REVIEW OF LITERATURE

Normal fasting plasma glucose level is normally maintained in a narrow range, usually 75-110 mg/dl and following a meal, it usually will not rise above 140mg/dl. As per the American Diabetes Association (ADA) guidelines, diagnosis of DM is established by noting elevation of blood glucose by any one of the three criterias5:

1. Glycated hemoglobin (Hb A1C) ≥ 6.5%. The test should be performed in a laboratory using a method that is NGSP certified and standardized to the Diabetes Complications and Control Trial (DCCT) assay.*

OR

2. Fasting plasma glucose (FPG) ≥126 mg/dl (7.0mmol/l). Fasting is defined as no caloric intake for at least 8 h.*

OR

3. 2-h plasma glucose ≥ 200 mg/dl (11.1mmol/l) during an oral glucose tolerance test (OGTT). The test should be performed as described by the WHO, using a glucose load containing the equivalent of 75 g anhydrous glucose dissolved in water.*

OR

4. In a patient with classic symptoms of hyperglycemia or hyperglycemic crisis, a random plasma glucose ≥ 200 mg/dl (11.1 mmol/l).

*In the absence of unequivocal hyperglycemia, criteria 1–3 should be confirmed by repeat testing.

Although all forms of DM share hyperglycemia as a common feature, the pathogenic processes involved in the development of hyperglycemia vary widely. The previous classification schemes of DM were based on the age of onset of the disease or on the mode of therapy; in contrast, the recently revised classification reflects our greater understanding of the pathogenesis of each variant. The vast majority of cases of DM fall into one of two broad classes:

Type 1 DM is characterized by an absolute deficiency of insulin caused by pancreatic β-cell destruction. It accounts for approximately 10% of all cases.

Type 2 DM is caused by a combination of peripheral resistance to insulin action and an inadequate secretory response by the pancreatic β-cells ("relative insulin deficiency"). Approximately 80% to 90% of patients have type 2DM.
DM is not a single disease entity, but rather a group of metabolic disorders sharing the common underlying feature of hyperglycemia. Chronic hyperglycemia and attendant metabolic dysregulation may be associated with secondary damage in multiple organ systems, especially the kidneys, eyes, nerves, and blood vessels. DM is a leading cause of end-stage renal disease, adult-onset blindness, and non-traumatic lower extremity amputations in the United States. In most cases, insulin resistance is the primary event, and is followed by increasing degrees of β-cell dysfunction. The complications of DM can be classified as follows:

**Acute complications of DM** - Diabetic Keto Acidosis (DKA)
- Hyper Glycemic Hyperosmolar State (HHS)

**Chronic complications of DM** –
- Microvascular complications → Eye diseases - Retinopathy
  - Macular oedema
  - Cataract
  - Glaucoma
  → Nephropathy
  → Neuropathy – Sensory and Motor
  - Autonomic
- Macrovascular complications → Coronary Artery Disease (CAD)
  → Peripheral Vascular Disease (PVD)
  → Cerebro Vascular Disease (CVD)
- Others → Gastrointestinal
  → Genitourinary
  → Dermatological

**Normal insulin physiology**: Glucose homeostasis is tightly regulated by three inter-related processes: glucose production in the liver; glucose uptake and utilization by peripheral tissues, chiefly skeletal muscle; and actions of insulin and counter-regulatory hormones, including glucagon, on glucose. Insulin and glucagon have opposing regulatory effects on glucose homeostasis. During fasting states, low insulin and high glucagon levels facilitate hepatic gluconeogenesis and glycogenolysis (glycogen breakdown) while decreasing glycogen synthesis, thereby preventing hypoglycemia. Thus, fasting plasma glucose levels are determined primarily by hepatic glucose output. Following a meal, insulin levels rise and glucagon levels fall in response to the large glucose
load. Insulin promotes glucose uptake and utilization by tissues. Skeletal muscle is the major insulin responsive site for postprandial glucose utilization, and is critical for preventing hyperglycemia and maintaining glucose homeostasis.

Regulation of Insulin Release:

Insulin gene is expressed in the β- cells of the pancreatic islets. Prepro-insulin is synthesized in the rough endoplasmic reticulum from insulin mRNA and delivered to the golgi apparatus. There, a series of proteolytic cleavage steps generate mature insulin and a cleavage peptide, C-peptide. Both insulin and C-peptide are then stored in secretory granules and secreted in equimolar quantities after physiologic stimulation; increasingly, C-peptide levels are being used as a clinical assay to measure endogenous insulin secretion. The most important stimulus that triggers insulin synthesis and release is glucose itself. A rise in blood glucose levels results in glucose uptake into pancreatic β- cells, facilitated by an insulin-independent, glucose-transporter protein, GLUT-2. Metabolism of glucose via glycolysis generates ATP, resulting in increase in cytoplasmic ATP/ADP ratio. This inhibits the activity of the ATP-sensitive K⁺-channel on the β- cell membrane, leading to membrane depolarization and the influx of extracellular Ca²⁺ through voltage-dependent Ca²⁺-channels. The resultant increase in intracellular Ca²⁺ stimulates secretion of insulin, presumably from stored hormone within the β-cell granules. This is the phase of immediate release of insulin. If the secretory stimulus persists, a delayed and protracted response follows that involves active synthesis of insulin. Other agents, including intestinal hormones and certain amino acids (leucine and arginine), stimulate insulin release but not synthesis.s

Insulin Action and Insulin Signaling Pathways:

Insulin is the most potent anabolic hormone known, with multiple synthetic and growth-promoting effects. Its principal metabolic function is to increase the rate of glucose transport into certain cells in the body. These are the striated muscle cells (including myocardial cells) and to a lesser extent, adipocytes, representing collectively about two thirds of the entire body weight. Glucose uptake by other peripheral tissues, most notably the brain, is insulin-independent. In muscle cells, glucose is then either stored as glycogen or oxidized to generate ATP. In adipose tissue, glucose is primarily stored as lipid. Besides promoting lipid synthesis, insulin also inhibits lipid degradation in adipocytes. Similarly, insulin promotes amino acid uptake and protein synthesis, while inhibiting protein degradation. Thus, the anabolic
effects of insulin are attributable to increased synthesis and reduced degradation of glycogen, lipids, and proteins. In addition, insulin has several mitogenic functions, including initiation of DNA synthesis in certain cells and stimulation of their growth and differentiation. Binding of insulin to its receptor triggers a complex signaling cascade of protein phosphorylation and dephosphorylation culminating in the metabolic and mitogenic effects of insulin described above. Insulin receptor is a tetrameric protein composed of two α- and two β-subunits. The β-subunit cytosolic domain possesses tyrosine kinase activity. Insulin binding to the α-subunit extracellular domain activates the β-subunit tyrosine kinase, resulting in both autophosphorylation of the receptor and phosphorylation of downstream signal transduction elements. The signaling pathways can be divided into two broad functional categories, mitogenic and metabolic, with the understanding that there may be considerable cross-talk between the protein intermediates. The mitogen activated protein kinase (MAP-Kinase) pathway is responsible for the mitogenic effects of insulin (and insulin-like growth factors), promoting cellular proliferation and growth. The metabolic effects of insulin are principally mediated by phosphatidylinositol-3-kinase (PI-3K). PI-3K-dependent signaling mediates several of the cellular effects of insulin described above.

**Pathogenesis of Type 1 DM**:  
This form of DM results from a severe lack of insulin caused by an immunologically mediated destruction of β-cells. Type I DM most commonly develops in childhood, becomes manifest at puberty, and progresses with age. Type 1 DM is an autoimmune disease in which islet destruction is caused primarily by T lymphocytes reacting against as yet poorly defined β-cell antigens. As in all autoimmune diseases, genetic susceptibility and environmental factors play important roles in the pathogenesis.  
**Mechanisms of β-Cell Destruction:**  
Although the clinical onset of type 1 DM is abrupt, this disease in fact results from a chronic autoimmune attack on β-cells that usually starts many years before the disease becomes evident. The classic manifestations of the disease (hyperglycemia and ketosis) occur late in its course, after more than 90% of the β-cells have been destroyed.  
Several mechanisms contribute to β-cell destruction:
- T lymphocytes react against β-cell antigens and cause cell damage.
- Locally produced cytokines damage β-cells.
Auto antibodies against islet cells and insulin are also detected in the blood of 70% to 80% of patients.

Genetic susceptibility: Type 1 DM has a complex pattern of genetic associations, and putative susceptibility genes have been mapped to at least 20 loci. Many of these associations are with chromosomal regions, and the particular genes involved are not known yet.

Environmental Factors: There is evidence that environmental factors, especially infections, are involved in triggering autoimmunity in type 1 DM and other autoimmune diseases. Epidemiologic studies suggest a role of viruses. Seasonal trends that often correspond to the prevalence of common viral infections have long been noted in the diagnosis of new cases, as has the association between coxsackie viruses of group B and pancreatic diseases, including diabetes. Other implicated viral infections include mumps, measles, cytomegalovirus, rubella, and infectious mononucleosis. In all these cases, the viruses are not thought to cause diabetes by directly damaging β-cells. Rather, two mechanisms, which are not mutually exclusive, have been proposed to explain how infections can trigger autoimmunity. One is that the infections induce tissue damage and inflammation, leading to the release of β-cell antigens and the recruitment and activation of lymphocytes and other inflammatory leukocytes in the tissue. The other possibility is that the viruses produce proteins that mimic self-antigens and the immune response to the viral protein cross-reacts with the self tissue. Although there is experimental evidence in support of both possibilities, neither has been established as being actually involved. It should also be pointed out that recent epidemiologic studies have shown that in the United States, the incidence of type 1 DM in children under 15 years of age has tripled since the 1960s. Similar trends are seen in Western Europe. These findings are often interpreted as suggesting that infections may actually be protective in this disease and the increased incidence reflects the reduction in common infections. Consistent with this possibility, infections also prevent disease development in the non-obese diabetic mouse model.

Pathogenesis of Type 2 DM:

While much has been learned in recent years, the pathogenesis of type 2 DM still remains enigmatic. Environmental factors, such as a sedentary life style and dietary habits, clearly play a role, as will become evident when obesity is considered. Nevertheless, genetic factors are even more important than in type 1 DM. Among identical twins, the concordance rate is 50% to 90%, while among first-degree
relatives with type 2 DM, the risk of developing the disease is 20% to 40%, compared to 5% to 7% in the population at large. Unlike type 1 DM, however, the disease is not linked to genes involved in immune tolerance and regulation, and there is no evidence to suggest an autoimmune basis for type 2 DM.

The two metabolic defects that characterize type 2 DM are
1) A decreased ability of peripheral tissues to respond to insulin (insulin resistance) and
2) β-cell dysfunction that is manifested as inadequate insulin secretion in the face of insulin resistance and hyperglycemia.

In most cases, insulin resistance is the primary event, and is followed by increasing degrees of β-cell dysfunction.

Insulin Resistance:
Insulin resistance is defined as resistance to the effects of insulin on glucose uptake, metabolism, or storage. Insulin resistance is a characteristic feature of most patients with type 2 DM and is an almost universal finding in diabetic individuals who are obese. The role of insulin resistance in the pathogenesis of type 2 DM can be gauged from the findings that
(1) Insulin resistance is often detected 10 to 20 years before the onset of diabetes in predisposed individuals (e.g., offspring of type 2 DM) and
(2) In prospective studies, insulin resistance is the best predictor for subsequent progression to diabetes.

Insulin resistance leads to decreased uptake of glucose in muscle and adipose tissues and an inability of the hormone to suppress hepatic gluconeogenesis. Functional studies in individuals with insulin resistance have demonstrated numerous quantitative and qualitative abnormalities of the insulin signaling pathway, including down regulation of the insulin receptor; decreased insulin receptor phosphorylation and tyrosine kinase activity; reduced levels of active intermediates in the insulin signaling pathway; and impairment of translocation, docking, and fusion of GLUT-4-containing vesicles with the plasma membrane. It is recognized that insulin resistance is a complex phenomenon.

Genetic Defects of the Insulin Receptor and Insulin Signaling Pathway: Loss or function abnormalities of either the insulin receptor or its downstream intermediates are obvious candidates for mediating insulin resistance in type 2 DM. In mice, tissue-
specific knockout of genes encoding various insulin signaling proteins has resulted in insulin resistance, hyperinsulinemia and hyperglycemia, recapitulating human type 2 DM. Unfortunately, the extrapolation of these single-gene knockout models to human disease has been less than gratifying. Point mutations of the insulin receptor are relatively rare, accounting for no more than 1% to 5% of patients with insulin resistance. Analysis of candidate genes involved in insulin secretion or insulin action, as well as whole genome linkage studies of affected families have yielded many polymorphisms that associate with the type 2 diabetic phenotype, but in most cases, the associations have been weak, or the studies were not reproducible. From these analyses, it appears that while the population risk associated with any particular genetic variant (polymorphism) may be significant, the increased risk for developing DM for a given individual harboring that variant is small at best. Suffice it to say that while no one questions a genetic component to insulin resistance, the high "noise" to signal ratio has hampered identification of the genes involved. The genetic basis of insulin resistance, and by extension type 2 DM, therefore, remains an enigma.

Obesity and Insulin Resistance: The association of obesity with type 2 DM has been recognized for decades, visceral obesity being a common phenomenon in the majority of type 2 diabetics. The link between obesity and DM is mediated via effects on insulin resistance. Insulin resistance is present even in simple obesity unaccompanied by hyperglycemia, indicating a fundamental abnormality of insulin signaling in states of fatty excess. The risk for DM increases as the body mass index (a measure of body fat content) increases. It is not only the absolute amount but also the distribution of body fat that has an effect on insulin sensitivity. Central obesity (abdominal fat) is more likely to be linked with insulin resistance than are peripheral (gluteal/subcutaneous) fat depots. Although many details of the so-called adipoin insulin axis remain to be elucidated, following are some of the putative pathways leading to insulin resistance:

- Role of free fatty acids (FFAs): Cross-sectional studies have demonstrated an inverse correlation between fasting plasma FFAs and insulin sensitivity. Furthermore, the level of intracellular tri aylglycerol (TG) is often markedly increased in muscle and liver tissues in obese individuals, presumably because excess circulating FFAs are deposited in these organs. Intracellular TG and products of fatty acid metabolism are potent inhibitors of insulin signaling and result in an acquired insulin resistance state.
These "lipotoxic" effects of FFAs are most likely mediated through a decrease in activity of key insulin-signaling proteins.

- Role of adipokines in insulin resistance: It is increasingly recognized that adipose tissue is not merely a passive storage depot for fat, but can also operate as a functional endocrine organ, releasing hormones in response to changes in the metabolic status. A variety of proteins released into the systemic circulation by adipose tissue have been identified, and these are collectively termed adipokines (or adipose cytokines). Dysregulation of adipokine secretion (either abnormally increased or decreased levels) may be one of the mechanisms by which insulin resistance is tied to obesity. Several adipokines have been implicated in insulin resistance, including leptin, adiponectin and resistin. Leptin acts on central nervous system receptors and other sites to reduce food intake and induce satiety. Leptin-deficient animals demonstrate severe insulin resistance that is reversed by administration of leptin. Whereas many of leptin's insulin-sensitizing actions are mediated by central nervous system receptors, some effects may be exerted directly at the level of insulin target tissues. The role of leptin in states of insulin resistance in humans is an area of active investigation.

- Role of the peroxisome proliferator—activated receptor gamma (PPAR γ) and thiazolidinediones (TZDs): TZDs are a class of antidiabetic compounds that were developed in the early 1980s as antioxidants. The target receptor for TZDs has been identified as PPAR γ, a nuclear receptor and transcription factor. PPAR γ is most highly expressed in adipose tissue, and activation of the receptor by TZDs results in modulation of gene expression in adipocytes, eventually leading to reduction of insulin resistance. The targets of PPAR γ activation include several of the adipokines discussed above. PPAR γ activation also decreases levels of FFAs, which, as mentioned earlier, contributes to insulin resistance in obesity. To summarize, insulin resistance in type 2 DM is a complex and multifactorial phenomenon. Genetic defects in the insulin signaling pathway are not common and, when present, are more likely polymorphisms with subtle effects rather than inactivating mutations. Insulin resistance is acquired in the overwhelming majority of individuals, and obesity is central to this phenomenon.

β-Cell Dysfunction: β-cell dysfunction in type 2 DM reflects the inability of these cells to adapt themselves to the long-term demands of peripheral insulin resistance and increased insulin secretion. In states of insulin resistance, insulin secretion is initially higher for each level of glucose than in controls. This hyperinsulinemic state
is a compensation for peripheral resistance and can often maintain normal plasma glucose for years. Eventually, however, β-cell compensation becomes inadequate, and there is progression to overt DM. The underlying basis for failure of β-cell adaptation is not known, although it is postulated that several mechanisms, including adverse effects of high circulating FFA (lipotoxicity) or chronic hyperglycemia (glucotoxicity), may play a role. β-cell dysfunction in type 2 DM manifests itself as both qualitative and quantitative defects:

- **Qualitative β-cell dysfunction** is initially subtle, and seen as loss of the normal pulsatile, oscillating pattern of insulin secretion and attenuation of the rapid first phase of insulin secretion triggered by an elevation in plasma glucose. Over time, the secretory defect affects all phases of insulin secretion, and even though some basal insulin secretion persists in type 2 DM, it is grossly inadequate to overcome the insulin resistance.

- **Quantitative β-cell dysfunction** is reflected by a decrease in β-cell mass, islet degeneration, and deposition of islet amyloid. Islet amyloid protein (amylin) is a characteristic finding in patients with type 2 DM and is present in more than 90% of diabetic islets examined. Islet amyloidosis is associated with a decrease in β-cell mass, although it is uncertain whether the amyloid is involved in or merely a consequence of the β-cell decrease. Although there are scant data in humans, studies from animal models of diabetes support the aforementioned sequence of events where in β-cell hyperplasia in the prediabetic state is followed by a decrease in β-cell mass that coincides with clinical progression to DM. In this context, it is important to note that even a "normal" β-cell mass in diabetic individuals may in fact indicate a relative reduction for the degree of insulin resistance.

**Biochemical basis of complications of DM**: Both types of DM are characterized by chronic hyperglycaemia and the development of diabetes-specific microvascular pathology in the retina, renal glomerulus and peripheral nerve. As a consequence of its microvascular pathology, DM is a leading cause of blindness, end stage renal disease and a variety of debilitating neuropathies. DM is also associated with accelerated atherosclerotic macrovascular disease affecting arteries that supply the heart, brain and lower extremities. As a result, patients with DM have a much higher risk of myocardial infarction, stroke and limb amputation. Large prospective clinical studies show a strong relationship between glycaemia and diabetic microvascular complications in
both type 1 and type 2 DM. Hyperglycaemia and insulin resistance both seem to have important roles in the pathogenesis of macrovascular complications. Diabetes-specific microvascular disease in the retina, glomerulus and vasa nervorum has similar pathophysiological features. Early in the course of diabetes, intracellular hyperglycaemia causes abnormalities in blood flow and increased vascular permeability. This reflects decreased activity of vasodilators such as nitric oxide (NO), increased activity of vasoconstrictors such as angiotensin II and endothelin-1, and elaboration of permeability factors such as vascular endothelial growth factor (VEGF). Quantitative and qualitative abnormalities of extracellular matrix contribute to an irreversible increase in vascular permeability. With time, microvascular cell loss occurs, in part as a result of programmed cell death, and there is progressive capillary occlusion due to extracellular matrix overproduction induced by growth factors such as transforming growth factor-β (TGF-β), and deposition of extravasated periodic acid–Schiff-positive plasma proteins. Hyperglycaemia may also decrease production of trophic factors for endothelial and neuronal cells. Together, these changes lead to oedema, ischaemia and hypoxia-induced neovascularization in the retina, proteinuria, mesangial matrix expansion and glomerulosclerosis in the kidney, and multifocal axonal degeneration in peripheral nerves. In diabetic arteries, endothelial dysfunction seems to involve both insulin resistance specific to the phosphatidylinositol-3-OH kinase pathway and hyperglycaemia. Pathway-selective insulin resistance results in decreased endothelial production of the anti-atherogenic molecule NO, and increased proliferation of vascular smooth muscle cells and production of plasminogen activator inhibitor-1 (PAI-1). Hyperglycaemia itself also inhibits production of NO in arterial endothelial cells and stimulates production of PAI-1. Both insulin resistance and hyperglycaemia have also been implicated in the pathogenesis of diabetic dyslipidaemia. Hyperglycaemia seems to cause raised levels of atherogenic cholesterol-enriched apolipoprotein B-containing remnant particles by reducing expression of the heparan sulphate proteoglycan perlecan on hepatocytes. Associations of atherosclerosis and atherosclerosis risk factors with glycaemia have been shown over a broad range of glucose tolerance, from normal to diabetic. Postprandial hyperglycaemia may be more predictive of atherosclerosis than fasting plasma glucose level or HbA1c. Whether postprandial hyperglycaemia is an independent risk factor is controversial and requires further study.
Mechanisms of hyperglycaemia-induced damage:

Four main hypotheses about how hyperglycaemia causes diabetic complications have generated a large amount of data, as well as several clinical trials based on specific inhibitors of these mechanisms. The four hypotheses are:

1) Increased polyol pathway flux,
2) Increased advanced glycation end-product (AGE) formation,
3) Activation of protein kinase C (PKC) isoforms and
4) Increased hexosamine pathway flux.

Until recently there was no unifying hypothesis linking these four mechanisms.

1) Increased polyol pathway flux:

AR (alditol:NAD(P)+ 1-oxidoreductase, EC 1.1.1.21) is the first enzyme in the polyol pathway. It is a cytosolic, monomeric oxidoreductase that catalyses the NADPH-dependent reduction of a wide variety of carbonyl compounds, including glucose. AR has a low affinity (high $K_m$) for glucose, and at the normal glucose concentrations as found in non-diabetics, metabolism of glucose by this pathway is a very small percentage of total glucose use. But in a hyperglycaemic environment, increased intracellular glucose results in its increased enzymatic conversion to the polyalcohol sorbitol, with concomitant decrease in NADPH. In the polyol pathway, sorbitol is oxidized to fructose by the enzyme sorbitol dehydrogenase (SDH), with NAD+ reduced to NADH. Flux through this pathway during hyperglycaemia varies from 33% of total glucose use in the rabbit lens to 11% in human erythrocytes. Thus, the contribution of this pathway to diabetic complications may be very much species, site and tissue dependent.
It has been proposed that oxidation of sorbitol by NAD+ increases the cytosolic NADH: NAD+ ratio, thereby inhibiting activity of the enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and increasing concentrations of triose phosphate. Raised triose phosphate concentrations could increase formation of both methylglyoxal, a precursor of AGEs, and diacylglycerol (DAG) (through a-glycerol-3-phosphate), thus activating PKC. Although hyperglycaemia does increase the NADH:NAD+ ratio in endothelial cells, this reflects a marked decrease in the absolute concentration of NAD+ as a result of consumption by activated poly(ADP-ribose) polymerase (PARP), rather than reduction of NAD+ to NADH. Activation of PARP by hyperglycaemia is mediated by increased production of reactive oxygen species. It has also been proposed that reduction of glucose to sorbitol by NADPH consumes NADPH. As NADPH is required for regenerating reduced glutathione (GSH), this could induce or exacerbate intracellular oxidative stress. Decreased levels of GSH have in fact been found in the lenses of transgenic mice that over express AR, and this is the most likely mechanism by which increased flux through the polyol pathway has deleterious consequences. This conclusion is further supported by recent experiments with homozygous knockout mice deficient in AR, which showed that, in contrast to wild-type mice, diabetes neither decreased the GSH content of sciatic nerve nor reduced motor nerve conduction velocity. Studies of inhibition of the polyol pathway in vivo have yielded inconsistent results. In a five-year study in dogs, AR inhibition prevented diabetic neuropathy, but failed to prevent retinopathy or thickening of the capillary basement membrane in the retina, kidney and muscle. Several negative clinical trials have questioned the relevance of this mechanism in humans. The positive effect of AR inhibition on diabetic neuropathy has, however, been confirmed in humans in a rigorous multi-dose, placebo controlled trial with the potent AR inhibitor zenarestat.

2) Increased intracellular formation of AGEs:
AGEs are found in increased amounts in diabetic retinal vessels and renal glomeruli. They were originally thought to arise from nonenzymatic reactions between extracellular proteins and glucose. But the rate of AGE formation from glucose is slower than the rate of AGE formation from glucose-derived dicarbonyl precursors generated intracellularly, and it now seems likely that intracellular hyperglycaemia is the primary initiating event in the formation of both intracellular and extracellular AGEs. AGEs can arise from intracellular auto-oxidation of glucose to glyoxal,
decomposition of the Amadori product (glucose-derived 1-amino-1-deoxyfructose lysine adducts) to 3-deoxyglucosone (perhaps accelerated by an amadoriase), and fragmentation of glyceraldehyde- 3-phosphate and dihydroxyacetone phosphate to methylglyoxal. These reactive intracellular dicarbonyls — glyoxal, methylglyoxal and 3-deoxyglucosone — react with amino groups of intracellular and extracellular proteins to form AGEs. Methylglyoxal and glyoxal are detoxified by the glyoxalase system. All three AGE precursors are also substrates for other reductases. The potential importance of AGEs in the pathogenesis of diabetic complications is indicated by the observation in animal models that two structurally unrelated AGE inhibitors partially prevented various functional and structural manifestations of diabetic microvascular disease in retina, kidney and nerve. In a large randomized, double-blind, placebo-controlled, multi-centre trial in type 1 diabetic patients with overt nephropathy, the AGE inhibitor aminoguanidine lowered total urinary protein and slowed progression of nephropathy, over and above the effects of existing optimal care. In addition, aminoguanidine reduced the progression of diabetic retinopathy. Production of intracellular AGE precursors damage target cells by three general mechanisms.
First, intracellular proteins modified by AGEs have altered function. Second, extracellular matrix components modified by AGE precursors interact abnormally with other matrix components and with the receptors for matrix proteins (integrins) on cells. Third, plasma proteins modified by AGE precursors bind to AGE receptors on endothelial cells, mesangial cells and macrophages, inducing receptor-mediated production of reactive oxygen species. This AGE receptor ligation activates the pleiotropic transcription factor NF-κB, causing pathological changes in gene expression. In endothelial cells exposed to high glucose, intracellular AGE formation occurs within a week. Basic fibroblast growth factor is one of the main AGE-modified proteins in endothelial cells. Proteins involved in macromolecular endocytosis are also modified by AGEs, as the increase in endocytosis induced by hyperglycaemia is prevented by overexpression of the methylglyoxal-detoxifying enzyme glyoxalase I. AGE formation alters the functional properties of several important matrix molecules like collagen.

3) Activation of PKC:
The PKC family comprises at least eleven isoforms, nine of which are activated by the lipid second messenger DAG. Intracellular hyperglycaemia increases the amount
of DAG in cultured microvascular cells and in the retina and renal glomeruli of diabetic animals. It seems to achieve this primarily by increasing de novo DAG synthesis from the glycolytic intermediate dihydroxyacetone phosphate, through reduction of the latter to glycerol-3-phosphate and stepwise acylation. Increased de novo synthesis of DAG activates PKC both in cultured vascular cells and in retina and glomeruli of diabetic animals. Hyperglycaemia may also activate PKC isoforms indirectly through both ligation of AGE receptors and increased activity of the polyol pathway, presumably by increasing reactive oxygen species. In early experimental diabetes, activation of PKC-b isoforms has been shown to mediate retinal and renal blood flow abnormalities, perhaps by depressing NO production and/or increasing endothelin-1 activity.

Abnormal activation of PKC has been implicated in the decreased glomerular production of NO induced by experimental diabetes, and in the decreased production of NO in smooth muscle cells that is induced by hyperglycaemia. Activation of PKC also inhibits insulin-stimulated expression of the messenger RNA for endothelial nitric oxide synthase (eNOS) in cultured endothelial cells. Hyperglycaemia increases endothelin-1-stimulated MAP-kinase activity in glomerular mesangial cells by activating PKC isoforms. The increased permeability of endothelial cells induced by high glucose in cultured cells is mediated by activation of PKC-a, however. Activation of PKC by raised glucose also induces expression of the permeability enhancing factor VEGF in smooth muscle cells. In addition to affecting hyperglycaemia-induced abnormalities of blood flow and permeability, activation of PKC contributes to increased microvascular matrix protein accumulation by inducing expression of TGF-b1, fibronectin and type IV collagen both in cultured mesangial cells and in glomeruli of diabetic rats. This effect seems to be mediated through inhibition of NO production by PKC. But hyperglycaemia-induced expression of laminin C1 in cultured mesangial cells is independent of PKC activation.

Hyperglycaemia-induced activation of PKC has also been implicated in the overexpression of the fibrinolytic inhibitor PAI-1, the activation of NF-kB in cultured endothelial cells and vascular smooth muscle cells, and in the regulation and activation of various membrane-associated NAD (P) H-dependent oxidases. Treatment with an inhibitor specific for PKC-b significantly reduced PKC activity in the retina and renal glomeruli of diabetic animals. Concomitantly, treatment significantly reduced diabetes induced increases in retinal mean circulation time,
normalized increases in glomerular filtration rate and partially corrected urinary albumin excretion.

4) Increased flux through the hexosamine pathway:
Shunting of excess intracellular glucose into the hexosamine pathway might also cause several manifestations of diabetic complications. In this pathway, fructose-6-phosphate is diverted from glycolysis to provide substrates for reactions that require UDP-N-acetylglucosamine, such as proteoglycan synthesis and the formation of O-linked glycoproteins. Inhibition of the rate-limiting enzyme in the conversion of glucose to glucosamine — glutamine:fructose-6-phosphate amidotransferase (GFAT) blocks hyperglycaemia-induced increases in the transcription of TGF-a, TGF-b1 and PAI-1. This pathway also plays an important role in hyperglycaemia induced and fat-induced insulin resistance. The mechanism by which increased flux through the hexosamine pathway mediates hyperglycaemia-induced increases in gene transcription is not certain, but the observation that binding sites for the transcription factor Sp1 regulate hyperglycaemia-induced activation of the PAI-1 promoter in vascular smooth muscle cells suggested that covalent modification of Sp1 by N-acetylglucosamine (GlcNAc) might explain the link between activation of the hexosamine pathway and hyperglycaemia-induced changes in transcription of the gene for PAI-1. Glucosamine itself was subsequently shown to activate the PAI-1 promoter through Sp1 sites in glomerular mesangial cells. The glycosylated form of Sp1 seems to be more transcriptionally active than the deglycosylated form. A fourfold increase in O-acetylglucosaminylaton of Sp1 caused by inhibition of the enzyme O-GlcNAc-b-N-acetylglucosaminidase resulted in a
A common element linking hyperglycaemia-induced damage:

Although specific inhibitors of AR activity, AGE formation, PKC activation and the hexosamine pathway each ameliorate various diabetes-induced abnormalities in cell culture and animal models, there has been no apparent common element linking the four mechanisms of hyperglycaemia-induced damage. This issue has now been resolved by the recent discovery that each of the four different pathogenic mechanisms reflects a single hyperglycaemia-induced process: overproduction of superoxide by the mitochondrial electron-transport chain. Many studies have shown that diabetes and hyperglycaemia increase oxidative stress, but neither the underlying mechanism nor the consequences for other pathways of hyperglycaemic damage were known. During the metabolism of glucose, most of its energy will be released as reducing equivalents, which undergo further oxidation and release their high energy by a process called oxidative phosphorylation in the inner mitochondrial membrane. As the reducing equivalents pass through various complexes in the electron transport chain, they undergo stepwise oxidation and release their energy. This generates an electrochemical potential gradient across the membrane. When the electrochemical potential difference generated by the proton gradient across the inner mitochondrial membrane is high, the lifetime of superoxide-generating electron-transport intermediates such as ubisemiquinone is prolonged.
There seems to be a threshold value above which superoxide production is markedly increased. Du, et al found that hyperglycaemia increases the proton gradient above this threshold value as a result of overproduction of electron donors by the TCA cycle. This, in turn, causes a marked increase in the production of superoxide by endothelial cells. Over expression of manganese superoxide dismutase (MnSOD), the mitochondrial form of superoxide dismutase, abolished the signal generated by reactive oxygen species, and over expression of uncoupling protein-1 (UCP-1) collapsed the proton electrochemical gradient and prevented hyperglycaemia-induced overproduction of reactive oxygen species. Inhibition by MnSOD or UCP-1 of hyperglycaemia-induced overproduction of mitochondrial superoxide completely prevented an increase in polyol pathway flux, increased intracellular AGE formation, increased PKC activation and an increase in hexosamine pathway activity in endothelial cells. As hyperglycaemia-induced overproduction of mitochondrial superoxide induces a 66% decrease in GAPDH activity, the effect of hyperglycaemia on polyol pathway flux may reflect the accumulation of glycolytic metabolites, including glucose, upstream of GAPDH.

**Pathophysiology of Diabetic Complications**:  
Diabetic Keto Acidosis (DKA): It is a state of severe metabolic decompensation characterized by the biochemical triad of hyperglycemia, metabolic acidosis and increased total ketone bodies or keto acids. These rearrangements result from a
combination of insulin deficiency and an increase in the counter regulatory hormones like glucagon, catecholamines, cortisol and growth hormone.

a) Hyperglycemia in DKA is the result of the following events:
   - Increased gluconeogenesis
   - Accelerated glycogenolysis
   - Impaired glucose use by peripheral tissues.

Increased hepatic production of glucose is due to high availability of gluconeogenic precursors and increased activity of the key gluconeogenic enzymes such as phosphoenol pyruvate carboxy kinase, fructose-1, 6- bisphosphatase and pyruvate carboxylase. In addition to increased glucose production, the combination of high levels of glucagon, catecholamines and cortisol with concurrent insulinopenia accelerate glycogenolysis and impair the glucose uptake in peripheral tissues by reducing the levels of GLUT-4.

b) The increased production of ketone bodies in DKA is caused, again by a decrease in effective circulatory insulin, associated with elevations in counter regulatory hormones, particularly epinephrine which causes the activation of hormone sensitive lipase in adipose tissue. The increased activity of the tissue lipase causes break down of TG to glycerol and FFA. The glycerol provide carbon- skeleton for gluconeogenesis while the elevated levels of FFA leads to increased production of ketone bodies via β- oxidation. Ketogenesis is enhanced further by decreased concentrations of malonyl Co A, which occurs as a result of the increased glucagon/insulin ratio. Malonyl Co A inhibits carnitine palmitoyl acyl transferase-1(CPT-1), the key enzyme of β-oxidation. Therefore, reduction in malonyl Co A leads to stimulation of CPT-1 and effective increase in ketogenesis, which leads to ketonemia. Accumulation of keto acids results in an increased anion gap metabolic acidosis.

c) Fluid and electrolyte abnormalities: Severe derangement of water and electrolyte balance occurs in DKA, resulting from hyperglycemia, insulin deficiency and ketonemia. Osmotic diuresis resulting from hyperglycemia promotes net loss of multiple minerals and electrolytes including sodium, calcium, magnesium, phosphate and chloride. Intracellular dehydration occurs with a shift of water out of the cells. This is associated with movement of potassium from the cell to extracellular compartment, a phenomenon that is aggravated by acidosis. Furthermore, insulin deficiency results in a reduction in the activity of Na⁺ - K⁺ ATPase, with reduced exchange of these ions across the cell membrane.
Hyperglycemic Hyperosmolar Syndrome (HHS):
HHS is a more serious but less common hyperglycemic emergency in DM. A typical HHS patient has undiagnosed DM, is between 55-70 years and with associated co-morbid condition like stroke or renal failure. The key difference between DKA and HHS is that ketosis is absent or minimal here; the cause of which is not completely understood. Presumably the insulin deficiency is only relative and less severe here than in DKA. Also the levels of counter-regulatory hormones and FFA are comparatively lower in HHS.

Microvascular Complications:
Hyperglycemia is the causative factor for microvascular complications. But few other incompletely defined factors suggested being responsible include genetic and environmental factors.

Ophthalmologic Complications of DM:
Diabetic retinopathy (DR) considered as the hallmark of DM, is the most common of the various ocular complications of DM. DR is clinically divided in to two major types or stages- proliferative and non-proliferative. It mainly affects the microvasculature of retina. Hyperglycemia exerts its effect via all the four proposed mechanisms, which leads to the death of retinal pericytes, microvascular cells and impairment of basement membrane function. These in turn are associated with formation of retinal capillary micro-aneurism, excessive vascular permeability and increased activity of vasoproliferative substances, all of which lead to retinal ischemia. Sorbitol accumulation in the crystalline lens increases with hyperglycemia. It leads to a rise in intracellular osmolality which causes absorption of water in to the cells and cellular swelling. Due to this osmotic lens swelling, there will be changes in the lens size leading to changes in refractive power.

Renal Complications of DM:
Diabetic nephropathy is the leading cause of end stage renal disease (ESRD) worldwide. Pathogenesis is again related to chronic hyperglycemia and the associated mechanisms, especially through the formation of AGEs and activation of cytokines causing hyperfiltration and renal injury. Genetic susceptibility has also been proposed to be an important factor in the development and progression of diabetic nephropathy. Development of systemic hypertension is an adverse factor. The deleterious effects of hypertension are likely to be directed at the vasculature and microvasculature. The natural history of diabetic nephropathy is characterized by a predictable sequence of
events which are almost similar in both the types of DM. In the first year after the
onset of DM, glomerular hypertension and renal atrophy occur and cause an increase
in glomerular filtration rate (GFR). After 5-10 years due to alterations in the
glomerular capillary wall and size selectivity, about 40% of the individuals develop
microalbuminuria. Later it may proceed to overt proteinuria and the GFR starts to
decline steadily. About 50% of the individuals reach ESRD by 7-10 years. The renal
lesions underlying renal dysfunction in the two types of DM may differ. Indeed,
although tubular, interstitial and arteriolar lesions are ultimately present in type 1 DM,
as the disease progresses, the most important structural changes involve the
glomerulus. In contrast, a substantial subset of type 2 diabetic patients, despite the
presence of microalbuminuria or proteinuria, have normal glomerular structure with
or without tubulo-interstitial and/or arteriolar abnormalities. The clinical
manifestations of diabetic nephropathy are strongly related to the structural changes,
especially with the degree of mesangial expansion in both type 1 and type 2 DM.
However, several other important structural changes are involved. A study on renal
function and renal biopsies in a large cohort of type 2DM patients with
microalbuminuria and proteinuria showed a marked heterogeneity in renal structure\textsuperscript{8}.
STZ-induced diabetic rodents also result in development of nephropathy similar to the
early stage of human diabetic nephropathy. In the diabetic animals, a significant
increase in the kidney weight was observed. The histological study performed on the
kidneys of diabetic rats showed damage to the glomerulus, thickened basement
membrane and edematous proximal convoluted tubule with increase in
mucopolysaccharide deposits\textsuperscript{9}.

Neuropathy of DM:
Diabetic neuropathy is one of the common complications of DM. It affects the
sensory, motor and autonomic neurons of the peripheral nervous system. As with
other microvascular complications of DM, chronic hyperglycemia is the main initiator
of neurovascular damage. Besides over activation of PKC-\(\beta\), increased AGES,
increased AR pathway flux and oxidative stress contribute to diabetic neuropathy.
Another biochemical alteration responsible is the decline in synthesis of neurotrophic
factors. These are proteins that promote survival of neurons by regulating gene
expression through second messenger systems. Their subsequent reduction can lead to
neuron loss, possibly through activation of apoptosis. The final impact of all these
derangements are nerve dysfunction and hypoxia, altered ion transport and neuron
Auto immunity also plays a role in neuropathy. Studies suggest that circulating autoantibodies against motor and sensory nerves are present in the serum of patients with DM. Autonomic neuropathy can delay gastric emptying which is known as gastroparesis, and genitourinary dysfunctions. In the cardiac vascular system, sudden deaths have been attributed to autonomic neuropathy. Morphological and electrophysiological analyses of human diabetic neuropathy have emphasized lesions involving peripheral nerve axons, Schwann cells, perineurial cells, or endoneurial vascular elements in the pathogenesis of diabetic neuropathy. Nerve biopsies from young diabetic patients characteristically exhibit ultrastructural lesions most consistent with an early primary distal axonal atrophy and degeneration. Endoneurial vascular abnormalities such as basement membrane thickening and reduplication, endothelial cell swelling and proliferation, and platelet aggregation resulting in vessel occlusion have been noted in sural nerve biopsies and at autopsy of diabetic patients.

Macrovascular complications:
Although all tissues may be exposed to hyperglycemia, it is important to recognize that glucose mediated damage is limited to cells that develop intracellular hyperglycemia. While hyperglycemia is the causative factor for microvascular complications, evidence implicating its causative role in the development of macrovascular complications is less conclusive. But poor glycemic control can accelerate the pathogenesis. Macrovascular complications are largely associated with accelerated atherosclerosis, another complex disorder with multiple causes.

DM and accelerated atherosclerosis:
The most common cause of mortality in DM is the atherosclerotic coronary artery disease (CAD). DM altogether changes the nature of peripheral arterial disease (PAD) which is very evident by it being the most common cause of non-traumatic amputation. Prevalence of calcified carotid atheroma is alarmingly high in diabetics, thereby increasing their risk of stroke. Factors unique to DM hasten the process of atherosclerosis and worsen its outcome. The pathophysiology is multifactorial, all which finally lead to three main abnormalities- dysfunction of endothelium, vascular smooth muscle and platelet function. The major contributors are chronic hyperglycemia, insulin resistance and dyslipidemia.

Macroangiopathy in DM consists mainly of an accelerated form of atherosclerosis and affects the coronary, carotid and peripheral arteries, thus increasing the risk of
myocardial infarction, stroke and diabetic foot disease. Endothelial dysfunction is thought to play an important role not only in the initiation of atherosclerosis, but also in its progression and clinical sequelae\textsuperscript{11}.

Hyperglycemia can inhibit the production of NO in arterial endothelial cells contributing to endothelial dysfunction. This NO along with growth factor, endothelin-1, can cause or exacerbate arterial injury by a variety of mechanisms including vascular permeability, apoptosis, recruitment of invasive leukocytes and the production of reactive oxygen species (ROS). One important implication between insulin resistance and atherosclerosis is that inflammation underlies both. Insulin has been shown to exert an anti-inflammatory effect by reducing ROS generation by suppressing NADPH oxidase expression and through many other cellular mechanisms. Insulin resistance promotes inflammation by impairing the anti-inflammatory effects of insulin. Individuals with insulin resistance and type-2 DM have elevated levels of plasminogen activator inhibitors (especially PAI-1) and fibrinogen, which enhance the coagulation process and impair fibrinolysis, thus favoring the development of thrombosis.

DM is associated with multiple disturbances in lipoprotein metabolisms and these are triggered by insulin resistance as well as hyperglycemia. The characteristic lipid abnormality seen in diabetics is elevated TG, low levels of high density lipoproteins (HDL-C), shifting the balance towards atherogenic activity. Impaired insulin action results in increased production of FFA by the adipocytes. The liver in turn responds by increased production of very low density lipoproteins (VLDL) which are TG rich particles. Increased plasma TGs are the driving force for low HDL-C and abnormal small dense low density lipoproteins (LDL). The latter is more atherogenic. In patients with DM, the LDL particles can also become glycated, in a process similar to the glycation of proteins. Glycation of the LDL lengthens its half life and therefore increases the ability of LDL to promote atherosclerosis. Paradoxically, glycation of HDL shortens its half life, rendering them less protective against atherogenesis!

The DCCT and United Kingdom Prospective Diabetes Survey (UKPDS) studies have shown that mere preservation of normoglycemia does not prevent diabetic complications and there remains a need for alternative methods. AR and α-glucosidase have been known to play a crucial role in the pathophysiology of diabetic complications. In this regard, AR inhibitors and α-glucosidase inhibitors have been tried without very conclusive results in humans.
DM has been treated by varieties of drugs. The therapeutic measurements include use of insulin and other agents like amylin analogs, alpha-glucosidase inhibitors like acarbose, miglitol and voglibiose, sulphonylureas, biguanides for the treatment of hyperglycemia. All the drugs used in the treatment of DM aim at a single factor of controlling the glycemic status of the individual. DCCT provided definitive proof that reduction in chronic hyperglycemia can prevent many of the early complications of type 1 DM and reduced nonproliferative and proliferative retinopathy (47% reduction), microalbuminuria (39% reduction), clinical nephropathy (54% reduction), and neuropathy (60% reduction). Improved glycemic control also slowed the progression of early diabetic complications. UKPDS studied the course of >5000 individuals with type 2 DM for >10 years. This study utilized multiple treatment regimens and monitored the effect of intensive glycemic control and risk factor treatment on the development of diabetic complications.

Diet:

A well-balanced, nutritious diet remains a fundamental element of therapy. The ADA recommends about 45–65% of total daily calories in the form of carbohydrates; 25–35% in the form of fat (of which less than 7% are from saturated fat), and 10–35% in the form of protein. The current recommendations for both types of diabetes continue to limit cholesterol to 300 mg daily, and individuals with LDL cholesterol more than 100 mg/dl should limit dietary cholesterol to 200 mg daily. High protein intake may cause progression of renal disease in patients with diabetic nephropathy; for these individuals, a reduction in protein intake to 0.8 kg/day (or about 10% of total calories daily) is recommended.

Dietary fiber:

Plant components such as cellulose, gum, and pectin are indigestible by humans and are termed dietary "fiber." Insoluble fibers such as cellulose or hemicellulose, as found in bran, tend to increase intestinal transit and may have beneficial effects on colonic function. In contrast, soluble fibers such as gums and pectins, as found in beans, oatmeal, or apple skin, tend to retard nutrient absorption rates so that glucose absorption is slower and hyperglycemia may be slightly diminished.
Exercise 14:

Exercise has multiple positive benefits including cardiovascular risk reduction, reduced blood pressure, maintenance of muscle mass, reduction in body fat, and weight loss. For individuals with type 1 or type 2 DM, exercise is also useful for lowering plasma glucose (during and following exercise) and increasing insulin sensitivity.

Artificial and other sweeteners14:

Aspartame (NutraSweet) consists of two major amino acids, aspartic acid and phenylalanine, which combine to produce a sweetener 180 times as sweet as sucrose. A major limitation is that it is not heat stable, so it cannot be used in cooking. Saccharin (Sweet 'N Low), Sucralose (Splenda), and Acesulfame potassium (Sweet One) are other "artificial" sweeteners that can be used in cooking and baking. Fructose represents a "natural" sugar substance that is a highly effective sweetener, induces only slight increases in plasma glucose levels, and does not require insulin for its metabolism. However, because of potential adverse effects of large amounts of fructose on raising serum cholesterol, triglycerides, and LDL cholesterol, it does not have any advantage as a sweetening agent in the diabetic diet.

Sugar alcohols, also known as polyols or polyalcohol, are commonly used as sweeteners and bulking agents. They occur naturally in a variety of fruits and vegetables but are also commercially made from sucrose, glucose, and starch. Examples are sorbitol, xylitol, mannitol, lactitol, isomaltitol, maltitol, and hydrogenated starch hydrolysates (HSH). They are not as easily absorbed as sugar, so they do not raise blood glucose levels as much. Therefore, sugar alcohols are often used in food products that are labeled as "sugar free," such as chewing gum, lozenges, hard candy, and sugar-free ice cream. However, if consumed in large quantities, they will raise blood glucose and can cause bloating and diarrhea.

Drugs for Treating Hyperglycemia14:

(1) Drugs that primarily stimulate insulin secretion by binding to the sulfonylurea receptor on the β-cells: Sulfonylureas remain the most widely prescribed drugs for
treated hyperglycemia. The meglitinide analog repaglinide and the D-phenylalanine derivative nateglinide also bind the sulfonylurea receptor and stimulate insulin secretion. Sulfonylureas are not indicated for use in type 1 DM since these drugs require functioning pancreatic β cells to produce their effect on blood glucose. Sulfonylureas are generally contraindicated in patients with severe hepatic or renal impairment. Idiosyncratic reactions are rare, with skin rashes or hematologic toxicity (leukopenia, thrombocytopenia) occurring in less than 0.1% of users. Glyburide has few adverse effects other than its potential for causing hypoglycemia, which at times can be prolonged. Flushing has rarely been reported after ethanol ingestion.

(2) Drugs that alter insulin action: Metformin works in the liver. The thiazolidinediones appear to have their main effect on skeletal muscle and adipose tissue. The most frequent side effects of metformin are gastrointestinal symptoms, which occur in up to 20% of patients. These effects are dose-related, tend to occur at onset of therapy, and often are transient. However, in 3–5% of patients, therapy may have to be discontinued because of persistent diarrheal discomfort. Lactic acidosis has been reported as a side effect but is uncommon with metformin in contrast to phenformin. Anemia occurs in 4% of patients treated with thiazolidinediones. Rosiglitazone has recently been reported as being associated with new onset or worsening macular edema.

(3) Drugs that principally affect absorption of glucose: The α-glucosidase inhibitors acarbose and miglitol are such currently available drugs. The principal adverse effect of acarbose, seen in 20–30% of patients, is flatulence. In 3% of cases, troublesome diarrhea occurs. If combined with insulin or sulfonylureas, acarbose might increase the risk of hypoglycemia from these agents.

(4) Drugs that mimic incretin effect or prolong incretin action: Exenatide and DPP IV inhibitors fall into this category. The main side effect of exenatide was nausea, affecting over 40% of the patients. The risk of hypoglycemia was higher in persons taking sulfonylureas along with these drugs. The main adverse effect of sitagliptin, a DPPIV inhibitor, appears to be a predisposition to nasopharyngitis or upper respiratory tract infection.
(5) Others: Pramlintide, a synthetic analog of islet amyloid polypeptide (IAPP or amylin), lowers glucose by suppressing glucagon and slowing gastric emptying. Hypoglycemia can occur, and it is recommended that the short-acting or premixed insulin doses be reduced by 50% when the drug is started. Nausea was the other main side effect, affecting 30–50% of persons but tended to improve with time.

Insulin\textsuperscript{14}:

Insulin is indicated for type 1 DM as well as for type 2 diabetic patients with insulinopenia whose hyperglycemia does not respond to diet therapy either alone or combined with other hypoglycemic drugs. With the development of highly purified human insulin preparations, immunogenicity has been markedly reduced, thereby decreasing the incidence of therapeutic complications such as insulin allergy, immune insulin resistance, and localized lipoatrophy at the injection site. However, the problem of achieving optimal insulin delivery remains unsolved with the present state of technology. It has not been possible to reproduce the physiologic patterns of intraportal insulin secretion with subcutaneous injections of short-acting or longer-acting insulin preparations. Known complications of continuous subcutaneous insulin infusion (CSII) include ketoacidosis, which occurs when insulin delivery is interrupted, and skin infections. Another disadvantage is its cost and the time demanded of physicians and staff in initiating therapy. Inhaled insulin, Exubera, showed a small decrease in pulmonary function was seen in the first few months of use, but in the phase 3 studies lasting 2 years, patients did not experience clinically significant effects on pulmonary function. Other side effects associated with Exubera therapy include cough, shortness of breath, sore throat, and dry mouth.

In spite of having varieties of drugs for the treatment of DM and the tremendous scientific advances witnessed in this century, still the medical science cannot claim that it knows all that needs to be known about this disease, including its management. Moreover, most of these drugs used in the treatment of DM have one or the other side effects or certain difficulties in their application. This drew the scientist’s interest towards finding out a new solution for this metabolic disorder. From the ancient Indian history, it could be studied that most of the diseases have been treated with a varieties of plant medicines. Currently we mention them as the “Alternative medicines”. Varieties of plant medicines have been used for treating DM as well.
“Alternative medicines” for DM:

The Indian word for DM is *madhumeha*, “madhu” meaning sweet/sweetness and “meha” excessive urination. Indians have known of this disease for several thousand years. The earliest description of madhumeha is found in the *Atharvaveda*, one of the four sacred *Vedas*, that dates to around 1500 to 1000 B.C. The etiology, symptomatology, pathology, prognosis, and management principles of diabetes are described in detail by the physician Charaka in the *Charaka Samhita* around the first century A.D.

The pharmacopoeia of India is especially rich in herbal treatments for DM. More than 100 medicinal plants are mentioned in the Indian system of medicines including folk medicines for the management of DM, which are effective either singly or in combinations\(^\text{15}\). Researches conducted in the last few decades on plants mentioned in ancient literature or used traditionally for DM have shown antidiabetic property. There have been several reviews on the hypoglycemic medicinal plants more particularly use of Indian botanicals for hypoglycemic activity\(^\text{16-21}\). Scientific validation of several Indian plant species has proved the efficacy of the botanicals in reducing the sugar level comparable to modern drugs in experimental animals\(^\text{15, 22-24}\). Quite a few of these plants have undergone clinical trials also\(^\text{25-28}\). In addition to having anti diabetic properties, a few of these plants have shown AR and α-glucosidase activity inhibiting potential\(^\text{29-32}\). Many of these plants have been studied in detail and the active principles bringing out the said effects have been identified from them, thus providing scientific explanation for their action\(^\text{33-39}\). The list, though extensive, is no way complete and the search for better phytomedicines continues!

These plants also have a wider therapeutic index, with lower chances of hypoglycemic attacks and other side effects. The phytomedicines, with a multitude of beneficial effects, have prompted scientists worldwide to take an interest in them. As more and more phytotherapeutic alternatives are being officially recognized, the question of whether these agents delay or completely prevent the manifestations of the disease should be taken seriously enough to prompt future long-term research on the subject.

**Few plants with hypoglycemic activity\(^\text{40}\):**

A preparation of the whole plant of *Phyllanthus amarus* was found to have hypoglycemic effects in nine human subjects, four of whom were diabetics.
In vitro studies carried out by Rizvi, et al. have shown that epicatechin, an active constituent of *Pterocarpus marsupium* (Vijaysar) exerted a protective effect on erythrocyte osmotic fragility, similar to insulin, but by a different mechanism of action. In STZ (streptozotocin) induced diabetic rats, of the three important phenolic constituents of the heartwood of *Pterocarpus marsupium* (viz. pterosupin, marsupin and pterostilbene) marsupin and pterostilbene significantly lowered the blood glucose levels and the effects were comparable to metformin. The hypoglycemic efficacy of *Pterocarpus marsupium* has been further evaluated in a multicentric (4 centres) flexible-dose open trial in newly-diagnosed patients of non-insulin-dependent diabetes mellitus. Control of blood glucose (both fasting and post-prandial levels) was attained in 67 of 97 patients (69%) studied in 12 weeks and the optimum dose was 2 g of the extract. HbA1c values also decreased significantly. No significant change was observed in the mean levels of lipids.

The alcoholic extract of *Inula racemosa* (Pushkarmula) lowered blood glucose and enhanced liver glycogen in rats. However, there was neither increase in plasma insulin levels nor an increase in the degree of degranulation of β- cells of pancreas. Its action may be at the peripheral level by potentiating insulin sensitivity.

Hot water extract of *Camellia sinensis* (Black tea leaf) significantly reduced the blood glucose level and was found to possess both preventive and curative effects in STZ induced diabetic rats.

The leaf extract of *Azadirachta indica* had no effect per se, on the peripheral utilization of glucose (determined by intravenous glucose tolerance tests) and on hepatic glycogen in normal and STZ induced diabetic rats. However, it blocked the effects of epinephrine on glucose metabolism and reduction in peripheral glucose utilization in diabetic rats and to some extent in normal rats, indicative of an anti-hyperglycemic potential of the plant.

Leaf extract of *Aegle marmelos* (Bilva) was found to significantly reverse the raised Km values, but not Vmax values of the enzyme malate dehydrogenase, an important enzyme in glucose metabolism, in STZ induced diabetic rats. Alteration in the qualitative and quantitative nature of the enzyme has been suggested to contribute to the pathological state of diabetes. The leaf extract was also effective in restoring blood glucose and body weight to normal values. In another study, leaf extract of *Aegle marmelos* significantly reversed the altered (histological and ultrastructural) parameters in tissues of STZ induced diabetic rats seen by light and electron
microscopy to near normal and improved the functional state of pancreatic beta cells. The hypoglycemic effect of this plant drug thus appears to be mediated through regeneration of damaged pancreas.

Oral administration of the methanolic extract (but not the water extract) of aerial parts of *Artemisia pallens* (Daman) led to significant blood glucose lowering in glucose fed hyperglycemic and alloxan induced diabetic rats. Increased peripheral utilisation of glucose is probably the mechanism responsible. Inhibition of renal proximal tubular reabsorption of glucose may also contribute.

Saxena *et al.*, compared the effects of mode of action of three structurally different hypoglycemic agents, tolbutamide, centipiperalon and a swerchirin- containing fraction (SW1) from the plant *Swertia chirata* (Chirayata) in normal and STZ induced mild and severe diabetes in rats. Except in rats with severe pancreatic damage, SW1 showed better blood glucose lowering effect compared to tolbutamide.

*Ocimum album* (Holy basil) leaves significantly decreased the fasting and post-prandial blood glucose levels in patients with NIDDM in a randomized, placebo-controlled, crossover, single blind trial. Administration of *Ocimum sanctum* leaf powder to normal and diabetic rats for a period of one month resulted in a significant reduction in fasting blood sugar, uronic acid, total amino acids, total cholesterol (TC), TG, phospholipids and total lipids. TC, TG and total lipids were significantly lowered in the liver, kidney and heart. They indicate the hypoglycemic and hypolipidemic effect of *Ocimum sanctum* in diabetic rats.

Chronic administration of *Prunus amygdalus* (Almond) seeds and its proportionate fractions viz. defatted seed and oil to rabbits demonstrated a definite hypoglycemic effect. The active factor seems to be a non-oil fraction which is only partly soluble in ethyl ether.

Significant hypoglycemic effect was observed with 1500 mg/kg dose of juice of leaves of *Lantana camara* in rats.

The protective effect of *Capparis deciduas* (Karir) powder on oxidative stress and diabetes in alloxan induced diabetic rats has been evaluated. The data indicate that *Capparis decidua* may have a potential use as an anti-diabetic agent, especially in chronic cases as it helps in lowering the oxidative stress in diabetes.

Dubey *et al.*, studied the effect of D-400, a polyherbal formulation, on blood glucose, blood urea and serum creatinine in alloxan-induced diabetic rabbits. D400 significantly prevented the rise in blood urea and serum creatinine levels at the end of
36 weeks, thus showing promise against alloxan induced renal damage. The rise in blood sugar too in the treated group was lower than the saline control. Further studies by Dhawan, et al demonstrated that D-400, in diabetic rats, brought the raised blood glucose levels to within normal limits and raised the suppressed glycogen levels.

Gymnema sylvestre (Retz.) R. Br. ex Schult (G. sylvestre) (Madhunashini, Meshashringi) is a woody climber, belongs to the family Asclepiadaceae, common in central and southern India. It is known as periploca of the woods in English. Its Hindu common name, gurmara or gurmar, means “sugar destroyer”. The first scientific confirmation of G. sylvestre use in human diabetics came almost a century back when it was demonstrated that the leaves of G. sylvestre reduce urine glucose in diabetics.

Warren et al., and Gent J et al. claimed that Gymnema preparations were known to have an action on the modulation of taste, particularly suppressing sweet taste sensations. It has been used in the treatment of DM, and in food additives against obesity and caries. Anti-allergic, antiviral, lipid lowering and other effects were also reported. A number of constituents have been isolated from this plant since the first chemical studies were done at the end of the nineteenth century. Most important for the treatment of diabetes are the gymnemic acids, which were reportedly first isolated by Hooper in 1889. The best studied extract of Gymnema, GS4, which contains a group of at least 15 triterpene sapinoids (the gymnemic acids) plus a polypeptide, gurmarin. The complete amino acid sequence of gurmarin was determined by Kamei, et al. Sequencing was done by the Edman analysis of peptides derived from digests obtained with Staphylococcus aureus V8 protease, pyrogglutamyl aminopeptidase, and lysyl endopeptidase. Gurmarin was found to consist of 35 amino acid residues with an amino-terminal pyroglutamyl residue and having a molecular weight of 4,209. Gurmarin was found to have no homology with other known proteins.

Liu H M et al., elucidated the structure of gymnemagenin, the saponin of the antisweet principles of G.sylvestre. Five antisweet principles, gymnemic acid-III, -IV, -V, -VIII and –IX, were isolated in pure states from hot water extract of leaves of G.sylvestre. They also determined the structure of gymnemic acids -VIII and –IX as \(3\beta\)-O-β-D-arabino-2-hexulopyranosyl gymnemic acid –III and –IV respectively. Yoshikawa et al., elucidated the chemical structure of gymnemosides. The study on the inhibitory effect of gymnemosides and principal triterpene glycosides from G. sylvestre on glucose uptake showed that gymnemic acids II, III and IV, gymnimosaponin V, and gymnmoside-f were found to exhibit the inhibitory activity.
In another study by the same authors\textsuperscript{53}, the structures of gymnemosides a and b were determined on the basis of chemical and physicochemical evidence. The inhibitory activity of each triterpene glycoside from gymnemic acid was examined to determine its impact on the increase of serum glucose level in oral glucose-loaded rats. Gymnemoside b and gymnemic acids III, V and VII were found to exhibit a little inhibitory activity against glucose absorption, but the principal constituents, gymnemic acid I and gymnemosaponin V, lacked this activity. Another study by Chowdhary F \textit{et al.}, \textsuperscript{54} also concluded that gymnemic acid isolated from \textit{G.sylvestre} was a glycoside and was triterpenoid saponin in character.

Shanmugasundaram \textit{et al.}, \textsuperscript{55} described in their communication that a scientific investigation of the biological effect of oral administration of the leaves revealed that it is able to increase the circulating insulin levels in alloxan diabetic rabbits and in a single maturity- onset diabetic patients. The hypoglycemic action of the leaf powder is not abrupt and severe, but slow. The blood glucose homeostasis during the \textit{G. sylvestre} therapy was suggested to be associated with lowering of the serum lipid levels.

\textbf{Animal studies on the effect of \textit{G.sylvestre} on hyperglycemia:}

1. Shanmugasundaram \textit{et al.}, \textsuperscript{55} in 1983 reported that the activity of key enzymes of insulin-dependent glucose utilization pathways, such as phosphorylases and gluconeogenic enzymes and SDH has been controlled in alloxan-treated rabbits. The uptake and incorporation of ($^{14}$C) glucose into glycogen and protein were shown to be increased in the liver, kidney and muscle in treated animals compared to the untreated controls. Also the pathological changes initiated in the liver during the hyperglycemic phase were reversed by controlling hyperglycemia by \textit{G.sylvestre}.

2. Prakash \textit{et al.}, \textsuperscript{56} in 1986 found that the powdered leaves of \textit{G.sylvestre} fed for 10 days prior and 15 days after i.v. beryllium nitrate significantly protected the animals from the full fall of blood glucose seen in rats received beryllium nitrate alone. The feeding of the leaves for 25 days to normal rats did not alter blood glucose significantly. It was also suggested by the authors that the leaves may contain a principle that could be useful as a prophylactic against beryllium toxicity.
3. Srivastava et al.,\textsuperscript{57} in 1986 demonstrated that alloxan diabetic rats treated with \textit{G. sylvestre} lived significantly longer than untreated rats. Here diabetes was induced by a single intraperitoneal dose of 120mg/kg body weight alloxan. The rats having blood sugar over 150mg/dl were classified as diabetic, which were subdivided in the frequency ranges of (1) 150-250mg/dl, (2) 250-400mg/dl and (3) above 400mg/dl blood sugar levels. Part of the hyperglycemic animals were treated orally (0.2g/2ml) by intubation twice daily for two weeks with an aqueous extract of \textit{G. sylvestre} leaves. This treatment corrected hyperglycemia in moderately diabetic rats and the effect of the extract persisted beyond a two months period after its discontinuation. The extract did not show reduction of blood sugar levels in severe and toxic groups of diabetic rats but the extract prolonged their survival time.

4. Shanmugasundaram et al.,\textsuperscript{58} in 1988 administered a leaf extract of \textit{G. sylvestre} which was extracted with ethanol (50/50, v.v.) at a dose of 20mg/day for STZ diabetic rats after 4 weeks of maintenance of hyperglycemia. A normal control and a diabetic control group were also maintained. The treatment was discontinued after eight weeks and at the end of 16 weeks, a glucose tolerance test was performed in all the rats and all animals were sacrificed. The plant could bring back the glucose homeostasis which was appreciated by increased serum insulin levels. Histological study showed a restoration or regeneration of the islets of langerhans. The increased glycoprotein was also brought under control, thus it showed to be preventing the onset of changes leading to micro- and macroangiopathy.

5. Shanmugasundaram et al.,\textsuperscript{45} in 1990 tested two water soluble extracts of \textit{G. sylvestre} leaves GS3 and GS4 in STZ diabetic rats for their effects on blood glucose homeostasis and pancreatic endocrine tissue. In the diabetic rats, fasting blood glucose levels returned to normal after 60 days of GS3 and after 20 days of GS4 oral administration. Both the extracts increased the serum insulin levels closer to normal fasting levels. In the pancreas, both the extracts were able to double the islet number and $\beta$-cell number. Thus it suggests the possible regeneration of the endocrine pancreas.

6. Okabayashi et al.,\textsuperscript{47} in 1990 studied the acute effect of GS4 in both non-diabetic and STZ – induced (30mg/kg) mildly diabetic rats. Administration of 1g/kg body weight of GS4 to 18-h fasted non-diabetic rats significantly
attenuated the serum glucose response to oral administration of 1g/kg glucose. The immunoreactive insulin (IRI) response in GS4 administered rats was lower, but not significantly, than that in control rats. A chronic effect of GS4 on mildly diabetic rats was examined by giving the extract for 4 weeks. At the end of the study, GS4 showed a tendency to reduce the serum glucose concentration in the fed state and to improve the glucose tolerance. Gain in body weight, food intake, pancreas weight and the pancreatic contents of IRI, protein, amylase and trypsinogen were unaltered in the GS4 treated group compared with the control.

7. Shimizu et al., 59 in 1997 demonstrated that the leaf extract of *G. sylvestre* containing gymnemic acids decreased absorption of glucose from rat intestine. Gymnemosides extracted from the leaves were found to be responsible for inhibition of glucose absorption from the intestine.

8. Persaud et al., 49 in 1998 studied the effect of GS4 on the isolated islets of langerhans of the rats. The exposed cells showed a dose dependent increase in insulin release. The different culture media showed different levels of increase in insulin levels, but the values were significant (p<0.001). The data obtained suggested that the increase in insulin in vitro may be by two mechanisms: 1) the major mode of action might be through permeabilisation of the β-cell plasma membranes, most likely resulting from the high saponin glycoside content of the extract, leading to unregulated loss of insulin from the cells; 2) there is a Ca²⁺-sensitive component, and at least part of this release of insulin may be dependent on channel-independent Ca²⁺ influx into the β-cells, perhaps through the pores formed by plasma membrane disruption.

9. Chattopadhyay 60 in 1998 studied the effect of a water soluble fraction of alcoholic extract of *G. sylvestre* leaves on glycogen content of isolated rat hemidiaphragm in normal and glucose fed hyperglycemic rats. The leaf extract by itself failed to alter the hepatic glycogen content in normal rats. In glucose fed rats, the extract lowered the glycogen content significantly (p<0.05) and this was further lowered when the leaf extract was given along with exogenous insulin.

10. Galletto R et al., 61 in 2004 in their study investigated the antidiabetic and hypolipidemic potential of dried powdered leaves of *G. sylvestre*. The acute effect of *G. sylvestre* administered by oral gavage on glucose blood level and
lipids in nondiabetic and alloxan-diabetic rats were investigated in the following conditions: a) after a balanced meal; b) after the ingestion of 1000 mg/kg amylose or 1000 mg/kg glucose; c) after the ingestion of a mixture of 12 ml/kg soybean oil + 1% cholesterol (SOC). In addition, the effect of the treatment with \textit{G.sylvestre} during two (sub-acute) or four weeks (chronic) on body weight, food and water ingestion, glucose blood level and lipids in nondiabetic and alloxan diabetic rats were measured. The dose of \textit{G.sylvestre} utilized in the majority of the experiments, i.e., 30 mg/kg, corresponds to that given to treat diabetes in Brazil. \textit{G.sylvestre} acutely (fed once) did not influence the elevation of glycemia promoted by a balanced meal or by the administration of amylose or glucose; but promoted more intense (P<0.05) elevation of serum lipids after the administration of SOC. Moreover, the sub-acute (two weeks) and chronic (four weeks) treatment with \textit{G.sylvestre} in nondiabetic and alloxan-diabetic rats did not change: a) the body weight gain; b) food and water ingestion; c) the blood level of glucose and lipids. They concluded that \textit{G.sylvestre}, at least in the form commercialized in the Brazil, i.e., dried powdered leaves, require further experimental and clinical trials before being recommended to treat diabetes and hyperlipidemia.

11. Gholap \textit{et al.}, \textsuperscript{62} in 2005 have studied the effect of three different doses (6.7, 13.4 and 26.8 mg/kg body weight, i.p.) of gymnemic acids from the leaves of \textit{G.sylvestre} in the regulation of dexamethasone induced hyperglycemia in mice. Thyroid hormone levels were also estimated by radioimmunoassay (RIA) in order to find out whether the effects are mediated through alteration in the thyroid function or not. They had done this because a prestandardised dose of 1mg/kg body wt. of intramuscular dexamethasone for 22 days could increase serum glucose, but it decreased the serum concentration of the thyroid hormones thyroxine (T4) and triiodothyronine(T3). The administration of all three doses could bring down the hyperglycemia induced by dexamethasone, but the decrease was significant in the two higher doses. These effects were comparable to a standard corticosteroid-inhibiting drug, ketoconazole. However, the percentage reduction was greater in the 13.4 and 26.8mg/kg of gymnemic acids-treated groups (28.76% and 21.71% respectively) as compared to that of ketoconazole, where it was only 9.07%. There were no significant changes in thyroid hormone concentrations by the administration of
any doses of gymnemic acid in dexamethasone-treated rats, which suggests that the effects of the tested material might not have been mediated through alterations in the thyroid function. The changes in hepatic lipid peroxidase (LPO), superoxide dismutase (SOD) and catalase (CAT) activity revealed a toxic effect at the highest dose (26.8mg/kg body wt) whereas the medium dose was found to be safe and antiperoxidative.

12. Sujin M et al., 63 in 2008 studied the anti-diabetic effect of G.sylvestre herbal powder in stomach of albino wistar rats. The histopatholoical and biochemical assays were carried out in organs and serum. The different concentration of G. sylvestre treated as 5, 10, 15, 20/gms/25 days. The effect of crude drugs in rats was assessed by the body weight and measuring the levels of selected blood parameters protein, glucose, cholesterol, insulin and triglycerides and the histopathology. Treatment with G. sylvestre reduced the stomach weight of animals and improved significantly the level of insulin, protein, TG,TC and glucose.

13. Liu B et al., 64 in 2009 studied the effect of G.sylvestre extract on insulin secretion from the MIN6 β-cell line and isolated human islets of langerhans. Insulin secretion from the MIN6 β-cells was stimulated by the extract in a concentration dependent manner, with low concentrations (0.06-0.25mg/ml) having no trytan blue uptake. The extract increased β-cell Ca2+ levels, an effect that was mediated by Ca2+ influx through voltage-operated calcium channels. The extract also reversibly stimulated insulin secretion from isolated human islets and its insulin secretagogue effects in MIN6 cells and human islets were partially dependent on the presence of extracellular Ca2+.

14. Mall G K et al., 65 in 2009 studied the effect of G.sylvestre in both normal and alloxan induced diabetic rats. The aqueous leaf extract of G.sylvestre at the dose of 400, 600 and 800 mg/ kg body weight was administered orally once a day to the groups for 30 days. The fasting blood glucose, TC, HDL-C and serum TG content were estimated in both normal and alloxan induced diabetic rats. The fasting blood glucose, TC and serum TG content were found to be significantly reduced (p<0.05) in treated rats where as the extract also showed the potent elevation in the level of serum HDL- C. The study revealed that G. sylvestre has significant antidiabetic activity and a hypolipidemic activity in
alloxan induced and normal fasting rats. It was concluded that the extract seems promising for the development of a phytomedicine for DM.

**Effect of *G.sylvestre* on lipid metabolism:**

1. Bishyee *et al.* in 1994 have orally administered a leaf extract of *G.sylvestre* at a dose of 25 – 100mg/kg body wt. to experimentally induced hyperlipidemic rats for 2 weeks. The elevated serum TG, TC, VLDL and LDL-C levels reduced in dose dependent manner. The decreased HDL-C and antiatherogenic index (AAI) in hyperlipidemia were found to reverse back to normal. The ability of the extract at 100mg/kg to lower the TG and TC in serum and its anti atherosclerotic potential were almost similar to that of a standard lipid lowering agent – clofibrate.

2. Oral administration of gymnemic acid for 22 days was found to increase fecal excretion of neutral steroids and bile acids in rats. This study was performed by Nakamura *et al.*, in 1999. Gymnemic acids extracted from the leaves fed at different doses to rats showed a decrease in the body weight gain and food intake in a dose dependent manner (p< 0.01). The column fractionate of the leaves which had gymnemic acid in the concentration of 161.6mg/g given at a dose of 1g/kg body weight significantly increased fecal excretion of cholesterol and cholic acid derived bile acids.

3. In the study by Nohiro *et al.* in 2001, oral administration of *G.sylvestre* leaf extract 33mg/kg body weight having a gymnemic acid content of 2.4% for 3 weeks did not influence the body weight gain or food intake. The fecal steroid excretion was considerably increased by the extract (p<0.05). The extract also showed to decrease the serum lipids like TC and TG significantly (p<0.05).

4. In another study by the same authors, same extract was fed for 10 weeks to the rats receiving a high fat diet or normal fat diet. Within the high fat diet group, the body weight gain was suppressed (p<0.05) and liver lipid contents were lowered (p<0.05). The intraperitoneal fat and fat drop vacuoles on the epithelium of renal tubules were scattered on treatment with the extract. Within the normal fat diet rats, plasma TG levels were decreased by the administration of extract.
Clinical trials on the effect of *G.sylvestre*:

1. Baskaran *et al.*,\(^4^8\) in 1990 have shown that 400mg/day *G.sylvestre* extract (GS4) given orally for 22 type 2 diabetic patients as a supplement to the conventional oral drug for 18-20 months brought a significant decrease in the blood glucose, HbA1c and glycosylated plasma proteins and the conventional drug dosage could be decreased. The data also suggested a possible regeneration or repair of β– cells which was supported by raised insulin levels in the serum.

2. Shanmugasundaram *et al.*, in 1990\(^4^6\) have shown that GS4, a water soluble extract of *G.sylvestre* leaves administered at a dosage of 400mg/day for 27 IDDM patients could decrease the insulin requirement along with decreased levels of fasting blood glucose, HbA1c and glycosylated plasma protein levels. The serum lipid levels were also reached to near normal levels. The glycosylated plasma protein levels remained higher than controls. It was also suggested by the authors that the extract appeared to enhance endogenous insulin, possibly by regeneration /revitalization of the residual β-cells in IDDM.

3. Preuss *et al.*,\(^7^0\) in 2004 assessed the efficacy of optimal doses of highly bioavailable (−)-hydroxycitric acid (HCA-SX) alone and in combination with niacin-bound chromium (NBC) and a standardized *G.sylvestre* extract on weight loss in moderately obese subjects by monitoring changes in body weight, body mass index (BMI), appetite, lipid profiles, serum leptin and excretion of urinary fat metabolites. HCA-SX has been shown to reduce appetite, inhibit fat synthesis and decrease body weight without stimulating the central nervous system. NBC has demonstrated its ability to maintain healthy insulin levels, while *G.sylvestre* has been shown to regulate weight loss and blood sugar levels. It was a randomized, double-blind, placebo-controlled human study, which was conducted in Elluru, India for 8 weeks in 60 moderately obese subjects (ages 21–50, BMI >26 kg/m²). Subjects were randomly divided into three groups. Group A was administered HCA-SX 4667 mg, group B was administered a combination of HCA-SX 4667 mg, NBC 4mg and *G.sylvestre* 400 mg, while group C was given placebo daily in three equally divided doses 30–60 min before meals. All subjects received a 2000
kcal diet/day and participated in supervised walking. At the end of 8 weeks, body weight and BMI decreased by 5–6% in both groups A and B. Food intake, TC, LDL-C, TG and serum leptin levels were significantly reduced in both groups, while HDL-C levels and excretion of urinary fat metabolites increased in both groups. A marginal or non-significant effect was observed in all parameters in group C. The authors concluded that optimal doses of HCA-SX and, to a greater degree, the combination of HCA-SX, NBC and G.sylvestre can serve as an effective and safe weight-loss formula that can facilitate a reduction in excess body weight and BMI, while promoting healthy blood lipid levels.

**Systematic reviews of G.sylvestre:**

1. In a systematic review of herbs and dietary supplements for glycemic control in diabetes, two non-randomised, open labeled clinical trials on G.sylvestre were reported to decrease the FBS, HbA1c, glycosylated plasma proteins, urine glucose and increase in insulin level71.

2. Matthew 72 in his systematic review supported the findings of the previous reference.

3. In another review on G.sylvestre 73, gymnemic acids were described to have antidiabetic, antisweetener and anti-inflammatory activities. The gymnemic acid was suggested to interfere with the absorption of glucose from the intestine. Other possible mechanisms of hypoglycemic activity were thought to be by increasing the secretion of insulin, promoting the regeneration of islets, increasing the utilization of glucose and inhibition of glucose absorption from the intestine.

4. Review by Saneja A et al.,41 explains that the leaves of the plant, G.sylvestre are widely used for the treatment of diabetes and as a diuretic in Indian proprietary medicines. Gymnemic acid is the main active chemical constituent isolated from the G.sylvestre plant. The plant is documented to possess beneficial effects as digestive, anti-inflammatory, diuretic, hypoglycemic and antihelmentic. It is believed to be used in dyspepsia, constipation, jaundice, haemorrhoids, cardiopathy, asthma, bronchitis and leucoderma. A scrutiny of literature revealed some notable pharmacological activities of the plant such as antidiabetic, antiobesity, hypolipidaemic, antimicrobial, free radical scavenging and anti-inflammatory. They also added that this review was an
attempt to highlight the various ethnobotanical and traditional uses as well as phytochemical and pharmacological reports on *G. sylvestre*.

**Toxicity study of *G. sylvestre***:

1. A 52-week dietary toxicity study in wistar rats was conducted by Ogawa *et al.*, 74. Here the rats were administered a graded dose of *G. sylvestre* extract at 0.01, 0.10 and 1.00% of basal powder diet, along with a group fed solely with the basal powder diet without the extract for 52 weeks. There were no adverse effects on physiological, haematological, biochemical and histopathological findings at the end of the study which said that *G. sylvestre* extract was safe at a dose of 504mg/kg/day for males and 563mg/kg/day for female rats as mean daily intake for 52 weeks.

**Few other studies on *G. sylvestre***:

1. TohrãoeFushiki *et al.*, 75 in 1992 studied gastric inhibitory peptide release into the portal vein in response to duodenal infusion of D-glucose in the presence of a leaf extract of *G. sylvestre*, purified gymnemic acid and inhibitors of some putative glucose sensors and carriers in the intestinal lumen. Intraduodenal infusion of D-glucose significantly increased the portal immunoreactive gastric inhibitory peptide concentration in a dose-dependent manner. The increase in the portal immunoreactive gastric inhibitory peptide induced by glucose was significantly depressed by concomitantly infused leaf extract of *G. sylvestre*, purified gymnemic acid and phlorizin but not by cytochalasin B. Mannohexulose, which inhibits glycolysis and procaine and lidocaine, which inhibit the vagal glucoreceptor in the lumen, did not affect portal immunoreactive gastric inhibitory peptide concentrations. These results suggest that a glucose receptor, which interacts with the leaf extract of *G. sylvestre*, purified gymnemic acid and phlorizin, exists for the release of immunoreactive gastric inhibitory peptide and that the glucose receptor for gastric inhibitory peptide release is not likely to be identical with a glucose transporter or a vagal glucoreceptor in the lumen.

2. In 1998, Preuss H *et al.*, 76 studied the effects of three agents chromium polynicotinate, bis(maltolato)oxovanadium (BMOV), and the herb, *G. sylvestre*, simultaneously on systolic blood pressure (SBP) in spontaneously hypertensive rats (SHR). Compared to starch, SHR consuming sucrose showed a significant elevation of SBP within days that was maintained for the duration
of study. Addition of chromium polynicotinate to the sucrose diet at the beginning of study prevented the sucrose-induced elevation of SBP for 2 weeks, but SBP rose significantly after that. BMOV at high concentrations overcame the sucrose-induced rise in SBP and even decreased SBP below values seen in SHR eating the starch diet, but marked weight loss was noted. A second study examined different concentrations of BMOV. At 0.01% w/w concentration of BMOV, SBP was still significantly decreased, eventhough SHR did not lose body weight (BW) early on. SHR consuming *G. sylvestre* showed no change or even elevated SBP. Hepatic thiobarbituric acid reacting substances (TBARS) formation, an estimate of lipid peroxidation, was decreased by chromium polynicotinate and BMOV, and renal TBARS by chromium polynicotinate. Circulating cholesterol concentrations were decreased in the SHR consuming *G.sylvestre*. It was concluded by the authors that chromium decreased the portion of SBP elevated by high sucrose intake, but high levels of sucrose ingestion can eventually overcome this. BMOV overcame sucrose-induced elevation of SBP as well as some of the “genetic hypertension.” Different from chromium, this decrease was not overcome by high levels of dietary sucrose. The significant lowering of cholesterol with *G.sylvestre* ingestion indicates some effect on metabolism, but *G.sylvestre* did not lower and even raised SBP.

3. Katsukawa H *et al.*, 1999 investigated preference for taste solutions and saliva composition in rats fed a diet containing *G.sylvestre*. Preference for 0.01M sucrose and a mixture of 0.03 M sucrose and 0.03mM quinine-HCl significantly decreased at 1-2 days after the start of the gymnema diet and subsequently returned closely to the control levels within about a week. There was no significant change in the preference for NaCl, monosodium glutamate and quinine-HCl during feeding trials. Submandibular saliva of rats fed the gymnema diet for 4 and 14 days showed an inhibitory effect on immunoreactions between gurmarin and antigurmarin serum. Analyses using electrophoresis and affinity chromatography indicated that the saliva contained gurmatin binding proteins with molecular weights of 15,16,45,60 and 66 kDa. These results suggested that reduction of preference for sucrose was probably caused by gurmarin contained in the gymnema diet and subsequent restoration.
of the preference may be due to suppression of the effect of gurmarin by salivary gurmarin-binding proteins induced by the gymnema diet.

4. In 2000, Harada S et al.,\textsuperscript{78} published an article on the inhibitory effect of gurmarin isolated from \textit{G.sylvestre} on palatal taste responses to amino acids in the rat. Gurmarin depressed the phasic responses to sugars and saccharin sodium, recorded from the greater superficial petrosal nerve innervating palatal taste buds in the rat. However, no significant effect of gurmarin was observed for taste responses to NaCl, HCl and quinine hydrochloride. Phasic responses to D-amino acids that taste sweet to humans (His, Asn, Phe, Gln) were also depressed, but gurmarin treatment was without significant effect on taste responses to D-Trp and D-Ala, six L-amino acids (His, Asn, Phe, Gln, Trp, Ala), and two basic amino acid HCl salts (Arg, Lys). With the exception of D-Trp, these inhibitory effects of gurmarin on greater superficial petrosal nerve taste responses were related to the rat’s preference for these substances.

5. Devi P et al.,\textsuperscript{79} in 2010 performed pharmacognostical and antimicrobial screening of \textit{G.sylvestre} and evaluation of gurmar herbal tooth paste and powder, composed of \textit{G.sylvestre} extracts in dental caries. The Macroscopic, Physiochemical parameters, phytochemical screenings were carried out to facilitate quick identification and selection of the drug from various adulterants. The extracts prepared using successive solvent extraction techniques were screened for its antimicrobial activity by Agar well diffusion method against \textit{Streptococcus mutans}, \textit{Staphylococcus aureus}, \textit{Streptococcus mitis} and \textit{Candida albicans} with the doses 25, 50 and 100 mg/ml. The methanol extract showed strong antimicrobial activity with the zone of inhibition ranges from 12-23mm at 25mg/ml. The successive extracts of \textit{G.sylvestre} was screened for its particle size, total microbial load, investigation with GC-MS and HPTLC studies. The \textit{G.sylvestre} hydro alcoholic extract and paste base, tooth powder base were used in the formulation of “Gurmar Herbal tooth paste” and “Gurmar Herbal Tooth powder” results found to be within the limits. This proves that the extract can be useful to treat the dental caries with the scientific documentation.

6. Trivedi P et al.,\textsuperscript{80} in 2011 developed a simple liquid chromatographic method for the determination of gymnemagenin in leaves of \textit{G.sylvestre}. Gymnemagenin was obtained after acidic hydrolysis followed by basic
hydrolysis of the sample and extraction into ethyl acetate. Analyte separation and quantitation were achieved by isocratic reversed-phase liquid chromatography and UV detection at 220 nm. The method involved the use of an RP-18e Lichrocart reversed-phase column (5 µm, 75 x 4 mm id) and a binary isocratic mobile-phase profile. Linearity was observed in the range of 9.18 to 720 µg mL-1 with correlation coefficient of 0.998. Relative standard deviation of linearity of the method was found to be 0.015%. Detection limit was 5.5 µg mL-1 and quantitation limit was 7.5 µg mL-1. Average recovery of 99.2 ± 0.54, was obtained by spiking pre-analyzed samples with standard solution at 3 different concentration levels (80, 100, and 120%). Three leaf samples of *G. sylvestre* from three different regions, one marketed *G. sylvestre* extract and an anti-diabetic polyherbal formulation containing *G. sylvestre* leaf powder were analyzed by this method. They were found to contain 0.30-0.34, 5.9 and 0.125% w/w gymnemagenin respectively. They concluded that the new method was comparatively simpler, reproducible and sensitive than the other reported methods.

*Salacia oblonga* Wall. ex Wight & Arn (*S. oblonga*) (Ponkoranti), a climber growing in the southern parts of our country, belongs to the family Celastraceae.

1. In 1995, Augusti *et al.*, 81 published a research paper, in which they have claimed that the chloroform eluted fraction of the petroleum ether extract of the root bark of *S. oblonga* and a fluorescent compound separated from it (by TLC) demonstrated hypoglycemic potency in rats when compared to tolbutamide.

2. Ismail *et al.*, 82 in 1997 studied the anti-inflammatory effect of *S. oblonga*. The anti-inflammatory activity of *S. oblonga* root bark powder and *Azima tetracantha* leaf powder was assayed in male albino rats using carrageenan-induced rat paw oedema (acute inflammation) and cotton pellet granuloma (chronic inflammation) methods. Both the crude drugs were maximally active at a dose of 1000 mg/kg. In the cotton pellet granuloma assay, these drugs were able to suppress the transudative, exudative and proliferative components of chronic inflammation. Furthermore, these drugs were able to lower the lipid peroxide content of exudate and liver, gamma-glutamyl transpeptidase (GGT) activity in the exudate of cotton pellet granuloma. The increased acid and
alkaline phosphatase activity and decreased serum albumin in cotton pellet granulomatous rats were normalised after treatment with these drugs. It is likely that these drugs may exert their activity by antiproliferative, antioxidative and lysosomal membrane stabilization.

3. Krishnakumar et al., 83 in 1999 reported in his article that the petroleum ether extract of the root of S.oblonga could significantly prevent STZ induced hyperglycemia and hypoinsulinemia. It was also said that a significant decrease in peroxidation products viz. TBARS (p<0.001), conjugated dienes(p<0.05), hydroperoxides(p<0.001) and increase in glutathione peroxidase and glutathione reductase was observed in the heart tissue of the diabetic rats treated with the extract. This study suggested the anti-diabetic and anti-oxidative activity of S.oblonga.

4. Matsuda et al., 84 in 1999, found that a hydroalcoholic root extract of S.oblonga showed inhibitory activity on the increase in serum glucose level in sucrose-and maltose-loaded rats. The water soluble and ethyl acetate soluble fractions from this hydroalcoholic extract showed inhibitory activities on alpha-glucosidase and AR respectively. From the water soluble portion, potent alpha-glucosidase inhibitors, salacinol and kotalanol, were isolated, together with nine sugar related components, while a new friedelane-type triterpene, kotalaganin 16-acetate, was isolated from the ethyl acetate-soluble portion along with known diterpenes and triterpenes. The structure of kotalagenin 16-acetate was elucidated on the basis of physicochemical evidence. Principal components from this natural medicine were examined in terms of inhibitory activity on AR, and the diterpene and triterpene constituents, including the new kotalagenin 16-acetate, were found to be responsible components for the inhibitory activity on AR.

5. Krishnakumar et al., 85 in 2000 published another article, where they have claimed that hypoglycemic and anti-lipid peroxidative activity of a petroleum ether extract of the root bark of S.oblonga was studied in STZ hyperglycemic rats. The extract showed significant (p<0.001) hypoglycemic activity, which was supported by insulin assay. A detailed biochemical study including TBARS, hydroperoxides, conjugated dienes, glutathione, superoxide dismutase, catalase, glutathione peroxidase and glutathione reductase in the renal tissue demonstrated promising anti-lipid peroxidative activity.
6. Wolf et al.,\textsuperscript{86} in 2004 evaluated the safety of a hot water extract of \textit{S.oblonga} supplemented to or precessed in to a medical food. Male rats were given one of these three treatments: 1) EN-0178(control, liquid diet), 2) EN-0178+ salacinol (as 1 plus 500mg of salacinol extract per 253g diet, which was added to product immediately prior to feeding), 3) EN-0195( as 1 plus 500mg of salacinol extract per 250g diet, which was added during product manufacture). After 14 days of free access to dietary treatments, rats were sacrificed, blood collected and organs weighed. Rats consuming salacinol extract had reduced (p<0.05) weight gain and feed intake. The relative (% of body weight) testicular weight was higher (p<0.05) for rats consuming salacinol extract, whereas, the relative liver and spleen weight was lower (p<0.05) for rats consuming salacinol extract. Of the serum chemistries analysed, blood urea nitrogen and alkaline phosphatase was lower (p<0.05) for rats consuming salacinol extract. No difference in the blood haematology was found.

7. Yu Hao et al.,\textsuperscript{87} in 2004 published that they have shown a chronic administration of an aqueous extract of \textit{S.oblonga} to obese Zucker rats markedly improved interstitial and perivascular fibrosis in the hearts. It also reduced plasma glucose levels in non-fasted obese Zucker rats, whereas it had little effect in the fasted animals, suggesting inhibition of postprandial hyperglycemia in type2 diabetic animals, which might play an important role in improvement of the cardiac complications. The extract markedly suppressed the over expression of mRNAs encoding transforming growth factor betas 1 and 3 in the hearts, which may be an important part of the overall molecular mechanisms. The extract dose-dependently inhibited the increase of plasma glucose in sucrose, but not in glucose-loaded mice. Moreover, the extract demonstrated a strong inhibition of \( \alpha \)-glucosidase activity in vitro, which is suggested to contribute to the improvement of postprandial hyperglycemia.

8. Colleen et al.,\textsuperscript{88} in 2005 in a publication mentioned that they have evaluated the postprandial glycemic, insulinemic, and breath hydrogen responses to a liquid nutritional product containing \textit{S.oblonga} extract on 43 healthy subjects. The subjects were give the following meals on separate days after overnight fasting: control (C; 480ml of the study product containing 82g of carbohydrate, 20g protein and 14g fat), control plus 3.5g each of phenylalanine and leucine (AA), control plus 1000g of \textit{S.oblonga} extract(S),
and control plus S and AA (SAA). Postprandially, fingerstick capillary plasma glucose and venous serum insulin levels were measured for 180 min, and breath hydrogen excretion was measured for 480 min. The baseline-adjusted peak glucose response was not different across meals. However, changes in plasma glucose areas under the curve (0 to 120 min and 0 to 180 min, respectively) compared with C were -9% and -11% for AA (p>0.05 each), -27% and -24% for S (p=0.035 and 0.137), and -27% and -29% for SAA (p<0.05 each). Changes insulin areas under the curve were +5% and +5% for AA (p>0.05 each), -35% and -36% for S (p<0.001 each), and -6% and -7% for SAA (p>0.05 each). Breath hydrogen excretion was associated with mild flatulence. Thus it was concluded that *S. oblonga* could decrease glycemia. Supplementation with aminoacids had no significant additional effect on glycemia.

9. Heacock *et al.*, 89 in 2005 determined the effect of different doses (0,500,700,1,000g) of *S. oblonga* extract on postprandial glycemia, insulinemia and breath hydrogen responses in healthy adults on four separate occasions. Capillary finger-prick plasma glucose and venous serum insulin concentrations were measured at baseline and hourly for 8 hours postprandially. The highest of the doses (1g) could reduce the plasma glucose and serum insulin incremental areas under the curve (0 to 120 minutes postprandial) by 23% (p=0.32) and 29% (p=0.01) respectively. The other doses did not impact glycemia or insulinemia. Breath hydrogen excretion increased linearly as the dose of the extract was advanced. This suggests a mechanism similar to prescription alpha-glucosidase inhibitors.

10. Huang *et al.*, 90 in 2006 published that they could demonstrate a chronic oral administration of *S. oblonga* root extract reduced the cardiac TG and FA contents and decreased the oil red O-stained area in the myocardium of diabetic fatty rats, which paralleled the effect on plasma TG and FA levels. The treatment could also suppress the cardiac over expression of both FA transporter protein-1 mRNA and protein in the animals, suggesting inhibition of increased cardiac FA uptake as the basis for decreased cardiac FA levels. This suggests that the extract improves the excess cardiac lipid accumulation and increases cardiac FA oxidation in diabetes and obesity.
11. Huang T H W et al., in 2006 demonstrated that chronic oral administration of aqueous extract of *S. oblonga* root to diabetic fatty rats lowered plasma TG, TC levels, increased plasma HDL –C levels, and reduced the liver content of TG, nonesterified fatty acids and the ratio of fatty droplets to total tissue. The extract had no effect on the plasma TG and TC levels of fasted fatty rats. After olive oil administration to fatty rats, the extract also inhibited the increase in plasma TG levels. This suggested that the extract improves postprandial hyperlipidemia and hepatic steatosis in fatty rats. Additionally, the extract enhanced the hepatic expression of PPAR-alpha mRNA and protein, and carnitine palmitoyltransferase-1 and acyl-CoA oxidase mRNA in fatty rats. In vitro, the extract and its main component mangiferin activated PPAR-alpha luciferase acticity in human embryonic kidney 293 cells and lipoprotein lipase mRNA expression and enzyme activity in THP-1 differentiated macrophages; these effects were completely suppressed by a selective PPAR-alpha antagonist MK-886. The findings from both invivo and in vitro suggested that the extract functions as a PPAR-alpha activator, proving a potential mechanism for improvement of postprandial hyperlipidemia and hepatic steatosis in DM and obesity.

12. As part of a safety evaluation of novel ingredients for use in blood glucose control, the potential genotoxicity of a *S. oblonga* root extract was evaluated by Flammang et al., in 2006 using the standard battery of tests (reverse mutation assay; chromosomal aberrations assay; mouse micronucleus assay) recommended by US Food and Drug Administration (FDA) for food ingredients. *S. oblonga* was determined not to be genotoxic under the conditions of the reverse mutation assay and mouse micronucleus assay, and weakly positive for the chromosomal aberrations assay. A reproducible, although weak, positive chromosomal aberrations response in human lymphocytes is of concern and further toxicity research was recommended by the authors. They concluded that the use of *S. oblonga* is presently expected to be safe, as anticipated intake is small compared to the doses administered in the genotoxicity assays and may, after further toxicity research, may prove be a useful ingredient in foodstuffs.

13. Williams et al., in 2007 in a publication claimed that they have evaluated the effect of a herbal extract of *S. oblonga* on postprandial glycemia and
insulinemia in patients with type 2 diabetes after ingestion of a high-carbohydrate meal. The subjects were asked to consume one of the following meals: a standard liquid control meal, a control meal + 240 mg S.oblonga extract, and a control meal+ 480 mg S.oblonga extract. Serum glucose and insulin were measured at baseline and at postprandial intervals up to 180 min. Both the doses of the extract significantly lowered the postprandial positive area under the glucose curve (14% for the 240mg and 22% for the 480mg) and the adjusted peak glucose response (19% for the lower dose and 27% for the higher dose) to the control meal. Both the doses significantly decreased the postprandial insulin response, lowering both the positive area under the insulin curve and the adjusted peak insulin response (14% and 9% respectively, for the 480mg extract) in comparison with the control meal.

14. Hertzler S et al., in 2007 evaluated the effect of a hydroalcoholic extract of S.oblonga on the postprandial glycemia following a solid, high starch meal. In a randomized, crossover study, 14 healthy subjects were fed a meal containing 106g carbohydrate, 18g protein, 4g fat with 480mg S.oblonga extract. Serum glucose concentrations were measured immediately before the meal and at 15, 30, 45, 60, 90 and 120 min postprandial. Satiety ratings were obtained at the intervals above and at hourly intervals for an additional 6 hours. Symptom ratings (headache, nausea, abdominal cramping, bloating/fullness, flatulence) were collected at hourly intervals for 8 h postprandial. The extract lowered the mean baseline-adjusted peak glucose by 27% (p<0.001) and the positive incremental AUC by 25% (p=0.033). Flatulence rating were increased from 2.5 to 9.4 (40=maximum severity) by the extract, but there were no changes the glycemic response to a high-starch meal. The development of mild flatulence supports α-glucosidase inhibition as the main mechanism of action.

15. Huang et al., in 2008 published that they have administered aqueous extract of S.oblonga to male Zucker diabetic fatty rats at a dosage of 100mg/kg body weight for 7 weeks. At the end-point of the treatment, the hearts and left ventricles were weighed, cardiomyocyte cross-sectional areas were measured, and cardiac gene profiles were analysed. On the other hand, angiotensin II-stimulated embryonic rat heart-derived H9c2 cells and neonatal rat cardiac fibroblasts were pretreated with the extract and one of the prominent components mangiferin, respectively. Atrial natriuretic peptide mRNA
expression and protein synthesis and $[^3]$H thymidine incorporation were determined. The extract could lower the cardiac hypertrophy. The cardiac overexpression of atrial natriuretic peptide, brain natriuretic peptide and AT1 mRNAs and AT1 protein in Zucker diabetic fatty rats were suppressed by the treatment. The extract (50-100µg/ml) and mangiferin (25µmol) suppressed angiotensin II-induced atrial natriuretic peptide mRNA overexpression and protein synthesis in H9c2 cells. They also inhibited angiotensin II-stimulated $[^3]$Hthymidine incorporation by cardiac fibroblasts. These findings demonstrated that the extract could decrease cardiac hypertrophy in zucker diabetic fatty rats, at least in part by inhibiting cardiac AT1 overexpression.

16. Rong X et al., 96 in 2008 shown that an aqueous extract of S.oblonga root (100,300 and 900mg/kg, once daily by oral gavage over a 28 day period) elicited dose-related increases in liver weight by 1.6%, 13.4% and 42.5% respectively, in male rats. These effects were less pronounced in females. The extract selectively increased the liver mass in male rats but Sudan red staining was not different, which indicates that hepatic lipid accumulation was similar in both genders. However, the extract even at the highest dosage did not influence serum ALT and AST activities in both the genders. The extract was also found to activate PPAR-alpha in human hepatoma-derived HepG2 cells, as evidenced by the upregulation of PPAR-alpha and acyl-CoA oxidase mRNA expression. Thus it was suggested by the authors that a S.oblonga extract-dependent PPAR-alpha activation may preceded the development of the gender difference in hepatic hypertrophy; this process may be influenced by sex hormone status.

17. Li et al., 97 in 2008 published a review article, where they have discussed about the use of S.oblonga root for DM and obesity. It was said that S.oblonga roots were used in Ayurveda since antiquity, have been consumed in Japan, the United States and other countries as a food supplement for the prevention of obesity and DM. recent pharmacological studies have demonstrated that S.oblonga roots modulate multiple targets: peroxisome proliferator-activated receptor-alpha-mediated lipogenic gene transcription, angiotensin II/angiotensin II type 1 receptor, α-glucosidase, AR and pancreatic lipase. These multi-target actions may mainly contribute to S.oblonga root-induced improvement of type 2 DM and obesity-associated hyperglycemia,
dyslipidemia and related cardiovascular complications seen in humans and rodents. The results of bioassay-guided identification indicate that mangiferin, salacinol, kotalanol and kotalagenin 16-acetate are at least in part responsible for these multi-target regulatory activities of S. oblonga roots. Thus this plant could be used in the prevention and treatment of DM and obesity. Although toxicological studies have suggested minimal adverse effects in rodents, a clinical trial is crucial to further confirm the safety of S. oblonga roots.

18. He et al., 98 in 2009 found that an aqueous extract of S. oblonga at a dose of 100mg/kg body weight p.o for 6 weeks diminished renal glomerulosclerosis and interstitial fibrosis in diabetic fatty rats, as revealed by van Giesen staining. The extract also reduced renal salt-soluble, acid-soluble and salt-insoluble collagen contents. These changes were accompanied by normalization of hypoalbuminemia and BUN. Gene profiling revealed that the increase in transcripts encoding the glomerulosclerotic mediators collagen I, collagen IV, fibronectin, angiotensin II type 1 receptor (AT1), transforming growth factor (TGF)-beta 1, plasminogen activator inhibitor (PAI)-1 observed in the rat kidney was suppressed by the extract. In rat-derived mesangial cells, similar to the effect of the AT1 antagonist telmisartan, the plant extract and its major component mangiferin suppressed the stimulatory effect of angiotensin II on proliferation and increased mRNA expression and/or activities of collagen I, collagen IV, fibronectin, AT1, TGF-beta 1 and PAI-1. Thus it was concluded that the extract attenuated diabetic renal fibrosis, at least in part by suppressing angiotensin II/AT1 signaling.

19. Singh et al., 99 in 2009 studied the anti-mutagenic activity of hydroalcoholic root bark extract of S. oblonga in two doses (0.5 and 1.0gm/kg,p.o) on Wistar rats. The rats were administered with the extract for 7 days and on the 7th day mitomycin-C (2mg/kg,i.p). The testicular toxicity was assessed by monitoring the sperm shape abnormality and sperm count after 48 and 72 hours of mitomycin administration. Antioxidant activity was evaluated by measuring the serum levels of superoxide dismutase and catalase. The results indicated that prior treatment of the extract had suppressed the changes produced by mitomycin-C. The higher dose of 1.0g/kg had shown significant (p<0.01) inhibition in the sperm shape abnormality and sperm count in both the time intervals, while the lower dose showed inhibitory effect mainly at 48 hr
duration compared to the mitomycin group. The results also indicated that the extract has also improved the status of serum antioxidant enzymes compared with the mitomycin group. Thus this study suggested that *S.oblonga* extract possesses antimutagenic effect against mitomycin-C and the activity could be due to its antioxidant potential.

20. Giron *et al.*, 100 in 2009 published that the antidiabetic properties of *S.oblonga* extract are mediated not only by inhibiting intestinal alpha-glycosidases but also by enhancing glucose transport in muscle and adipose cells. *S.oblonga* extract effects on 2-deoxy-D-glucose uptake were assayed in muscle L6-myotubes and 3T3-adipocytes. In L6-myotubes, the amount and translocation of glucose transporters were assayed. A fractionation of the extract was carried out to identify the active compounds. Furthermore, we analyzed the phosphorylation status of key components of signaling pathways that are involved in the molecular mechanisms regulating glucose uptake. *S.oblonga* extract increased 2-deoxy-D-glucose uptake by 50% in L6-myotubes and 3T3-adipocytes. In L6-myotubes, the extract increased up to a 100% the GLUT4 content, activating GLUT-4 promoter transcription and its translocation to the plasma membrane. Mangiferin was identified as the bioactive compound. Furthermore, mangiferin effects were concomitant with the phosphorylation of 5'-AMP-activated protein kinase without the activation of PKB/Akt. The effect of mangiferin on 2-deoxy-D-glucose uptake was blocked by GW9662, an irreversible PPAR-gamma antagonist. They have concluded that *S.oblonga* extract and mangiferin may exert their antidiabetic effect by increasing GLUT-4 expression and translocation in muscle cells. These effects are probably mediated through two independent pathways that are related to 5'-AMP-activated protein kinase and PPAR-gamma.

21. In a review article by Donga *et al.*, 101 in 2011 about medicinal plants for the treatment of diabetes, a mention was made about *S.oblonga*. It was said that aqueous extract of the root bark of *S.oblonga* has shown hypoglycemic activity. Two biologically active fractions from the petroleum ether extract of the root bark has been shown to exert hypoglycemic effect of about 60 and 76% potency of an equal dose of tolbutamide (250 mg/kg) in albino rats. Petroleum ether extract of the bark of the root has been shown to prevent STZ (65 mg/kg) induced hyperglycemia and hypoinsulinemia in rats. The aqueous-
methanolic extract of the roots inhibited increase in serum glucose level in sucrose and maltose loaded rats. The water-soluble and ethyl acetate soluble portions of the same extract showed inhibitory activities on alphaglucosidase and AR. Further, salacinol and kotalanol with nine other sugar related component were isolated from the water soluble portion while, a new triterpene, kotalagenin 16-acetate along with known diterpene and triterpenes isolated from the ethyl acetate portion were found to be responsible component for the inhibitory activity on AR. In addition, the extract has shown significant anti-oxidant activity.

22. Sahayam S et al., \textsuperscript{102} in 2011 performed in vivo testing of the anti-diabetic activity of hydroalcoholic extract of various roots of \textit{Salacia} species, \textit{S.oblonga}, \textit{S.fruticosa}, \textit{S.reticulata} and \textit{S.chinensis} in Wistar albino rats. The results indicated that the various species of \textit{Salacia} have anti diabetic property. Comparatively \textit{S.oblonga} and \textit{S.reticulata} were more potent to the other species.

23. Nakata K et al.,\textsuperscript{103} in 2011 studied the beneficial effects on plasma glucose and lipids of a tea (SI tea) consisting of IP-PAI, a lipopolysaccharide derived from a Gram-negative bacteria and \textit{S.oblonga} (which contains an inhibitor of \(\alpha\)-glucosidase) in the KK-Ay/TaJcl type 2 diabetic model mice and in human subjects with premetabolic syndrome in a double-blind, randomized study. SI tea significantly decreased plasma glucose levels in KK-Ay/TaJcl mice. A clinical trial of SI tea was performed with 41 subjects between the ages of 40 and 69, who belonged either to a high plasma glucose group (HG: FPG 100-125 mg/dl) or to a hyperlipidemia group (HL: TG \(\geq\) 150 mg/dl, or LDL \(\geq\) 120 mg/dl, or HDL < 40 mg/dl). These subjects ingested either \textit{S.oblonga} without IP-PAI (the control) or SI tea. Blood samples were collected at 0, 30, and 60 days after initiating SI tea treatment, and were measured for FPG, HbA1c, TG, LDL, and HDL. These results showed that SI tea reduced FPG and HbA1c more rapidly than the control in the HL group, and also significantly improved LDL and HDL levels in the HG group. The conclusion was that, SI tea may be helpful in preventing lifestyle-related diseases.

24. Kim M S et al., \textsuperscript{104} demonstrated hypolipidemic and hypoglycemic effects of \textit{S.oblonga} on diabetic fatty rats at a dose of 100mg/kg body weight. There was a significant decrease in plasma TG, TC and liver TG content. They concluded
that the extract and its content, mangiferin, activate luciferase-linked PPAR-alpha promoter gene activity in HEK293 cells cotransfected with human pBI-G-hPPAR-alpha plasmid, tk-PPREx3-Luc reporter plasmid and betagalactosidase control vector. PPAR-alpha gene expression was also enhanced by the extract and mangiferin in liver derived HepG2 cells. They also have found that the extract is a potent alpha-glucosidase inhibitor, leading to a delay in the postprandial carbohydrate digestion.

25. A review article on ethanolic extract of *S. oblonga*, by Williams\textsuperscript{105}, Senior Abbott Nutrition Research Scientist, said that the extract decreased the postprandial glucose followed by a maltodextrin meal on rats. Toxicity was evaluated for 90 days on rats, and found that there was no treatment–related changes. Two studies conducted in healthy subjects tested the effect of *S. oblonga* on postprandial glycemia (PPG) when consumed as part of a high-carbohydrate liquid meal. *S. oblonga* significantly lowered PPG compared with a control meal in both clinical trials. The short-term efficacy of *S. oblonga* has also been demonstrated in patients with type 2 diabetes. One study tested the dose response of the extract in this specific population with a high-carbohydrate liquid meal; adjusted postprandial peak glucose was lowered by 14\% and 22\% (*P*<0.0001) and positive area under the curve for plasma glucose was lowered 19\% and 27\% (*P*<0.0001) respectively for the two doses (240 and 480 mg *S. oblonga*) versus a control meal. Second study demonstrated the efficacy of *S. oblonga* on PPG by showing its effect when consumed with a carbohydrate-rich pasta meal in patients with type 2 diabetes.\textsuperscript{16} A 240-mg dose of *S. oblonga* consumed along with a pasta meal lowered the positive area under the curve for glucose 19\% (*P*=0.0002) and the adjusted peak glucose by 19\% (*P*=0.0001), compared with the pasta meal alone. It was concluded by the author that *S. oblonga* is an excellent example of a novel ingredient for lowering PPG in people with diabetes.

Even after obtaining this much detail about the action of these two plants, a complete study of *G. sylvestre* and *S. oblonga* on their effect on ameliorating the complications of DM is not met with. So in the present study, a step has been taken towards this direction.
Experimental DM can be induced in laboratory animals by several methods. The generally effective method is to take the pancreas out of the body. The second method for creating DM in animals is injecting drugs such as alloxan (ALX) or streptozotocin (STZ). These materials inflate and ultimately degenerate the Langerhans islets beta cells. A less reliable method is injection of the anterior hypophysis extract.

STZ is a broad-spectrum antibiotic isolated from *Streptomyces achromogenes*. It has an unexpected property of highly specific diabetogenic effect. Rakieten and his associates first demonstrated the diabetogenic property of STZ in dogs and rats in 1963\(^1\). Chemically it has 1-methyl-1-nitrosourea linked to position C\(_2\) of D-glucose. It is freely soluble in water. It is unstable at room and even refrigerator temperatures and has to be stored below 20\(^{0}\)C. It is available as a dry-frozen, pale yellow, sterilized product. Pure STZ has alkaline pH. It is very unstable upon solution in saline or distilled water at room temperature and at neutral pH, where it decomposes within few minutes with visible formation of gas. Its stability in solution is optimal at pH 4 and low temperature. A single intravenous injection of 50 mg/kg was reported to yield 100% DM \(^1\). Because of low stability of STZ, the rapid intravenous injection appears to be the only dependable route of administration. Like ALX, STZ also known to show a triphasic pattern of blood glucose level fluctuations. Initial hyperglycemia is observed within 45 to 60 min of injection of STZ, then the animals go to a hypoglycemic state at around 10 hours and then onwards the blood glucose starts increasing again and reaches a peak at around 48 hours \(^1\). STZ induced DM in the rats was described as a specific form of hyperglycemia with virtually no ketosis or elevation of plasma FFA. Like ALX, it causes hyperglycaemia mainly by its direct cytotoxic action on the pancreatic beta cells. The evidences are accumulating on the mechanisms associated with diabetogenecity of STZ. Its nitrosourea moiety is responsible for beta cell toxicity, while deoxyglucose moiety facilitates transport across the cell membrane. Like ALX, the involvement of free radicals generation and resulting alteration of endogenous scavengers of these reactive species have been reported in STZ diabetogenecity. Further, STZ causing alkylation or breakage of DNA strands and a consequent increase in the activity of poly-ADP-ribose synthetase, an enzyme depleting NAD in beta cells finally leading to energy deprivation and death of beta cells is reported. These hypotheses have been confirmed by different studies in which the administrations of various chemicals such as antioxidants
(superoxide dismutase; SOD), free radical scavenger (alpha-phenyl-tertbutyl nitronate), NAD and poly ADP-ribosyl synthase inhibitors, concomitantly or before STZ injection have been shown to either prevent or lessen the severity of the induction of diabetes, respectively. There are wide varieties of reports available in the literatures on doses and development of hyperglycaemia with STZ since the susceptibility of animals to STZ appears to depend on age, species and even within strain107. ALX and STZ diabetic animals are most widely used for screening the compounds including natural products for their insulinomimetic, insulinitropic and other hypoglycaemic/antihyperglycaemic activities107. STZ diabetic rats mimic many chronic complications observed in diabetic human108. STZ is a preferred agent to induce experimental diabetes since it has some advantages over ALX such as, relatively longer half-life (15 min), sustained hyperglycaemia for longer duration and the development of well characterized diabetic complications with fewer incidences of ketosis as well as mortality107.