major determinant of diabetic macrovascular disease, what about the constellation of risk factors associated with insulin resistance and the metabolic syndrome? In order to separate increased macrovascular disease risk due to insulin resistance and its associated abnormalities from increased risk due to hyperglycemia, the San Antonio Heart Study, demonstrated high insulin resistance increases cardiovascular risk by 2.5-fold. What is surprising, though, is that after adjustment for 11 known cardiovascular risk factors, including LDL, HDL, triglycerides, systolic blood pressure, and smoking, the insulin-resistant subjects still had a twofold increased risk of cardiovascular disease. This suggests that a large part of cardiovascular disease risk due to insulin resistance.

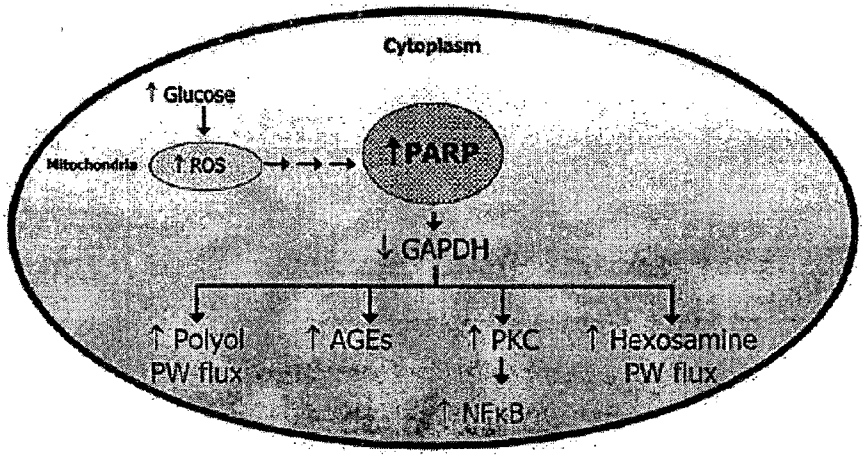


Figure 1.12: The Unifying mechanism of hyperglycemia-induced cellular damage.

The unappreciated consequence of insulin resistance is increased free fatty acid (FFA) flux from adipocytes into arterial endothelial cells, (Fig. 1.13). In macrovascular, but not in microvascular endothelial cells, increased flux results in increased FFA oxidation by the mitochondria. Since both β-oxidation of fatty acids and oxidation of FFA-derived acetyl CoA by the TCA cycle generate the same electron donors (NADH and $FADH_2$) generated by glucose oxidation, increased FFA oxidation causes mitochondrial overproduction of ROS by exactly the same mechanism described above for hyperglycemia. And, as with hyperglycemia, this FFA-induced increase in ROS activates the same damaging pathways: AGEs, PKC, the hexosamine pathway (GlcNAc), and NFκB. In insulin-resistant non diabetic animals models, inhibition of either FFA release from adipocytes or FFA oxidation in arterial endothelium prevents the increased production of ROS and its damaging effects. Thus, the unifying mechanism plays major role in the pathogenesis of diabetic macrovascular, as well as microvascular, complications.

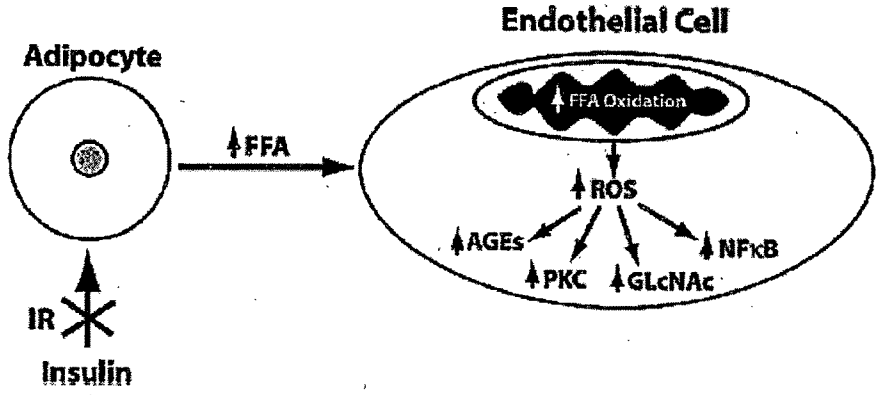


Figure 1.13: Insulin resistance causes mitochondrial overproduction of ROS in macrovascular endothelial cells by increasing FFA flux and oxidation.

Source: Hofmann S, Brownlee M: Biochemistry and molecular biology of diabetic complications: a unifying mechanism. In Diabetes Mellitus: A fundamental and Clinical Text. 3rd ed. LeRoith D, Taylor SI, Olefsky JM, Eds. Piladelphia, Lippion-cott Williams and Wilkins, p. 1441-1457, 2004.

1.18. Therapies for diabetes mellitus

1.18.1 Sulfonylureas

Sulfonylureas work by stimulating insulin release from the beta cells of the pancreas and may slightly improve insulin resistance in peripheral target tissues (muscle, fat). On average, this class reduces glycosylated hemoglobin A_{1c} (HbA_{1c}) levels by 0.8 to 2.0 percent and fasting plasma glucose (FPG) concentrations by 60 to 70 mg per dL (3.3 to 3.9 mmol per L), with the greatest reductions observed in patients with the highest FPG concentrations at the initiation of therapy (DeFronzo, 1999). Hypoglycemia is the most worrisome side effect of the sulfonylureas. It is of particular concern with agents that are metabolized to an active metabolite with significant renal excretion. These agents include chlorpropamide (Diabinese) and glyburide, both of which should be avoided in the setting of impaired renal function and used with caution in elderly patients. Glipizide and glimepiride are associated with a lower incidence of hypoglycemia. All sulfonylureas have been associated with weight gain and thus, may not be the optimal first choice for obese patients.

1.18.2 Meglitinides

Repaglinide (Prandin) is a new non-sulfonylurea insulin secretagogue agent, the first available from the meglitinide class. Nateglinide (Starlix), the newest member of the class. The mechanism of action of the meglitinides closely resembles that of the sulfonylureas. The meglitinides stimulate the release of insulin from the pancreatic beta cells. However, this action is mediated through a different binding site on the "sulfonylurea receptor" of the beta cell, and the drug has somewhat different characteristics. Unlike the commonly used sulfonylureas, the meglitinides have a very short onset of action and a short half-life. Repaglinide has shown similar effects on HbA_{1c} and FPG levels when compared with glyburide, 0.5 to 2 percent and 65 to 75 mg per dL (3.6 to 4.2 mmol per L), respectively (Luna et al., 1999). Some potential advantages of this class of agents include a greater decrease in postprandial glucose and a decreased risk of hypoglycemia.

1.18.3 Biguanides

Metformin works by reducing hepatic glucose output and, to a lesser extent, enhancing insulin sensitivity in hepatic and peripheral tissues. Metformin has been shown to reduce HbA_{1c} levels by approximately 1.5 to 2.0 percent and FPG levels by 50 to 70 mg per dL (2.8 to 3.9 mmol per L) (DeFronzo, 1999). Other effects include a reduction in plasma triglyceride levels and low-density lipoprotein (LDL) cholesterol levels. On the whole, metformin has a favorable side effect profile. Most of the related side effects (including metallic taste, gastrointestinal discomfort and nausea) are transient and commonly reported only during initiation of therapy. Slow-dosage titration is recommended to lessen these effects. Taking the drug with meals may also lessen the severity of the gastrointestinal side effects. Because metformin does not affect insulin secretion, it is not associated with hypoglycemia when used as monotherapy, but can potentiate hypoglycemia when used in combination with a sulfonylurea or insulin. A rare, but more worrisome potential adverse effect is that of lactic

acidosis. Metformin is unusual instead of weight gain it causes weight loss in some overweight patients (Hermann et al., 1994). Metformin also improves glycemic control in patients who are not overweight.

1.18.4 Thiazolidinediones

The thiazolidinediones work by enhancing insulin sensitivity in both muscle and adipose tissue and to a lesser extent by inhibiting hepatic glucose production. These agents have a notable effect on improving insulin resistance, particularly when used in combination with other antidiabetic drugs, but have no effect on insulin secretion. Monotherapy with these agents has been associated with a 0.5 to 1.5 percent reduction in HbA_{1c} levels and 25 to 50 mg per dL (1.4 to 2.8 mmol per L) reduction in FPG levels (DeFronzo, 1999). As a class, the thiazolidinediones have also been shown to alter lipid profiles in patients with type 2 diabetes. Results from studies with troglitazone consistently show a decrease in triglyceride levels--in some cases by as much as 33 percent (Saltiel & Olefsky, 1996). Patients treated with pioglitazone have displayed mean decreases in triglyceride levels, mean increases in HDL cholesterol levels, and no consistent mean changes in LDL and total cholesterol levels. Thiazolidinediones have been shown to interfere with expression and release of mediators of insulin resistance originating in adipose tissue (e.g., increased free fatty acids, decreased adiponectin) in a way that results in net improvement of insulin sensitivity (i.e., in muscle and liver). A direct or indirect effect on AMP-dependent protein kinase may also be involved. Prevention of lipid accumulation in tissues critical to glycaemia such as visceral adipocytes, liver, muscle and beta-cells at the expense of lipids accumulating at the less harmful subcutaneous site may be central to their net metabolic effect. Moreover, their anti-inflammatory properties also make them interesting in the prevention and treatment of atherosclerosis and possibly other inflammatory conditions (e.g., inflammatory bowel disease) (Stumvoll, 2003).

1.18.5 Alpha-glucosidase inhibitors

Acarbose (Precose) and miglitol (Glycet) are the two agents available in this class. Alpha-glucosidase inhibitors act by inhibiting the enzyme alpha-glucosidase found in the brush border cells that line the small intestine, which cleaves more complex carbohydrates into sugars. Because they inhibit the breakdown and subsequent absorption of carbohydrates (dextrins, maltose, sucrose and starch; no effect on glucose) from the gut following meals, the largest impact of these drugs is on postprandial hyperglycemia. Their effect on FPG levels is modest. They have been associated with a reduction in HbA_{1c} by 0.7 to 1.0 percent and FPG levels by 35 to 40 mg per dL (1.9 to 2.2 mmol per L) (DeFronzo, 1999). Thus, these agents are most useful in patients who have mild FPG elevations or in patients with predominant postprandial hyperglycemia. The most bothersome side effects observed with these agents are gastrointestinal, including abdominal discomfort, bloating, flatulence and diarrhea but are reversible with discontinuation. Therapy with acarbose has been linked to elevations in serum transaminase levels and the use of this agent is contraindicated in patients with liver cirrhosis.

1.18.6 Insulin

Insulin treatment is necessary in case of IDDM to control hyperglycemia and development of the ketoacidosis. Maximum decline occurs in plasma glucose at 30 minute following intravenous insulin administration and at 2-3 hours after subcutaneous insulin administration. Various forms of insulin like rapid acting, intermediate and long acting are commercially available. Insulin administration is also associated with some side effects like hypoglycemic shock, weight gain and an increased risk of atherogenesis (Sinha et al., 1996; USKPD, 1998).

1.18.7 Gene and Islet therapy

Gene and islet transplantation therapy can provide an ideal solution for the treatment of IDDM. Tremendous experimental efforts are in progress to make transplanted islets more viable and functional for the longer period of time. Scientists are trying to make human/non-human engineered insulin producing

cells suitable for graft within special immunoisolation barrier membranes. A significant number of animal studies have demonstrated the potential of islet cell transplantation in restoring the normoglycemia in context of immuno-regulation achieved by gene transfer of immuno-regulatory genes to allo- and xenogenic islets ex vivo. Gene and cell therapy is also used to induce tolerance to auto- and allo-antigens and to generate the tolerance state in autoimmune rodent model of type 1 diabetes. For human diabetics, islet transplantation is still under experimental stage. Successful clinical trails are being conducted with these advance strategies to achieve the final goal i.e. the cure of IDDM. The achievement of gene and cell therapy in type 2 diabetes is less evident. Type 2 diabetes will likely require a better understanding of the processes that determine insulin sensitivity in the periphery (Giannoukakis and Robbins, 2002).

1.18.8 Exercise

-Helps insulin to work better and lower your blood glucose.

-Lowers the blood pressure and cholesterol levels.

-Strengthens the heart and improves blood circulation.

-Reduces body fat and control body weight.

1.18.9 Vitamin E

Diabetes produces a state of increased free radical activity. The purported effects of vitamin E on glucose control relate to the vitamin's potent lipophilic antioxidant activity, with possible influences on protein glycation, lipid oxidation, and insulin sensitivity and secretion. Through unknown mechanisms, it may also affect nonoxidative glucose metabolism (Mooradian et al., 1994).

1.18.10 α-Lipoic acid

Also known as thioctic acid, a disulfide compound synthesized in the liver, α-lipoic acid is a potent lipophilic antioxidant. It is a cofactor in many multienzyme complexes and may also play a role in glucose oxidation (Konrad et al., 1999).

Table 1.1: Treatments available for diabetes (Fig. 1.16)

Type	Example	Action	Side effect
1. Sulfonylureas	Tolbutamide Glibenclamide (Daonil) Glipizide (Minidiab) Gliclazide (Diamicron)	Make the pancreas release insulin and lowers blood sugar	Hypoglycemia Weight gain
2. Biguanides	Metformin (Glucophage)	Help cells in the body respond more effectively to insulin i.e. Reduce insulin resistance.	Lactic acidosis. Sometimes it can cause some abdominal discomfort, nausea or diarrhoea. To avoid this, patients can start with a lower dose and take the tablets together with meals initially. Not appropriate for people with renal / liver
3.Alpha-glucosidase Inhibitors	Acarbose (Glucobay)	Slow sugar absorption in the intestine. Prevent sudden increase in blood glucose that occurs after eating.	GI disturbance e.g. flatus (gas) diarrhoea.

Experimental in vitro data have shown possible effects in enhancing glucose uptake in muscle and preventing glucose-induced protein modifications.

Certain metals like Chromium, Vanadium, Selenium, Manganese, Zinc and Potassium etc. have also shown the hypoglycemic activities and some of these metals are being used for the treatment of diabetes.

1.18.11 Chromium species

Chromium (Cr3), a trace element in its trivalent form, is required for the maintenance of normal glucose metabolism. Experimentally, chromium deficiency is associated with impaired glucose tolerance, which can be improved with supplementation (O'Connell, 2001). Most individuals with diabetes, however, are not chromium deficient. In addition to glucose control, the supplement has been studied for its effects on weight control, lipids, and bone density. Its action is linked with glucose tolerance factor (GTF), and has been shown to increase the number of insulin receptors, to enhance receptor binding, and to potentiate insulin action. Some suggest that chromium picolinate is the preferred form because it is utilized more efficiently (Trow et al., 2000).

1.18.12 Magnesium

Hypomagnesemia is common in patients with diabetes, especially those with glycosuria, ketoacidosis, and excess urinary magnesium losses. Deficiency of magnesium can potentially cause states of insulin resistance. Studies have examined magnesium's potential role in the evolution of such complications as neuropathy, retinopathy, thrombosis, and hypertension. However, its role in glycemic control is unknown. Magnesium is a cofactor in various enzyme pathways involved in glucose oxidation, and it modulates glucose transport across cell membranes. It may increase insulin secretion and/or improve insulin sensitivity and peripheral glucose uptake. It has been shown to have no effect on hepatic glucose output and nonoxidative glucose disposal (Mooradian et al., 1994).

1.18.13 Vanadium

Vanadium has been described as either a nonessential nutrient or a nutrient that is required only in minute quantities, as no physiological role of the trace element has yet to be found (Goldwaser et al., 2000) Human deficiency has not been documented. Vanadium exists in several valence forms, with vanadyl (+5) sulfate and sodium metavanadate (+4) being the most common supplement forms. Its mechanism of action in glycemic control is thought to be primarily insulin-mimetic with upregulation of insulin receptors. In animal models, it has been shown to facilitate glucose uptake and metabolism and to enhance insulin sensitivity. Clinically, it may enhance glucose oxidation and glycogen synthesis, and it may modulate hepatic glucose output (O'Connell, 2001).

1.19. Hypolipidemic drugs

The National Cholesterol Education Program Adult Treatment Panel (ATP) has identified low-density lipoprotein (LDL) cholesterol as the primary target for evaluating and treating dyslipidemia. For many patients with several lipid abnormalities to reach the lipid lowering goals may require the use of combination therapy. Patients with diabetes mellitus are at high risk of cardiovascular diseases. Dyslipidemia is an important risk factor for cardiovascular complications in diabetes. Increased triglyceride and reduced HDL-cholesterol plasma concentrations are common features of dyslipidemia in type 2 diabetes. The LDL particles are small and dense and have an increased atherogenity. Abnormalities in lipoprotein composition are observed in diabetes mellitus, especially in type 2. There are increasing indications that dyslipidemia in diabetes mellitus deserves aggressive treatment and that lipid target levels should be very low (Niemeijer-Kanters, 2001). Reducing elevated levels of low-density-lipoprotein cholesterol (LDL-C) significantly reduces the incidence of coronary heart disease (CHD) events and mortality in hypercholesterolemic patients. Based on their lipid-lowering abilities, safety, and tolerability profiles, the HMG-CoA reductase inhibitors (statins) are the first-line pharmaco-therapeutic agents for hypercholesterolemia. The statins thus, have ability to

reduce CHD events. For combined dyslipidemia, statin monotherapy is a reasonable initial approach in patients with moderate hypertriglyceridemia. Fibrates or niacin is effective therapies for severe hypertriglyceridemia. Resins are moderately effective in isolated hypercholesterolemia, and are a useful alternative to statins in pregnant women or patients with liver disease. For severe hyperlipidemia that does not respond to single drug therapy, combination drug therapy may be required (McKenney, 2001).

Several classes of hypolipidaemic drugs are currently available in the market:

1.19.1. Statins (HMG-CoA reductase inhibitors):

Statins are widely used to reduce LDL-C and thus blocking regression of atherogenic plaques and reduction of CHD mortality; side effects include myalgia, liver dysfunction. These classes of drugs exert their major effect i.e. reduction of LDL levels through a mevalonic acid like moiety that competitively inhibits HMG-CoA reductase by product inhibition (Nakamura et al., 1980). Statins affect blood cholesterol levels by inhibiting cholesterogenesis in the liver, which results in increased expression of the LDL receptor gene. The greater number of LDL receptors on the surface of hepatocytes results in increased removal of LDL from the blood (Bilheimer et al., 1983), thereby lowering LDL-C levels. Statins lower 35% to 45% triglycerides and 20% to 55% LDL-C and increase 5% to 10% HDL-C levels depending on dose and statin used. eg. Atorvastatin, Flavostatin and Cerivastatin etc. Hepatotoxicity and myopathy are the commonly found adverse effects of statins.

1.19.2. Bile acid sequestrants (resins)

Bile acid sequestrants including cholestyramine, colestipol, and colesevelam low toxicity due to low absorbance, may increase serum triglycerides, not often used. They are highly positively charged and bind negatively charged bile acids. Because of their large size, resins are not absorbed, and the bound bile acids are excreted in stool. Since over 95% of bile acids are normally reabsorbed,

interruption of this process depletes the liver's pool of bile acids, and hepatic bile acid synthesis increases. As a result, hepatic cholesterol content declines, stimulating the production of LDL receptors, an effect similar to that of statins (Bilheimer et al., 1983).

1.19.3. Nicotinic acid (Niacin)

This is the best agent available for increasing HDL-C (increment of 30% to 40%); it also lowers triglycerides by 35% to 45% (as effectively as fibrates and more potent statins) and reduces LDL-C levels by 20% to 30% (Knopp et al., 1985). In adipose tissue, niacin inhibits the lipolysis of triglycerides by hormone sensitive lipase, which reduces transport of free fatty acids to the liver and decreases hepatic triglyceride synthesis (Grundy et. al., 1981). Niacin raises HDL-C levels by decreasing the fractional clearance of apo A-I in HDL rather than by enhancing HDL synthesis (Blum et al., 1977). Two of niacin's side effects, flushing and dyspepsia, limit patient compliance. Hepatotoxicity, produce severe hyperglycemia in diabetes mellitus patients, elevate uric acid levels, atrial tachyarrhythmias, atrial fibrillation, etc. are the other side effects observed. It increases risk of myositis and liver dysfunction when used with HMG-CoA reductase inhibitors.

1.19.4. Fibrates

Fibrates lower triglycerides, LDL-C and increase HDL-Cholesterol levels; improves glycemic control and often used with HMG-CoA reductase inhibitors. Effects of these compounds on blood lipids are mediated by their interaction with peroxisome proliferator-activators (PPARs) (Kesten et al., 2000), which regulate gene transcription, fatty acid oxidation, increases LPL synthesis, and reduces expression of apo C-III. An increase in LPL would enhance the clearance triglyceride-rich lipoproteins. A reduction in hepatic production of apo C-III, which serves as an inhibitor of lipolytic processing and receptor-mediated clearance, would enhance the clearance of VLDL. PPARα also stimulates apo A-I and apo A-II expression (Staels & Auwerx, 1998), which increases HDL levels.

Usually well tolerated, gastrointestinal side effects, rash, utricaria, hair loss, myalgia, fatigue, headache, impotence, anaemia, flu like syndrome, renal failure, hepatotoxicity are the side effects.

1.19.5. MTP inhibitor

MTP transfer triglycerides and other nonpolar lipids to the apoproteins of nascent lipoproteins as they form in the intestine and liver and is required for the synthesis and secretion of chylomicrons and VLDL. An MTP inhibitor targeted to the liver would decrease VLDL production, thereby decreasing plasma triglyceride levels and ultimately reducing LDL production from VLDL.

1.19.6. Dietary and biliary cholesterol absorption inhibitor

Ezetimibe is an azetidione-based cholesterol absorption inhibitor that blocks the intestinal absorption of cholesterol, resulting in lowered plasma total cholesterol and LDL-C levels (Van, 2000).

1.19.7. ACAT inhibitor

ACAT-1 is expressed in several tissues, including macrophages (Chang et al., 1993). Avasimibe, an inhibitor of ACAT enzyme, appears to reduce macrophage and cholesteryl ester contents of lesions in cholesterol-fed rabbits and could affect atherosclerotic lesion development, an effect that could stabilize lesions (Bocan, 2000).

1.20. Combination Therapy

Diabetes mellitus is defined as a "metabolic syndrome" and can be precisely said that "type 2 diabetes mellitus represents a syndrome of metabolic and haemodynamic abnormalities as a result of complex pathophysiological interactions between hyperglycemia, β-cell dysfunction, hypertension, insulin resistance, dyslipidaemia, endothelial cell dysfunction and proteinuria which underlie the aetiology and progression of micro- and macro-angiopathy" (Das,

2002). Keeping this in view, combination therapy for diabetes mellitus has become inevitable. In recent years it has been shown that the control of the latter has to be strict and that in many cases monotherapy does not achieve the established aims. At the same time, new oral anti-diabetic medicines have recently appeared with different mechanisms of action. The possible combinations of treatment with different oral anti-diabetics, or else with oral anti-diabetics with insulin, are very numerous and have shown their effectiveness in reducing glycemia and the glycosylated haemoglobin. Selection of the type of association necessary will depend on the individual aims of control, on the physiopathological mechanism presumably involved in each case, on the efficacy, cost and secondary effects of each medicine, as well as on the characteristics of each patient (Menendez, 2002). Several of the available oral agents have been studied in combination and have been shown to further improve glycemic control when compared to monotherapy (Riddle, 2000). Reasonable combinations of agents include a sulfonylurea plus metformin, a sulfonylurea plus an alpha-glucosidase inhibitor, a sulfonylurea plus a thiazolidinedione, metformin plus repaglinide, biguanide plus alpha-glucosidase inhibitor, and metformin plus a thiazolidinedione. Some physicians advocate therapy combining three oral agents, (sulfonylurea, metformin, thiazolidinedione or sulfonylurea, metformin, alpha-glucosidase inhibitor), although this approach has not been extensively studied (Ovalle & Bell, 1998).

Combination therapy is a valuable treatment option for patients with persistent lipoprotein disorders and for those requiring more than 1 agent to effectively reach their LDL cholesterol goals. In addition, some physicians may be hesitant to use high doses of a lipid-lowering agent because of the potential adverse effects. Fibrates are the treatment of choice for reducing triglycerides but, like monotherapy, are not effective for normalizing LDL cholesterol. However, fibrate-statin combinations are widely used to enhance reductions of triglycerides and LDL cholesterol along with increases in HDL cholesterol. Reports of an increased risk of myopathy, including rhabdomyolysis, may cause some

clinicians to limit the use of fibrate-statin combinations. Such combination should be avoided in patients predisposed to myopathy because of renal or liver impairment, increased age, debilitated status, surgery, trauma, or heavy exercise. Ezetimibe is the first in a new class of lipid-lowering agents known as selective cholesterol absorption inhibitors, which inhibit intestinal absorption of sterols, particularly dietary and biliary cholesterol. An ezetimibe-statin product currently in development may be beneficial for patients who need additional LDL cholesterol lowering despite statin therapy. However, ezetimibe only minimally affects HDL cholesterol and has no significant effect on triglyceride levels; therefore, its use will be limited for patients with multiple lipid abnormalities.

1.21. Herbal Therapy

Hypoglycemic herbs are widely used as non-prescription treatment for diabetes. However, few herbal medicines have been well characterized and demonstrated the efficacy in systematic clinical trials as those of Western drugs.

Anti-diabetes herbs

Certain herbs may lower blood glucose (Yin et al., 2008; Kuriyan et al., 2008); however, their test results are subject to several factors. Firstly, each herb contains thousands of components, only a few of which may be therapeutically effective (Angelova et al., 2008). Secondly, different parts of an herb have different ingredient profiles. Moreover, different extraction methods may yield different active ingredients (Shan et al., 2007). Thirdly, herbal formulae containing multiple herbs may have synergistic effects (Liu et al., 2004; Kawase et al., 2000).

1.21.1 *Ginseng*

The therapeutic potency of ginseng mainly relies on its geographical locality, dosage, processing and types of diabetes. *Panax ginseng* (Chinese or Korean ginseng) has the highest therapeutic potency. *Panax quinquefolius* (American ginseng) is the medium potency grade ginseng, while *Panax japonicus* (Japanese ginseng) is considered the low potency grade ginseng. Thus, the most commonly

used therapeutic ginseng is *Panax ginseng*. The anti-tumor, angiomodulating and steroid-like activities of ginseng have been recently delineated (Yue et al., 2007). The anti-diabetic effects of ginseng have been investigated with aqueous or ethanol ginseng extracts. A proposed action mechanism has been tested on various animal models (Kang et al., 2008). Korean red ginseng (0.1–1.0 g/ml) significantly stimulated insulin release from isolated rat pancreatic islets at 3.3 mM glucose concentration (Kim et al., 2008). The treatment with oral administration of H-AG (heat-processed American ginseng) at a dose of 100 mg/kg of body weight for 20 days decreased serum levels of glucose and glycosylated proteins and hemoglobin A_{1C} in streptozotocin (STZ)-induced diabetic rats. The treatment also improved the decreased creatinine clearance level and decreased the accumulation of N (ε)-(carboxymethyl) lysine and its receptors for advanced glycation end product (AGE) expressions in kidney (Kim et al., 2008). *Radix Ginseng Alba* improved hyperglycemia in KKAy mice, possibly by blocking intestinal glucose absorption and inhibiting hepatic glucose-6-phosphatase, while *Radix Ginseng Palva* has a similar effect through the up-regulation of adipocytic PPAR-y protein expression and inhibition of intestinal glucose absorption (Chung et al., 2001), (Fig. 1.17).

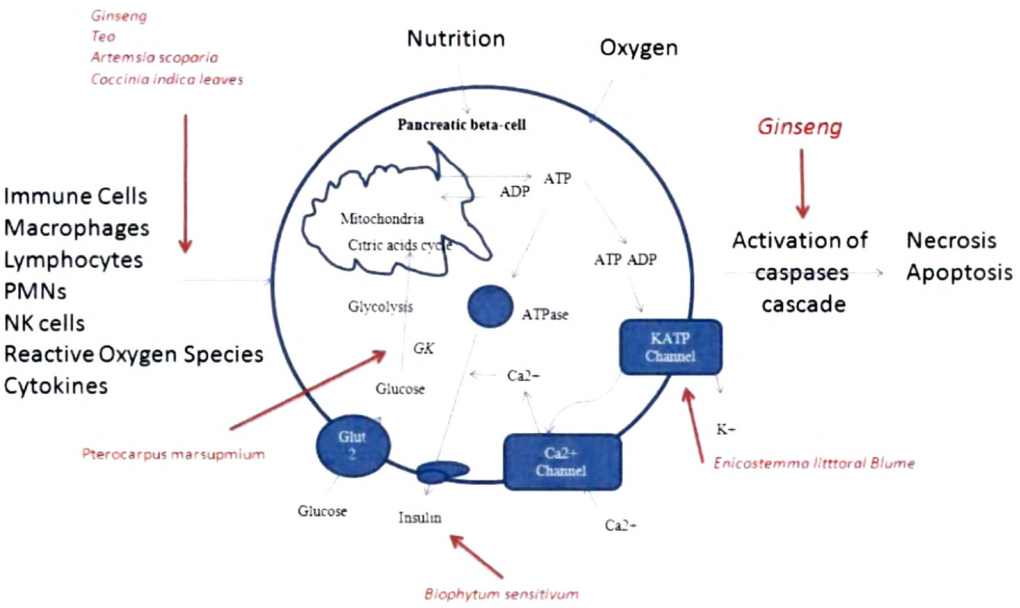


Figure 1.14: Insulin secretion and pancreatic-β-cell apoptosis.

Glucose is taken up into β-cells via glucose transporters. It is metabolized in glycolysis and Krebs cycle, resulting in an increased ratio of ATP to ADP in the cytoplasm. This closes ATP-sensitive potassium channels (KATP channels), leading to cell membrane depolarization and subsequently opening voltage-gated Ca^{2+} channels. These changes increase free Ca^{2+} concentration ($[Ca^{2+}]i$) in cytoplasm and eventually triggers insulin secretion. In apoptosis, stimuli promotes the release of caspase activators from mitochondria and result in the activation of caspases procedure, by cleaving the effector caspases, which interacts with a variety of cellular proteins, resulting in directly or indirectly the morphological and biochemical characteristics of cell apoptosis. The action sites of hypoglycemia herbs are indicated with a narrow (Fig. 1.14).

The treatment of the C57BL/Ks *db/db* mice with *Panax ginseng* berry extract (150 mg/kg of body weight) significantly lowered the fasting blood glucose levels on day 5 and achieved euglycemia on day 12 (Vuksan et al., 2008). Berry extract showed marked anti-obesity effect in obese *ob/ob* and *db/db* mice (Xie et al., 2002). Red ginseng lowered hemoglobin A_{1C} to normal range and improved insulin sensitivity (Vuksan et al., 2008). Similarly, extract of American ginseng berry also lowered fasting blood glucose levels significantly in diabetic *ob/ob* mice receiving daily berry juice at 0.6 ml/kg. This hypoglycemic effect continued for at least ten days after the treatment. In addition, reduction of body weight was also observed (Xie et al., 2007).

The inner part of IR reveals a tyrosine kinase activity and coupled with proteins of Src-homology-collagen-like protein (SHC) and multifunctional docking proteins IRS-1 and IRS-2. The interaction of insulin and IR activates its tyrosine activity and phosphorylates the coupled SHC and subsequently activates, in turn, a series of signal proteins, including the growth factor receptor-binding protein 2 (Grb2), and the ras small guanosine 5'-triphosphatebinding

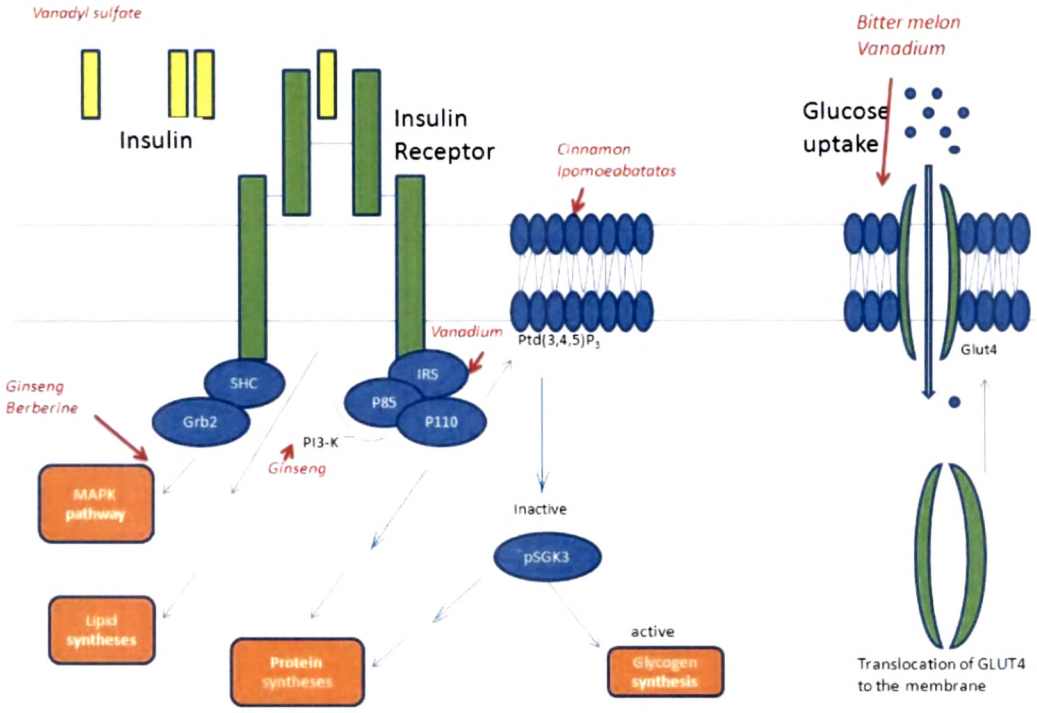


Figure1. 15: Insulin signal pathway and insulin insensitive.

protein. The in turn signaling leads to an activation of the MAPK cascade involved in mitogenesis and the open status of a hexose transporter protein (GLUTs), which is located in the cell membrane and is the only pump to take into glucose for cells. The decreased serine/threonine phosphorylation of IR, inactivates hexokinase and glycogen synthase, as well as defects in the phosphorylation of glucose transporter protein (GLUT4) and genetic primary defect in mitochondrial fatty acid oxidation, leading to insulin resistance and an increase of triglyceride synthesis contribute to this insulin insensitivity. The action sites of hypoglycemia herbs are indicated with an arrow. While both ginseng root and berry possess anti-diabetic effects (Dey et al., 2003), ginseng berry seems to be more potent in antihyperglycemic activity (Dey et al., 2002). Furthermore, only ginseng berry showed marked anti-obesity effects in *ob/ob* mice (Dey et al., 2003; Vogler et al., 1999).

A total of 705 components have been isolated from ginseng, such as ginsenosides, polysaccharides, peptides and polyacetylenic alcohols, among which ginsenosides are believed to be responsible for ginseng's efficacy (Kimura et al., 1996).Pharmacological sequential trials of three components, i.e. (1) fat-soluble components, (2) ginseng saponins and (3) a third component with hypoglycemic activity identified the most active components (100-fold more effective than the original water-soluble extract of the ginseng root). Ginseng's clinical efficacy is thought to be medicated by multiple factors (Kimura et al., 1996; Ng et al., 1985): the component panaxans (panaxans A to E) elicits hypoglycemia in both normal and diabetic mice; the component adenosine inhibits catecholamine-induced lipolysis; both components of carboxylic acid and peptide 1400 inhibit catecholamine-induced lipolysis in rat epididymal fat pads; and the component DPG-3-2 provokes insulin secretion in fraction related to DPG-3-2, also exhibits an anti-lipolytic activity related to anti-obesity effects. Ginsenoside Rg3 inhibits adipocyte differentiation via PPAR-γ pathway in rosiglitazone-treated cells and activates AMPK, a pathway involved in the control of nutritional and hormonal modulation (Hwang et al., 2009). Ginsenoside Rh2 improves insulin sensitivity in rats fed with fructose rich chow (Lee et al., 2007). Thus the whole extract of ginseng contains multiple biologically active components that stimulate insulin secretion, blocking intestinal glucose absorption and enhancing glucose peripheral utilization in diabetic and glucose-loaded normal mice (Hwang et al., 2009). EPG-3-2, a Ginseng treatment for type 2 diabetes has been tested in both animal models and human clinical trials. *Panax quinquefolius* (10 g/1 kg diet) increases body weight and decreases cholesterol levels, PPAR actions and triglyceride metabolism in male Zucker diabetic fatty (ZDF) rats (Banz et al., 2002). In human clinical trials, *Panax quinquefolius* improves post-prandial glycemia in type 2 diabetic patients (Wu et al., 2007). Single intravenous injection of ginsenoside Rh2 decreases plasma glucose concentrations within 60 minutes in a dose-dependent manner in rats fed with fructose rich chow and STZ-induced insulin resistant rats (Hwang et al., 2009). A possible mechanism is that ginsenoside Rh2 promotes the release of ACh from

nerve terminals which stimulate muscarinic M (Yin et al., 2008) receptors in pancreatic cells to increase insulin secretion (Lee et al., 2006). Ginseng is also used to treat type 1 diabetic patients. Ginsenosides at 0.1–1.0 g/mL inhibited cytokine-induced apoptosis of β-cells. The action mechanism involves the reduction of nitric oxide (NO), production of reactive oxygen species (ROS) (Kim et al., 2007), inhibition on p53/p21 expression and inhibition on cleavage of caspases and poly (ADP-ribose) polymerase (PARP) (Xiang et al., 2008). Alternation expression of NOS gene is implicated in the pathogenesis of numerous secondary complications in diabetic patients. In animal models, enhanced NOS expression was detected in the hippocampus of diabetic rats and the administration of ginseng root suppressed NOS expression (Wu et al., 2007). Pharmacological studies confirmed that ginseng possesses multiple actions (central nervous system, neuroprotective, immunomodulation and anticancer effects. Ginsenosides have antioxidant, anti-inflammatory, anti-apoptotic and immuno-stimulant properties (Xiang et al., 2008). Side-effects of ginseng include insomnia, diarrhea, vaginal bleeding, breast pain, severe headache, schizophrenia and fatal Stevens-Johnson syndrome (Kiefer et al., 2003). The recommended dosage of ginseng application is 1–3 g of root or 200–600 mg of extract (Vuksan et al., 2000). Ginseng has the potential to prolong bleeding time and therefore should not be used concomitantly with warfarin. Moreover, ginseng may cause headache, tremulousness, and manic episodes in patients treated with phenelzine sulfate (Abd et al., 2006). Ginseng may interfere with the actions of estrogens or corticosteroids and may impede digoxin metabolism or digoxin monitoring (Miller, 2000).

Hypoglycemic medicines restore euglycemia via several types, including insulin secretagogues (sulfonylureas, meglitinides), insulin sensitizers (biguanides, metformin, thiazolidinediones), alpha-glucosidase inhibitors (miglitol, acarbose).

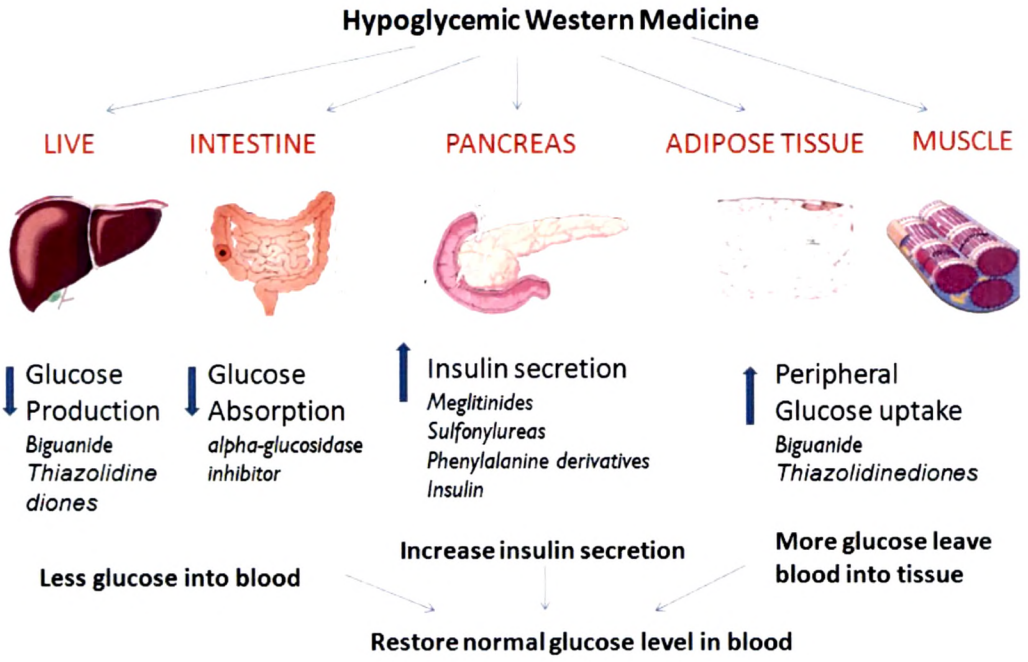


Figure 1.16: Action sites of western medicine in diabetes treatment.

The efficacy of hypoglycemia herbs has been mediated by increasing insulin secretion (ginseng, bitter melon, aloes, biophytum sensitivum), enhancing glucose uptake by adipose and muscle tissues (ginseng, bitter melon and cinnamon), inhibiting glucose absorption from intestine (myrcia and sanzhi) and inhibiting glucose production from heptocytes (berberine, fenurgreek leaves).

1.21.2 *Momordica charantia (bitter melon)*

Hypoglycemic effects of bitter melon were demonstrated in cell culture, animal models (McCarty, 2004) and human studies (Krawinkel et al., 2006). The anti-diabetic components in bitter melon include charantin, vicine, polypeptide-p, alkaloids and other non-specific bioactive components such as anti-oxidants. The major compounds in bitter melon methanol extract, including 5-β, 19-epoxy-3-β, 25-dihydroxycucurbita- 6,23(E)-diene (4) and 3-β,7-β,25- trihydroxycucurbita

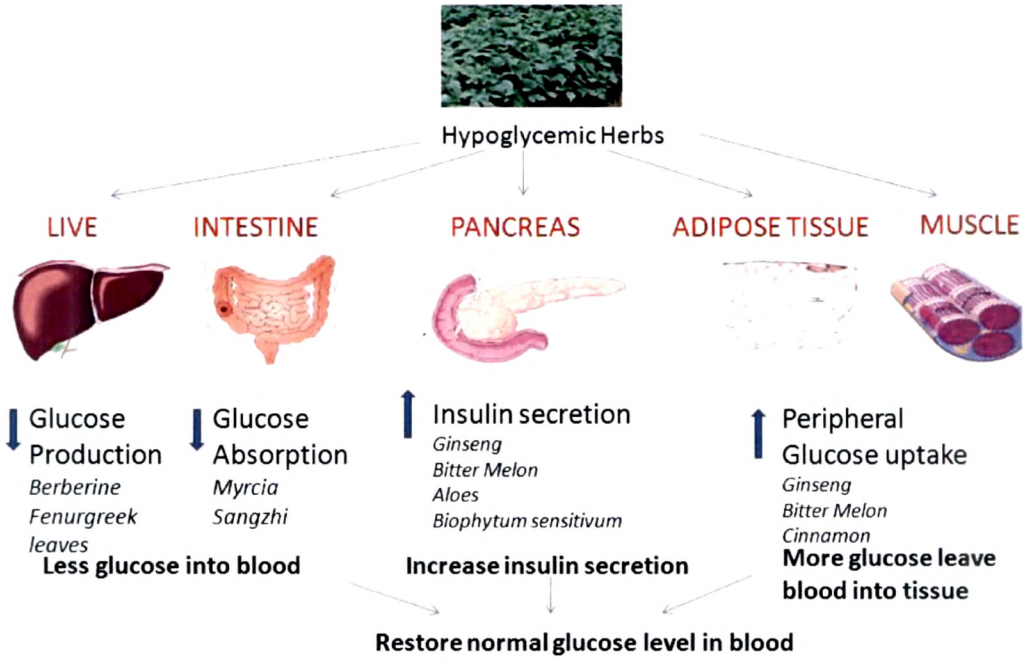


Figure 1.17: Action sites of herbs in diabetes treatment.

-5,23(E)-dien-19-al (5) showed hypoglycemic effects in the diabetic male *ddY* mice at 400 mg/kg (Harinantenaina et al., 2006).Oleanolic acid glycosides, compounds from bitter melon, improved glucose tolerance in Type 2 diabetics by preventing sugar from being absorbed into intestines. Saponin fraction (SF) extracted from bitter melon with PEG/salt aqueous two-phase systems showed hypoglycemic activity in alloxan-induced hyperglycemic mice (Han et al., 2008). Bitter melon increased the mass of β cells in the pancreas and insulin production (Shetty et al., 2005; Chao et al., 2003). With edible portion of bitter melon at 10% level in the diet STZ-induced diabetic rats, an amelioration of about 30% in fasting blood glucose was observed (Shetty et al., 2005). Biochemical studies indicated that bitter melon regulated cell signaling pathways in pancreatic β-cell, adipocytes and muscles. Ethyl acetate (EA) extract of bitter melon activates peroxisome proliferator receptors (PPARs) α and γ (Chao and Huang, 2003; Chuang et al., 2006), modulates the phosphorylation of IR and its downstream signaling pathway, thereby lowering plasma apoB-100 and apoB-48 in mice fed with high-fat diet (HFD). The momordicosides (Q, R, S and T) stimulate GLUT4

translocation of the cell membrane and increase the activity of AMP-activated protein kinase (AMPK) in both L6 myotubes and 3T3-L1 adipocytes, thereby enhancing fatty acid oxidation and glucose disposal during glucose tolerance tests in both insulin-sensitive and insulin-insensitive mice (Tan et al., 2008). Bitter melon can be used as a dietary supplement herbal medicine for the management of diabetes and/or metabolic syndromes (Cefalu et al., 2008). Reported adverse effects of bitter melon include hypoglycemic coma, convulsions in children, reduced fertility in mice, a favism-like syndrome, increased enzyme activities of γ-glutamyl transferase and alkaline phosphotase in animals and headaches in humans.

1.21.3 *Coptis chinensis (Huanglian)*

Coptis chinensis is commonly used to treat diabetes in China. Found in plant roots, rhizomes, stems and barks, berberine is an isoquinoline alkaloids and the active ingredient of *Coptis chinensis*. Intragastric administration of berberine (100 and 200 mg/kg) in diabetic rats decreased fasting blood glucose levels and serum content of TC, TG, LDL-c, increased HDL-c and NO level, and blocked the increase of SOD and GSH-px levels (Yu et al., 2005; Tang et al., 2006). Multiple mechanisms may be responsible for weight reduction and increased insulin response induced by berberine. Glucose's uptake by adipocytes is enhanced by berberine via GLUT1, adenosine monophosphate-activated protein kinase and acetylcoenzyme A carboxylase phosphorylation (Zhou et al., 2007). Berberine also increases the PPAR α/δ/γ protein expression in liver (Zhou et al., 2008), increases insulin receptor expression in liver and skeletal muscle cells and improves cellular glucose consumption in the presence of insulin (Kong et al., 2009). Berberine increases GLUT4 translocation in adipocytes and myotubes (Lee et al., 2006), increases AMPK activity, decreases glucose stimulated insulin secretion (GSIS) and palmitate-potential insulin secretion in MIN6 cells and rat islets (Zhou et al., 2008). Furthermore, berberine decreases significantly the enzyme activity of intestinal disaccharidases and β-glucuronidase in STZ-induced diabetic rats (Liu et al., 2008). Recently, dihydroberberine (dhBBR), an

identified BBR berberine derivative, demonstrated *in vivo* beneficial effects in rodents fed with high-fat (Turner et al., 2008). Berberine may also relieve some diabetic complications. Studies showed that berberine restored damaged pancreas tissues in diabetic rats induced by alloxan (Liu et al., 2008). Berberine ameliorates renal dysfunction in rats with diabetic nephropathy through controlling blood glucose, reduction of oxidative stress and suppressing the polyol pathway (Liu et al., 2008). Berberine ameliorates renal injury in STZ-induced diabetes, not by suppression in both oxidative stress and aldose reductase activities (Liu et al., 2008). As berberine is an oral hypoglycemic agent in clinical studies, the hypoglycemic effect of berberine was similar to that of metformin in 36 adult patients of recently diagnosed type 2 diabetes (Yin et al., 2008). Berberine also lowered fasting blood glucose and postprandial blood glucose in 48 adult patients of poorly controlled type 2 diabetes during a 3-month period (Yin et al., 2008). In the same trials, the fasting plasma insulin, insulin insensitivity index, the total cholesterol and low-density lipoprotein cholesterol reduced significantly (Yin et al., 2008).

1.22. Chinese herbal preparations for diabetes

1.22.1 ADHF (anti-diabetes herbal formulation)

ADHF was studied in diet-induced type 2 diabetic animals (C57BL/6J mouse model). The blood glucose level dropped markedly in the mice fed with a diet containing 4% or 8% ADHF. Other diabetic parameters such as insulin insensitivity, histopathological changes in the pancreas and liver were also improved significantly in the mice fed with ADHF (Winters et al., 2003).

1.22.2 *BN (Byakko-ka-ninjin-to)*

BN contains *Radix Ginseng* (*Renshen*), *Rhizoma Anemarrhena* (*Zhimu*), *Radix Glycyrrhizae Uralensis* (*Gancao*), gypsum (*Shigao*) and rice. BN lowered blood glucose levels in diabetic mice. Furthermore, ginseng-anemarrhena (or ginseng-licorice) reduced the blood glucose levels more than any individual component did. The study results indicate that the anti-hyperglycemic effect of BN relies on

the cooperation of four crude therapeutic components and Ca^{2+} (Kimura et al., 1999). The major goal in treating diabetes is to minimize elevation of blood glucose without causing abnormally low levels of blood glucose. The action mechanisms for hypoglycemic herbs are multiple (Fig. 1.17), such as increasing insulin secretion, enhancing glucose uptake by adipose and muscle tissues, inhibiting glucose absorption from intestine and inhibiting glucose production from heptocytes. Our literature search (Yoshikawa et al., 1998; Ziegenfuss et al., 2006; Anderson, 2008; Dannemann et al., 2008) reveals some commonly used herbs for the management of diabetes mellitus (Table 1.1).

1.23. Antidiabetic Indian medicinal plants

India has a rich history of using various potent herbs and herbal components for treating diabetes. Many Indian plants have been investigated for their beneficial use in different types of diabetes. The ethnobotanical information reports about 800 plants that may have anti-diabetic activities (Grover et al., 2002). Indian medicinal plants such as *Azadirachta indica, Gymnema sylvestre, Momordica charantia, Murraya koenigii, Mangifera indica, Swertia chirayita, Syzigium cumini, Trigonella foenum graecum* have been shown to possess hypoglycemic activities (Halim et al., 2002; Shanmugasundaram et al., 1983; Jaspreet et al., 2003; Achyut et al., 2007; Aderibigbe et al., 1999; Chandra et al., 1987).

1.23.1 *Murraya koeingii (curry leaves)*

Curry leaves powder supplementation (12 g providing 2.5 g fiber) for a period of 1 month in 30 NIDDM patients showed reduction in fasting and post-prandial blood sugar levels at 15-day period with no appreciable changes in serum glycosylated protein levels, glycosylated low density lipoprotein cholesterol fraction, serum lipids, lipoprotein cholesterol levels, uronic acid and total amino acids (Grover et al., 2002; Iyer and Mani, 1990). Oral feeding of M. koeingii leaves diet (10%, w/w) for 60 days to normal rats resulted in significant hypoglycemic action associated with increased hepatic glycogen content owing to increased glycogenesis and decreased glycogenolysis and gluconeogenesis

(Grover et al., 2002; Khan et al., 1995). Dietary supplementation with curry leaves has been shown to have a hypolipidemic effect in rats (Khan et al., 1996). Diet containing various doses of curry leaves (5, 10 and 15%) was fed to normal rats for 7 days as well as mild diabetic (blood glucose levels>175 mg/dl) and moderate diabetic rats (>250 mg/ dl) for 5 weeks. In normal rats reduction in blood glucose was almost negligible (4% with 10 and 15% diet). In mild and moderate diabetic rats feeding of 5, 10 and 15% diet caused a maximal reduction in blood sugar by 13.1, 16.3 and 21.4% and 3.2, 5.58 and 8.21%, respectively (Yadav et al., 2002). A single oral administration of 300 mg/kg of aqueous extract of leaves led to 14.68% fall of blood glucose level in normal and 27.96% in mild diabetic after 4 h. The same dose also showed improvement in glucose tolerance of 46.25% in sub diabetic and 38.5% in mild diabetic rabbits in glucose tolerance test after 2 h (Kesari et al., 2005).

1.23.2 *Mangifera indica: mango*

Leaf extract.

Oral administration of aqueous extract of the leaves (1 gm/kg) failed to alter the blood glucose levels in normoglycemic or STZ-induced diabetic rats. However, the extract exhibited anti-diabetic activity when given 60 min before or concurrently with glucose and this action could be attributed to the reduction in intestinal absorption of glucose (Aderibigbe et al., 1999). However, possibility of other mechanism cannot be excluded (Grover et al., 2002). M. indica has also been shown to exert powerful antioxidant activity in vitro (Grover et al., 2002, Martinez et al., 2000). The aqueous extract of leaves of Mangifera produced a reduction in blood glucose level in normoglycemic and glucose induced hyperglycemia but was ineffective on streptozotocin induced diabetic mice (Aderibigbe et al., 2001).

Stem bark extract.

Stem bark aqueous extract (50–800 mg/kg intraperitoneal) caused significant hypoglycemic effects in STZ diabetic rats. The different chemical constituents of

the plant especially the polyphenolics, flavonoids, triterpenoids, mangiferin may be involved in the hypoglycemic effects of the extract (Ojewole, 2005) Mangiferin (10 and 20 mg/kg), intraperitoneal administered once daily for 28 days to STZ-induced diabetic rats caused lowering of blood glucose and improved oral glucose tolerance in glucose loaded normal rats upon chronic administration (10 and 20 mg/kg) intraperitoneal for 14 days. Probably the pancreatic and extra pancreatic mechanisms were involved in the effect (Muruganandan et al., 2005).

1.23.3 *Syzigium cumini (Eugenia jambolana): black berry*

Extract. Oral feeding of E. jambolana (170, 240 and 510 mg/rat for 15 days) caused 50% reduction in blood glucose of normal fasted rats while chlorpropamide showed 52% reduction. In addition, there was a 2.4–6.8-fold and 9.2-fold increase in cathepsin B activity (proteolytic conversion of proinsulin to insulin) by plant extract and chlorpropamide, respectively (Bansal et al., 1981). Decoction of dry leaves of S. cumini has shown hypoglycemic effect (Coimbra et al., 1992). Daily administration of lyophilized powder of E. jambolana (200 mg/kg) showed maximum reduction of 73.51, 55.62 and 48.81 as compared to their basal values in mild (plasma sugar >180 mg/dl, duration 21 days), moderate (plasma sugar >280 mg/ dl, duration 120 days) and severe (plasma sugar >400 mg/dl, duration 60 days) diabetic rats. In addition, the treatment also partially restored altered hepatic and skeletal muscle glycogen content and hepatic glucokinase, hexokinase, glucose-6-phosphate and phosphofructokinase levels (Grover et al., 2000).

Oral feeding of Eugenia extracts (200 mg/kg) daily for 40 days to STZ-diabetic rats reduced the plasma glucose concentrations by 20.84%, prevented polyuria and partially but significantly prevented renal hypertrophy (Grover et al., 2001). The plant extract at 200 mg/kg upon administration for 50 days in STZ-induced diabetic mice caused considerable reduction in the plasma glucose concentration (Grover et al., 2002). Oral administration of alcoholic S. cumini extract to diabetic rats at a dose of 100 mg/kg body weight resulted in a

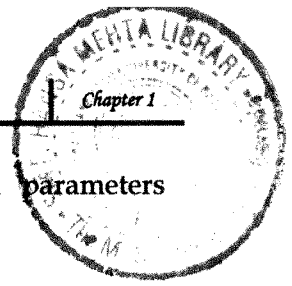
significant reduction in blood glucose and urine sugar and lipids in serum and tissues. The extract also increased the total hemoglobin (Prince et al., 2004). Ethanolic whole seeds, kernel (100 mg/kg of body weight) and seed coat extracts fed to STZ-diabetic rats displayed prominent hypoglycemic effect (Ravi et al., 2004).

Fruit

Oral administration of pulp extract of the fruit of S. cumini to normoglycemic and STZ-induced diabetic rats showed hypoglycemic activity in 30 min while the seeds of the same fruit required 24 h. The action was possibly mediated by insulin secretion (Achrekar et al., 1991). Treatment of alloxan diabetic and severely diabetic rabbits daily once with 25 mg/kg, body weight with F-III (hypoglycemic fraction) of water extract of fruit pulp of Eugenia for 7 and 15 days reduced the fasting glucose (38% diabetic; 48% severely diabetic). Further there was increase in the plasma insulin levels in both diabetic (24.4%) and severely diabetic rabbits (26.3%). The mechanism of action of F-III appeared to be both pancreatic by stimulating release of insulin and extra pancreatic by directly acting on the tissues (Sharma et al., 2006).

Seeds

Preliminary studies on S. cumini seeds have also shown hypoglycemic effect (Mahapatra et al., 1985). Oral administration of dried alcoholic extract of the seeds caused hypoglycemia and reduced glycosuria (Indira et al., 1992). Oral administration of the aqueous extract of seeds of S. cumini (2.5 and 5.0 g/kg body weight for 6 weeks) to alloxan-diabetic rats exhibited hypoglycemic (>glibenclamide) and antioxidant activity. The hypoglycemic effect was most prominent at the dose of 5.0 g/kg while no significant effect was observed at 7.5 g/kg dose (Prince et al., 1998). Blood glucose lowering response of seed powder in STZ female albino Wistar rats was observed at oral doses of 250, 500 or 100 mg/kg (Sridhar et al., 2005). Oral feeding of ethanolic extract (100 mg/kg body weight) of seeds of Eugenia to alloxandiabetic rabbits showed hypoglycaemic



and hypolipidemic effect along with normal histopathological parameters (Sharma et al., 2003).

1.23.4 *Trigonella foenum graecum (fenugreek)*

Defatted seed fraction. Administration of defatted fraction (comprising mainly of the fibers) of fenugreek seeds to normal and diabetic dogs for 8 days exhibited prominent antidiabetic effect (Ribes et al., 1984). The hypo-cholesterolemic effect of defatted portion of fenugreek seeds in dogs has been investigated (Valette et al., 1984). Defatted seed material of fenugreek which is rich in fibers, saponins and other proteins given with meals to alloxan-diabetic dogs for 21 days showed significant antihyperglycemic and anti-glycosuric effect along with reduction in high plasma glucagon and somatostatin (Ribes et al., 1986). Defatted ethanolic extract of fenugreek seeds fed to rats for a 4-week period showed hypocholesterolemic activity (Stark and Madar, 1993).

Extract

A single A single 0.5 ml oral dose of 40–80% decoctions to normal as well as alloxanized mice was followed by hypoglycemia developed over a 6-h period, which was maximum at 6 h and was dose-dependent. The hypoglycemia caused by the ethanol extract (200–400 mg/kg) in alloxanized mice was also dose-dependent and 200 mg/kg was comparable in effect to 200 mg/kg tolbutamide (Ajabnoor et al., 1988). Oral administration of 2 and 8 g/kg of plant extract produced dose-dependent fall in blood glucose both in the normal as well as diabetic rats (20, Khosla et al., 1995). Oral administration of aqueous leaf extract (0.06, 0.2, 0.5, 1 g/kg, IP and 1, 2, 8 g/kg, PO) to normal and alloxanized diabetic rats showed significant hypoglycemic and antihyperglycemic effect while (50%) ethanolic extract significantly reduced blood glucose concentration ($P < 0.02$) at 2 and 24 h when given IP (0.8 g/kg) (20, Abdel-Barry et al., 1995).

Seed powder

A similar glucose lowering effect of powdered fenugreek seed (15 g) soaked in water was observed in noninsulin dependent diabetics (Madar et al., 1988). Fifteen non-insulin dependent diabetic patients given diets with 100 g defatted fenugreek seed powder for 10 days exhibited significant hypoglycaemic effect (Sharma and Raghuram, 1980). Administration of fenugreek seed powder (50 g given with the meals) to Type I diabetic patients for 10 days significantly reduced fasting blood sugar and improved OGTT along with 54% reduction in glycosuria. Significant hypolipidemic effect was also observed (Sharma et al., 1990). Seed powder normalized the altered creatinine kinase activity in heart, skeletal muscle and liver of diabetic rats to almost control values (Genet et al., 1999). It also normalized alteration in hepatic and renal glucose-6-phosphatase and fructose-1,6-bisphosphatase activity (Gupta et al., 1999). Disrupted free radical metabolism in diabetic rats was found to be normalized by fenugreek seed supplementation in the diet (Ravikumar and Anuradha et al., 1999).

Adjunct use of fenugreek seeds (1 g/day hydroalcoholic extract) for 2 months improved glycemic control and decreased the insulin resistance in mild 12 type-2 diabetic patients. There was also a favorable effect on hypertriglyceridemia (Gupta and Lal, 2001). Alcoholic extract (1 g/kg, PO) of fenugreek seeds orally administered to normal and alloxan-diabetic rats for 21 days daily reduced the blood sugar levels from 74.33 ± 4.77 to 60.56 ± 1.9 in normal rats and 201.25 ± 7.69 to 121.25 ± 6.25 in diabetic rats (Vats et al., 2002). A 26% improvement in fasting blood glucose and 30% improvement in glucosuria were observed in STZ-diabetic rats given fenugreek seed mucilage (Kumar et al., 2005).

Diet

In a metabolic study diets with 25 g fenugreek given to 10 non-insulin dependent diabetics for 15 days significantly reduced the area under the plasma glucose curve, half-life and increased the metabolic clearance rate with an

increase in the erythrocyte insulin receptors. Thus, fenugreek may exert its hypoglycemic effect by acting at the insulin receptors as well as at the gastrointestinal level (Raghuram et al., 1994). Fenugreek given in a dose of 2.5 g twice daily for 3 months to coronary artery disease patients also with NIDDM, significantly decreased the blood lipids (total cholesterol and triglycerides) without affecting the HDL-c. When administered in the same daily dose to NIDDM (non-CAD) patients (mild cases), fenugreek reduced significantly the blood sugar (fasting and post prandial). However, in severe NIDDM cases no such effect was observed (Bordia et al., 1997). Ingestion of an experimental diet containing 25 g fenugreek seed powder resulted in a significant reduction of total cholesterol, LDL and VLDL cholesterol and triglyceride levels (Sharma et al., 1998).

Amino acid

4-hydroxyisoleucine, a novel amino acid has been extracted and purified from fenugreek seeds. It increased glucose-induced insulin release (ranging from 100mmol/1 to 1mmol/l) through a direct effect on the isolated islets of langerhans in both rats and humans. This pattern of insulin secretion was biphasic, glucose dependent, occurred in the absence of any change in pancreatic alpha and delta cell activity and without interaction with other agonists of insulin secretion (such as leucine, arginine, tolbutamide, glyceraldehyde) (Sauvaire et al., 1998).

1.22.5 *Azadirachta indica(Neem)*

Neem (*Azadirachta indica*) is an evergreen tree, cultivated in various parts of the India subcontinent. Neem has been extensively used in ayurveda, unani and homoeopathic medicine and has become a cynosure of modern medicine. *Azadirachta indica* leaf extract (200mg/kg) due to epinephrine action showed reduction of peripheral utilization of glucose and glycogenolytic effect (Chattopadhyay, 1996). It also blocked the inhibitory effect of serotonin on insulin secretion mediated by glucose (Chattopadhyay, 1999).

1.22.6 *Allium sativum* (Lahasun)

Garlic (*Allium sativum L.*, Liliaceae) is a common spicy flavoring agent used since ancient times. Oral administration of garlic ethanolic extract (0.1, 0.25, 0.5 g/kg of body weight) showed anti-diabetic effect in diabetic rats (Eidi et al., 2006). *In vivo* treatment with aqueous garlic extract (100mg/kg/day; intra-peritoneal, for 8 weeks) inhibited the development of abnormalities in vascular reactivity induced by diabetes in diabetic rats (Tourandokht and Mehrdad, 2003). Daily Oral administration of 1 ml of either onion or garlic juices/100mg body weight for four week showed hypoglycemic effects in alloxan diabetic rats (El-Demerdash et al., 2005).

1.22.7 *Enicostemma littorale* Blume

It is a small perennial herb of family Gentianaceae, the whole plant is used for medicinal purposes. It is commonly known as "chota chirayita" in Hindi or "mamejua" in Gujarati. It is very bitter and pungent mainly due to the presence of the alkaloid gentianine and the glycoside swertiamarin (Govindachari, 1966; Natarajan et al., 1972; Ghosal & Jaiswal, 1980). Secoiridoids and their glucosides like swertiamarin, sweroside, gentiopicroside, erythrocentaurin etc. are widely distributed in gentianaceae (Jensen, 1992). Isoflavone genistein and c-glycoflavones like swertisin, isovitexin, genkwanin, apigenin, swertisin-5-O-glucoside and isoswertisin-5-O-glucoside were also isolated from *Enicostemma hyssopifolium,* a synonym of *E. littorale* (Ghosal and Jaiswal, 1980) (Fig. 1.10).Chemical investigation of *E. littorale* by Natarajan and Prasad showed four chloroform soluble alkaloids and one water-soluble alkaloid, two sterols and a volatile oil (Natarajan and Prasad, 1972). Iridoid glucoside, swertiamarin isolated from the same plant showed central nervous system (CNS) depressant and cardiostimulant activity (Bhattacharya et al., 1976). Also its hypoglycemic activity has been reported by Vyas et al., (1979) in alloxan induced diabetic rabbits.

Table 1.2: Herbs commonly used in diabetes management

Herbs	Components	Anti-diabetics Mechanisms	Models of experiments or tests	Application and recommend dosage	References
Myrcia	Flavanone glucosides (myrciacitrins) and acetophenone glucosides myrciaphenones)	Inhibit activity of aldose reductase and alphaglucosidase	Streptozotocin diabetic rats	Type II DM	Yoshikawa *et al.*; 1998.
Cinnamon	Cinnulin PF(R)	Improve insulin sensitivity, Decrease fasting blood glucose	Human	Type II DM Type I	Anderson, 2008, Dannemann *et al* .; 2008, Ziegenfuss *et al.*; 2006.
Enicostemma littorale Blume		Increase the serum insulin through K(+)-ATP channel dependent pathway but did not require Ca2+ influx	Alloxan-induced diabetic rats	Type II DM	Maroo *et al.*; 2002.
Biophytum sensitivum		Stimulating the synthesis/ release of insulin from the beta cells of Langerhans	Alloxan-induced diabetic Rabbits	Type II DM	Puri 2001.
Ipomoea batatas	Caiapo (ipomoea batatas)	Decrease insulin insensitivity, increase adiponectin and decrease fibrinogen levels	Type II diabetic patients	Type II DM (4 g/d)	Ludvik *et al.*; 2003, 2008.
Tithonia diversifolia (Hemsl) A. Gray	Nitobegiku	Reducing insulin insensitivity	KK-Ay-mice	Type II DM	Miura *et al.*; 2002.

Sangzhi	Ramulus mori, SZ	Alpha-glucosidase inhibitory effects	Alloxan induced diabetic rats	Type II DM	Ye *et al.*; 2002.
Galega officinalis		Hypoglycemic effects is independent on a reduction of food intake		Type II DM	Palit *et al.*; 1999.
Fenugreek leaves		Similar to glibenclamide, hypoglycemic property and an anti-hyperlipidemic via inferenceiing carbohydrate metabolic enzymes	Streptozotocin induced diabetic rats, human	Type II DM	Devi *et al.*; 2003, Basch *et al.*; 2003.
Pterocarpus marsupium		Decrease HK (hexokinase), GK (glucokinase) and PFK (phosphofructokinase)	Human, alloxan-induced diabetic rats	Type II DM	Grover *et al.*; 2002, Dhanabal *et al.*; 2006.
Vanadium		Regulate activity of carbohydrate-metabolizing enzymes, and enhance expression of IRS-1 and GLUT4 mRNA in adipocytes	STZ-induced diabetic rats, dexamethasonei nduced insulin insensitivity in 3T3-L1 adipocytes	Type II DM	Ramachandr an *et al.*; 2003, Zuo *et al.*; 2008.
Artemisia scoparia	Scoparone (6,7-Dimethoxycouma rin	Anti-atherogenic effect; free radical scavenging properties; inhibited iNOS gene expression and inhibited NFkappaB activation.	Hyperlipidaemi c diabetic rabbits, cytokine-induced beta-cell dysfunction	Type I DM, Type II DM	Chen *et al.*; 1994, Kim *et al.*; 2007
Gymnema sylvestre	Gymnemic acids	Controls the activities of phosphorylase, gluconeogenic enzymes and sorbitol dehydrogenase	Alloxan diabetic rabbits	Type II DM complication	Shanmugasu ndaram *et al* .;1981, Kanetkar *et al* .; 2007.
Daio (Rhei Rhizoma)		Improve kidney function	Patients	Diabetic nephropathy	Goto *et al.*; 2003
Lupinus termis	Lupinus termis	Regulates acetyl cholinesterase activity, AST (Aspartate aminotransferase), ALT (alanine aminotransferase)	Alloxan-induced diabetes, patients	Type II DM	Mansour *et al.*; 2002, Knecht *et al.*; 2006.

		and LDH (lactate dehydrogenase)			
Tea	EGCG	Reduction of IL-1beta and IFNgamma-induced nitric oxide (NO) production and levels of NO synthase (iNOS	STZ-treated islets	Type I DM, Type II DM	Kim *et al.*;2003, Bhathena *et al.*; 2002
Coccinia indica Leaves	Coccinia indica leaf ethanoliextract (CLEt)	Antioxidant property of CLEt	Streptozotocin-diabetic rats	Type II DM	Venkateswaran *et al* .; 2003.
Clausena anisata (Willd) Hook [family: Rutaceae	Terpenoid and coumar	Similar to glibenclamide	Diabetic rats	Type II DM	Ojewole 2002.
Hovenia dulcis Thunb (HDT)		Similar to glibenclamide, lower blood sugar and hepatic glycogen	Alloxan, induced diabetes rats	Type II DM	Ji *et al.*; 2002.
Aloes		Similar to glibenclamide	Patients, alloxan induced Swiss albino diabetic Mice	Type II DM	Ghannam *et al.*; 1986, Okyar *et al* .; 2001.
Vanadyl sulfate	bis(maltolato) oxovanadium (IV), BMOV, bis(ethylmaltolato)oxovanadium (IV), BEOV, and bis(isopropylmaltolato)oxovanadium (IV), BIO V,	Insulin-mimetic	Patients, streptozotocin (STZ)-induced type 1 diabetic mice	Type II DM, Type I DM, 100 mg per day	Yanardag *et al.*; 2003, Thompson *et al.*; 2003, Karmaker *et al* .; 2008.

Several new properties attributed to this plant have been reported in recent past. Our earlier studies has shown its hypoglycemic potential in alloxan-induced diabetic rats (Vijayvargia et al., 2000; Maroo et al., 2002, 2003a, 2003b. and also reported its hypoglycemic, antioxidant and hypolipidaemic potential in newly-diagnosed NIDDM patients (Vasu et al., 2003). We have also reported it's protective in diabetic neuropathy rat model (Bhatt et al., 2009). It is also used as an antidiabetic herbomineral preparation (Babu & Prince, 2004). This herb is also known for its anti-inflammatory (Sadique et al., 1987) and anticancer property (Kavimani & Manisenthlkumar, 2000). Swertiamarin is a secoiridoid glycoside

present in EL, has been reported for its number of activity namely, hepatoprotective, antiedematogenic, free radical scavenging activity, antispastic activity (Vaijanathappa and Badami, 2009). Swertiamarin also showed antihyperlipidaemic effects in P-407-induced hyperlipidaemic rats (Vaidya et al., 2009) and aerial part of *Enicostemma littorale* was reported to show hypolipidaemic effect in p-dimethylaminobenzene (p-DAB) induced hepatotoxic animals (Gopal et al., 2004).

Aims and objectives

Hyperglycemia leads to dislipidemia, generation of oxidative stress in diabetic condition. Hyperglycemia activates four major metabolic pathways 1) polyol pathway 2) Advanced glycation end product (AGEs) formation, 3) protein kinase C activation, 4) increased hexos amine pathway activity. These metabolic alterations are responsible for the development of diabetic complications like neuropathy, nephropathy, retinopathy, cardiomyopathy and diabetic reproductive dysfunctions.

Obesity and type 2 diabetes are occurring at epidemic rates in the United States and many parts of the world. The "obesity epidemic" appears to have emerged largely from changes in our diet and reduced physical activity. An important but not well-appreciated dietary change has been the substantial increase in the amount of dietary fructose consumption from high intake of sucrose and high fructose corn syrup, a common sweetener used in the food industry has been correlated with increased incidence of type 2 diabetes; with varying degree of insulin resistance. Progressive loss of β-cells occurs in both type 1 and type 2 diabetes thus leads to decreased availability of insulin which is responsible for the development of hyperglycemia.

India is a rich sourc of herbal medicines and can be used as an alternative and complimentary medicine for the treatment of diabetes. Our lab is dealing with one such herbal medicine *Enicostemma littorale* Blume. It is having good hypoglycemic, antioxidant, and hypolipidemic activity. In light of this, focus of present study was to evaluate efficacy of EL in diet induced insulin resistance

condition as well as to evaluate its protective role against oxidative stress induced loss of β-cells at cellular level and diabetic complications in rat models.

Objectives of the study –

1. Evaluation of efficacy of *E. littorale* aqueous extract in diet induced insulin resistance rat model.
2. Evaluation of protective effect of *E. littorale* methanolic extract against H_2O_2 induced apoptosis of islets of langerhans.
3. Evaluation of efficacy of *E. littorale* methanolic extract in nephropathic condition in rat models.
4. Evaluation of efficacy of *E. littorale* methanolic extract in diabetic complications in alloxan induced rat model.

1.24. References

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