CHAPTER 1

INTRODUCTION

1.1 Diabetes Mellitus

Diabetes Mellitus (DM) is a group of metabolic disorders characterized by hyperglycemia resulting from the inability of the body to produce or properly use insulin. Essentially there are three types of diabetes that exist, gestational, insulin dependent (Type I) and non-insulin dependent (Type II). Despite many ongoing studies on this condition, the exact cause is unknown. However, genetics, environmental factors, obesity and lack of exercise appear to play a role in the development of DM. Diabetes affects approximately 171 million people worldwide and is likely to more than double by 2030 to reach 366 million (WHO, 2006). Gestational diabetes occurs in pregnant women without prior indication of DM and is characterized by high blood sugar (glucose) levels during pregnancy. Gestational diabetes affects about 4% of all pregnant women (ADA, 2006). The exact mechanism of development is not known, however, one theory is that placental hormones, which assist in fetal development, block the action of insulin in the mother's body accounting for high levels of sugar in the blood an action called insulin resistance (ADA, 2006). Insulin-Dependent Diabetes Mellitus (Type I), occurs from a cellular-mediated auto-immune destruction of pancreatic β-cells, and is usually diagnosed in children and young adults (JDC, 2006; JDRF, 2006).

The most common type of DM is Non-Insulin Dependent Diabetes Mellitus (Type II). In this type of disorder, either the pancreas does not produce enough insulin or the cells cannot

utilize the insulin (ADA, 2006; JDC, 2006). Most patients with Type II diabetes and obesity are responsible for some degree of insulin resistance (ECDCDM, 2003). While insulin resistance may improve with weight reduction and/or pharmacological treatment of hyperglycemia it is seldom restored to a normal status. Gestational diabetes, Type I and II diabetes are often associated with lifestyle, possible environmental triggers, and strong genetic predisposition that vary in frequency with different racial/ethnic subgroups (ECDCDM, 2003). Symptoms commonly seen in diabetics include excessive thirst, constant hunger, excessive urination, rapid, hard breathing, vision changes, drowsiness or exhaustion. More severe complications associated with DM can occur, such as heart disease, allowing an increased susceptibility for a stroke. DM can also cause irregular kidney function causing valuable nutrients to be lost in the urine (microalbuminuria) (JDC, 2006). While this may be treatable if diagnosed early, under severe circumstances, a kidney transplant must occur (JDC, 2006). Other complications include hepatic malfunction, damaged nerve endings resulting in amputations, and pregnancy complications (ADA, 2006; JDC, 2006). Treatments for DM depend on the specific type; however there are some general guidelines that individuals suffering these conditions should follow. This includes regular exercise, following a healthy meal plan, and in some cases the use of pharmaceutical agents for the reduction of blood glucose levels (ADA, 2006).

Diabetes is ranked as the sixth leading cause of death based on 73,249 death certificates that were filed during the year. These figures are likely to double by 2030. The U.S. is also ranked among the top ten countries in number of sufferers, along with India, China, Indonesia, Japan, Pakistan, Russia, Brazil, Italy and Bangladesh. Much of the increased prevalence of DM will arise in developing countries due to growth in population, ageing, inadequate diets, obesity, and sedentary lifestyles (WHO, 2006).

Treatment of DM consists of fundamental factors such as: education bestowed on the patient concerning the disease; physical exercise; diet; and hypoglycemic (pharmacological) agents. People with Type 1 diabetes are usually completely dependent on a daily administration of insulin injections for survival. Treatment of individuals with the Type 2 form of DM, though not dependent on insulin for survival, about one third of those afflicted need insulin for reduction of their blood glucose levels (ADA, 2006).

Medications that are commonly used to control glucose levels work either to improve pancreatic insulin secretion, increase the body's sensitivity to insulin, or are involved in

weight loss. These medications include sulfonylureas, thiazolidinediones derivatives, alpha-glucosidase inhibitors, biguanides, and orlistat, among others (Freemark, 2003). Despite the extensive and continuous use of these medications because of their favorable effects in controlling hyperglycemia, these agents have not acquired an adequate control over DM nor been able to suppress the chronic and acute complications associated with diabetes. Moreover, many of these pharmacons, including insulin, are unavailable and/or unaffordable in many economically challenged countries.

DM is a very costly disease that can put an individual in financial distress. The cost for treatment of DM is increasingly outstretching the health-care resources. For an individual with DM, it is estimated that the total health care costs are between twice and three times as much as oppose to individuals without the condition (WHO, 2006). Direct cost involved with medical care from the part of the family and/or individual are the purchase of medications, insulin and syringes, physician services, and blood-testing equipment (to name a few) and in many situations bare the increased payments for health, life, and automobile insurance (ADA, 2006). In developing countries, diabetes can absorb as much as 25% of a low-income family with an adult suffering from DM. Due to the chronic nature of DM, for most countries the largest single factor for diabetes expenditure are hospital admissions for treatment of long-term complications, such as heart disease and stroke, kidney failure and foot problem (WHO, 2006). Due to the cost of DM care many people have turned to alternative or traditional medicine (TM) to ease their ailments.

1.1.1 Mechanisms of Action

Found within the pancreas are *islets of Langerhans* which contain specialized β- and and α-cells. These cells secrete insulin and glucagon, two peptide hormones that work together to maintain a stable blood glucose level. The β-Cells release insulin in order to reduce the level of blood glucose, whereas glucagon is released by α-cells which act to increase blood glucose, essentially having the opposite effect (Figure 2.1) (Webb et al.,2000; Lodish et al., 2004).

The insulin hormone contains of two polypeptide chains linked by disulfide bonds, is the essential hormone for the initiation of multiple signaling pathways, inducing immediate and long term cellular effects (King, 2004). The acute effects of insulin include an increase in the rate of glucose uptake from the blood into tissue specific cells and the activation of various

enzymes involved in glucose metabolism. Chronic exposure time results in increased expression of liver enzymes, glycogen synthesis and of adipocyte enzymes that synthesize triaclyglycerols. Insulin also acts as a growth factor for many cells (Lodish et al., 2004), and has other important roles in stimulating lipogenesis, diminishing lipolysis, increasing amino acid transport into cells, modulating transcription, altering the cell content of numerous mRNAs and numerous other processes (Sleight,2002; Ramm et. al., 2000;Belke et al., 2002).

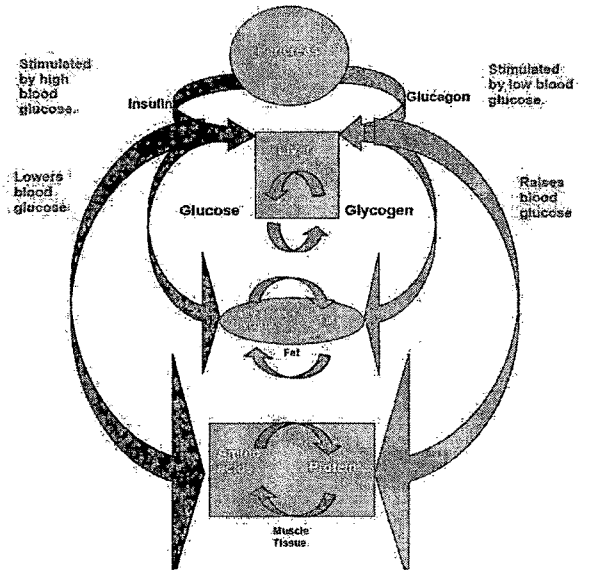

Figure 1 Homeostatic physiological function of insulin and glucagons.

The utilization of glucose for cellular metabolism is regulated during periods of abundance (such as after a meal) or shortages (during fasting) by adjustment of insulin and glucagons concentrations in the blood (King, 2004). After a meal, when blood glucose rises above normal levels, the pancreatic β-cells respond to the rise in glucose levels by releasing insulin into the blood, directly infusing it via the portal vein to the liver, where it exerts profound metabolic effects throughout the body (King,2004; Lodish et al., 2004). As insulin is distributed, it binds to insulin receptors that are found on cellular surfaces throughout the body. This binding causes glucose to be removed from the blood and stored in tissue specific cells as glycogen (Weinstein et al., 1994). This process is facilitated by the insulin receptor. The insulin receptor is a heterotetramer $\alpha 2\beta 2$ transmembrane glycoprotein whose intracellular signaling events are activated once insulin is bound (Schaffer et al., 2003).

Glucose transport is initiated upon binding of insulin to the insulin receptor (figure 2) (Schaffer et al., 2003). This binding leads to a conformational change and

autophosphorylation of the receptor. A polypeptide, called insulin receptor substrate 1 (IRS1), is recruited and binds to a phosphotyrosine residue, phosphorylating IRS1 (Chen et al., 1996).

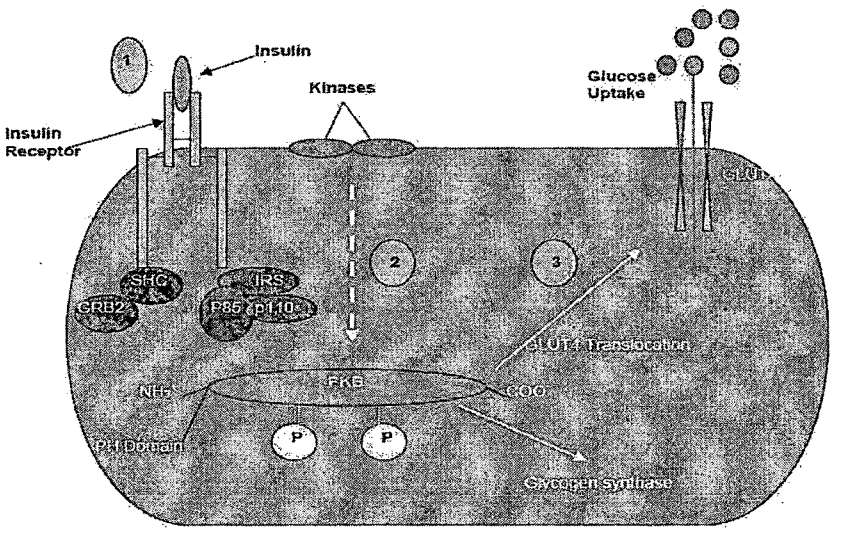

Figure 2 Insulin Pathway (Ras-Independent Pathway).

Phosphoinositide 3 Kinase (PI-3) is bound by IRS1, causing stimulation in kinase activity, which accounts for the rapid rise in phosphoinositides observed in insulinstimulated cells (Chen et al., 1996; Eldar et al., 1997). This increase in activity leads to recruitment of *Protien Kinase B* (PKB) to the membrane, which contains a *Pleckstrin Homology* (PH) domain on its N-terminal and binds to the plasma membrane bound phosphoinositides (Eldar et al., 1997; Lodish et al., 2004).

Once localized in the membrane, PKB is phosphorylated (activated) by two membrane bound kinases, then released into the cytosol where it mediates numerous effects of insulin, including stimulation of glucose uptake and glycogen synthesis. This sequence of events is called the Ras-independent pathway (Foulds et al., 2004; Lodish et al., 2004).

1.1.2 Possible Environmental Etiological Factors to the Onset of Diabetes Mellitus

Much of the evidence surrounding the etiological factors associated with the development of Diabetes Mellitus is inconclusive. In many cases it has been attributed to hereditary elements across age, different ethnic groups, as well as the interactions of gene elements with environmental contaminants (Longecker et. al., 2001).

As previously mentioned, Type I diabetes or Insulin-Dependent Diabetes Mellitus (IDDM), occurs from a decrease in the insulin production by the pancreatic β-cells, usually resulting from an autoimmune process (JDC, 2006; JDRFI, 2006). Thus, autoimmunity against β-cells is believed to be induced by a combination of genetic predisposition and environmental factors such as infectious agents or dietary elements which stimulate an immune reaction (Longnecker et. al. 2001). Coxsackie-B4 (CVB) and encephalomyocarditis M are two examples of viral agents that are associated with the induction of IDDM in rodents (Kraine et. al. 1999). Both viral agents have been documented to play a role in lymphocyte destruction of acutely infected islet cells as well as in the lyses of chronically infected islets by macrophages (Kraine et. al. 1999). Therefore, the destruction of islets results in a prolonged hyperglycemia, which in most cases revert within a period of 6-12 months (Kraine et. al. 1999). In recent years, rubella, coxsackie, and retroviruses have been extensively studied in humans; with rubella being the only virus for which conclusive evidence exists demonstrating that an infection significantly increases the development of IDDM (Kraine et. al. 1999). Heavy metals exposure, in particular arsenic has been linked as a possible inducer in both Type I and Type II diabetes. In a study by Walton et al. 2004, examination of the effects of trivalent arsenicals on the components of the insulin – stimulated signaling pathway determined that all trivalent arsenicals-(arsenite (iAs^{III}), methylarsine oxide ($MAs^{III}O$), and iododimethylarsine ($DMAs^{III}O$))-suppressed the expression and possibly phosphorylation of PKB/AKt leading to the disruption of insulin-stimulated glucose uptake by GLUT4 transporters in adipocytes (figure 3) (Walton et. al. 2004).

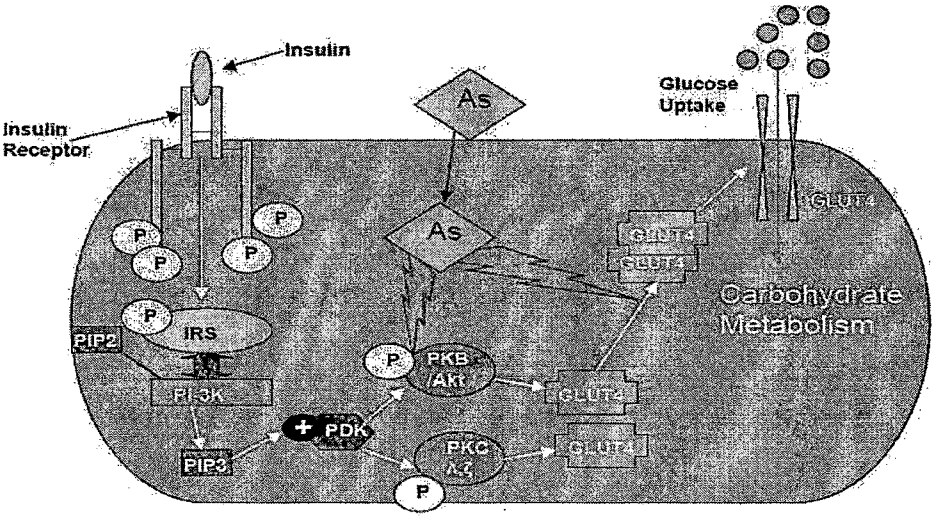

Figure 3 Possible mechanism by which trivalent arsenicals disrupt phosphorylation of the PKB/Akt complex which in turn disrupts GLUT4 uptake of glucose.

1.2 Glucose Metabolism

1.2.1 Glucose Transporter Structure and Function

Maintenance of blood glucose levels (homeostasis) within the body is of importance. Thus, through endocrine function, the body can initiate multiple signaling pathways that will regulate the levels of glucose, usually from about 70 mg/dl to perhaps 110 mg/dl (3.9 - 6.1 mmol/litre) (Wikipedia/Insulin, 2007). Insulin and Glucagon are two such hormones that are essential in glucose regulation. In humans, both peptide hormones are synthesized in the pancreas, insulin by β-cells and glucagon by α-cells within the *islets of Langerhans* (Lodish et al., 2001). As the concentration of blood glucose increases, there is a pancreatic release of insulin which acts as a means of reducing the blood glucose by binding to tissue specific membrane receptors on the cell surface and stimulating an increase in glucose uptake (Lodish et al., 2001; Wikipedia/Insulin, 2007).Glucose is then stored, mainly in the liver, as glycogen (Voet et al. 2002). In a similar mechanism, as blood glucose levels fall, such as during the times that an individual fasten, glucagon binds to tissue specific receptors, predominantly in the liver, and activates adenylyl cyclase which leads to the cAMPmediated cascade and in turn causes conversion of glycogen to glucose and its eventual release into the blood stream through glycolysis and gluconeogenesis pathways (Lodish et al., 2001, Voet et al., 2002).

A constant supply of glucose is essential for tissues such as the brain and red blood cells, which depend almost entirely on glucose as an energy source. Transport of glucose through the membrane is achieved through the use of integral membrane proteins that contain a 12 membrane-spanning helices with an amino ($-NH_3^+$) and a carboxyl ($-COO^-$) terminus in the inner, cytoplasmic side of the plasma membrane (Wikipedia/Glucose Transporter, 2007).

Generally, the cell employs two mechanisms to import glucose across the plasma membrane, depending on its tissue specificity. The first is the use of a sodium-linked glucose symporter (SGLT) in the apical membrane of epithelial cells, such as in kidney and intestine (Lodish et al., 2001; Aghayan et. al. 1992). The movement of sodium (Na^+) from the exterior of the cell into the cytosol is driven by 1) a Na^+ concentration gradient and 2) by the negatively charged inner membrane electric potential (Lodish et.al. 2001). Thus, the utilization of 2 sodium ions (instead of 1 Na^+ ion) per 1glucose molecule creates a much stronger concentration gradient allowing a more efficient transport (Lodish et.al. 2001).The second transport mechanism which is more ubiquitous throughout the body is a facilitated carrier system that catalyzes

movement of glucose down a concentration gradient, these transporters are known as the GLUT gene family of transporters (Pascual et.al 2004; Aghayan et. al. 1992).

There are currently 13 known isoforms within the glucose transporter (GLUT) family, all which differ in their expression in different tissues, in their kinetic characteristics, i.e. K_m values, and in their substrate specificity (Table 1) (Aghayan et. al. 1992; Navarete Santos et. al. 2004). For example, GLUT1 mediates glucose transport into erythrocytes and through the blood-brain barrier, and appears to provide a basal supply of glucose for most cells whereas GLUT4 is exclusively found in muscle and adipose tissue; its sub cellular localization is controlled by insulin (Dugani et. al. 2005).

Table 1 Mammalian family of facilitative glucose transporters (Gluts). Adapted from P.-H. Ducluzeau et al. 2002.

Name	Tissue distribution	Function
GLUT 1	Wide distribution, abundant in red blood cells, endothelial cells and tissue culture cell lines	Basal glucose uptake in many cells (incl. insulin sensitive cells), transport in growing cells and across blood-brain barrier
GLUT 2	Limited to pancreatic β bells, hepatocytes, intestine, kidney	Glucose-sensing in β cells, high capacity transport, trans-epithelial transport, major transporter in liver
GLUT 3	Wide distribution in humans, limited to brain in some species	Basal transport, uptake from cerebral fluid
GLUT 4	Largely expressed in insulin-responsive tissue of skeletal and cardiac muscle, and adipose	Insulin-sensitive glucose uptake, vital in postprandial disposal
GLUT 5	Primarily intestine, small amounts in adipose, muscle, brain, kidney	Absorption of fructose in intestine
GLUT 7	Gluconeogenesis tissues: hepatocytes	Release of glucose from gluconeogenesis from ER lumen
GLUT 8	Blastocyt, possible other tissues	Insulin-stimulated glucose uptake into blastocyt and possible other tissues lacking GLUT 4
GLUT X1	High in testis, moderate in central nervous system, low in insulin responsive tissues	Sequestered intracellularly therefore may play a role in regulatable glucose uptake

1.2.2 Transporter Deficiencies and Disease

Aside from Diabetes Mellitus, there exist a number of metabolic disorders that are associated with glucose transport malfunction which include De Vivo's Disease, Glucose-Galactose

Malabsorption Syndrome, and Fanconi-Bickel Syndrome (FBS) (Pascual et. al 2004). De Vivos's Disease, is the result of an autosomal-dominant loss-offunction mutation of the glucose transporter type 1 gene (GLUT1) leading to brain energy failure and epileptic encephalopathy (Wong et. al. 2006; Pascual et. al. 2004). Glucose-Galactose Malabsoption Syndrome (GGMS), is an autosomal recessive disorder that results from the inheritance of two faulty copies of the sodium-linked glucose symporter 1 (SLGT1) (NIH Genes and Disease, 2007). Defects of this gene causes improper transport of glucose and galactose from the lumen to the small intestine and as a result these molecules drag water out of the body into the intestinal lumen, bringing about diarrhea (NIH Genes and Disease, 2007; Pascual et. al. 2004). Fanconi-Bickel Syndrome, an autosomal recessive disorder of facilitative transporter GLUT2, was first described in 1945, and characterized by hepatorenal glycogen build up as it fails to properly transport glycogen and galactose (Sakamoto et. al., 2000). The transporter's malfunction in the pancreas and liver of those afflicted with FBS has lead to hepatomegaly (enlagement of the liver) due to the accumulation of glycogen and fat in the liver as well as ketotic hypoglycemia prepandially and hyperglycemia postprandially (Pascual et. al. 2004). The latter is considered to belong to a category of enzyme abnormalities that are related to glucose transport deficiencies known as glycogen storage diseases (GSD) (Pascual et. al 2004; Hendrickx et al., 1999; Chen et al., 1987).

GSDs are defined as inborn metabolic errors attributed to enzymatic defects that affect normal glycogen synthesis or degradation within specific tissues (Kikiuchi et al.1998; Hendrickx et al., 1999; Chen et al., 1987). The enzymes in question normally play a role in the conversion of glycogen to glucose, but deficiencies result in glycogen accumulation in tissues (Kikiuchi et al.1998). Common clinical symptoms present themselves as myopathy, such as muscle weakness and cramps; however more severe symptoms like hypoglycemic seizures or cardiomegaly may occur (Chen et al., 1987; Bogusky et al., 1986).

1.2.3 Novel Proteins for Diabetic Therapeutic Function.

Peroxisome Proliferator Activated Receptors (PPARs).

There are essentially three PPAR isoforms located within the cell nucleus that interact with peroxisome proliferators (PPAR-α, PPAR-ß, PPAR-γ) (Semple et al., 2006)

Each isoform (Table 3.3) is tissue specific, and once bound by the appropriate ligand such as fibric acid, glitazones as well as by certain fatty acids, they act as a transcription factors for specific genes (Tenenbaum et al., 2005; Barish et. al., 2006).

Table 2 Types of Glycogen Storage Disease with its respective enzyme deficiency and common name.

Type	Enzyme Deficiency	Common Name
GSD Type I	Glucose-6-Phosphatase	Von Gierke's Disease
GSD Type II	Acid Maltase	Pompe's Disease
GSD Type III	Glycogen Debrancher	Cori's or Forbe's Disease
GSD Type VI	Glycogen Branching	Andersen Disease
GSD Type V	Muscle Glycogen Phosphorylase	McArdle Disease
GSD Type VI	Liver Phosphorylase	Hers's Disease
GSD Type VIII	Muscle Phosphofructokinase	Tarui's Disease
GSD Type IX	Phosphorylase Kinase	
GSD Type XI	Glucose Transporter	Fanconi-Bickel

Table 3 demonstrates how different isoforms of PPAR are tissue specific.

Peroxisome Proliferator Activated Receptors	Tissue Specification
PPAR-α	Liver, Kidney, Heart, Muscle, Adipose
PPAR-ß	Brain, Adipose, Skin
PPAR-γ	Heart, Muscle, Kidney, Adipose,Pancrease, Spleen, Colon

All isoforms dimerize along with another receptor, the retinoid X receptor (RXR), forming a complex (Tenenbaum et al., 2005). Once binding of the ligand has occurred, it is consequently translocated into the cell nucleus where it binds to specific DNA sequences (near the promoter region) known as peroxisome proliferator response elements (PPREs) where expression or transrepression of gene products are dictated (Shi et al., 2002)

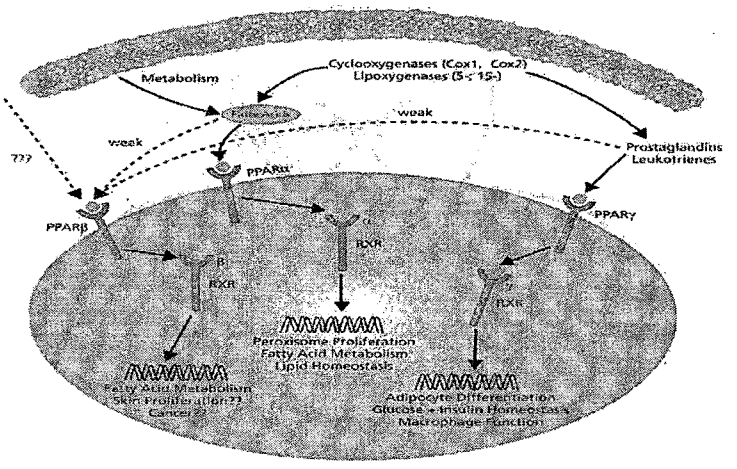

Figure 4 Signaling pathways of the PPAR isoforms (PPAR-α, PPAR-ß, PPAR-γ).

As mentioned in Table 3, PPAR-α isoform is predominately present in skeletal muscle, heart, liver and kidney as well as being linked to regulation of beta-oxidation of fatty acids (Semple et al., 2006). PPAR-α is activated by free fatty acids including linoleic, arachidoni, and oleic acids, thus leading to a reduction in blood triglyceride levels and a suppression of hepatocyte apoptosis (Sigma-Aldrich/Cell Signaling, 2007). Defects in the PPAR-γ isoform can lead to development of type 2 diabetes, hypertension, and also influence in the genetic predisposition towards obesity (Tenenbaum et al., 2005). The isoform plays an important role in adipocytes differentiation and lipid metabolism and as such appears to be activated very early in the differentiation of several cultured adipocyte cell lines (Tenenbaum et al., 2005; Semple et al., 2006). The β-isoform is weakly activated by fatty acids, prostaglandins, and leukotrienes; while its physiological role is still obscured (Sigma-Aldrich/Cell Signaling, 2007).

1.3 *In Vitro* Models Used in Diabetes Research

A range of *in vitro* and *in vivo* models are available to study potential antidiabetic activity in plant extracts. They are base on the primary need to control hyperglycemia in diabetic and the various means of achieving this goal. *In vitro* models may be used to screen randomly or ethnobotanically selected material for a specific activity that would result in lowering of blood glucose levels. Alternatively the models may be used to determine the mechanism of action of plant extract with traditional use and/or human *in vivo* data to support an antidiabetic effect.

It is relevant in the context of *in vitro* test for antidiabetic activity, to examine the source and fate of glucose in the body in the normal and diabetic states. Glucose is derived primarily

from the digestion of dietary carbohydrates in the gastro intestinal tract from which it is absorbed in to the blood by passive and active mechanisms. In the fed state, a rise in blood glucose normally stimulates insulin secretion from the pancreas. This hormone initiates glucose uptake in to specific target tissue, primarily liver, muscle and fat cells (adipocytes). It promotes glucose oxidation and glycogen deposition in liver and muscle and the incorporation of glucose (as glycerol) in to triglycerides in adipocytes. These combined activities have the effect of lowering elevated plasma glucose resulting from the intake of a meal. In the fasted state, insulin and glucose level decrease. Glucose is then metabolized from glycogen stored in liver (glycogenolysis). Another important source of glucose in the fasted state is gluconeogenesis – the *de novo* formation of glucose from smaller, unusual precursor molecules. This occurs in the liver and to a lesser extent, kidney and is under the control of glucagons, a common counter hormone whose level rise as those of insulin fall and vice versa. When glucagons levels are high and those of insulin are low gluconeogenesis and glycogenolysis are stimulated and glucose enters the blood stream.

In diabetes insulin is absent (Type I diabetes) or insufficient (Type II diabetes). In Type II diabetes insulin target tissues are generally less responsible to insulin (insulin resistant) than normal. The fine balance between glucose uptake in to target organs and release of hepatic glucose is impaired, resulting in abnormal high fasting glucose levels as well as poor glucose tolerance following a meal.

From these, following mechanism has been proposed for an agent that could lower or control plasma glucose level.

- Inhibition of carbohydrate digesting enzymes, reducing the amount of rate of glucose release from the diet.
- Impairment of glucose uptake from small intestine.
- Stimulation of insulin secretion from β cells of pancreas.
- Insulinomimetic or insulin secreting activity at insulin target tissue i.e, liver, skeletal muscle or adipocyte.
- Antagonism of glucagons activity.

Animal models have been used extensively in diabetes research. Early studies used pancreatectomised dogs to confirm the central role of the pancreas in glucose homeostasis, culminating in the discovery and purification of insulin. Selective inbreeding has produced several strains of animal that are considered reasonable models of Type I diabetes, Type II

diabetes and related phenotypes such as obesity and insulin resistance. Today, animal experimentation is contentious and subject to legal and ethical restrictions that vary throughout the world. Various cell lines have been used to evaluate the new chemical entities (NCEs) for their hypoglycemic effects. These cell lines have vast applications in diabetes research in screening of NCEs for type I and type II diabetes. In addition, it especially describes the appropriate selection and usefulness of different cell line in preclinical testing of various NCEs for the treatment of diabetes.

1.3.1 Screening for antidiabetic effect in cell cultures

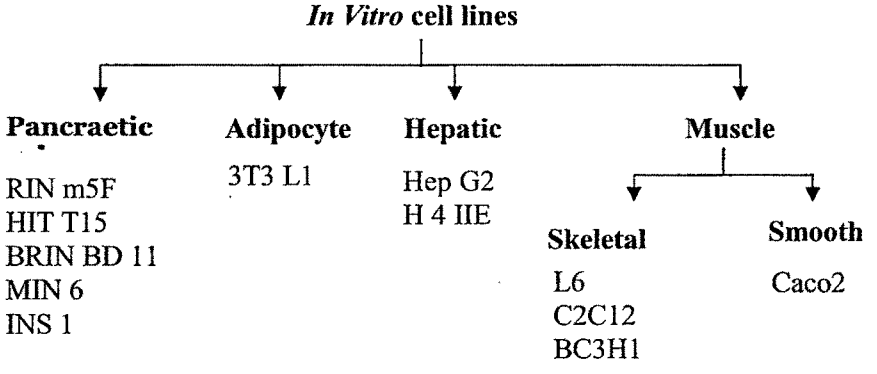

Figure 5 Cell lines used in Diabetes Research

1.4 Treatment of Diabetes mellitus

1.4.1 Lifestyle modification

Medical nutritional therapy and exercise is an integral part of the treatment plan for both type I and type II diabetes.

1.4.2 Diet

The incidence of diabetes mellitus is highly correlated with the fiber depleted high refined carbohydrate diet. Reestablishing a traditional diet and lifestyle reverses the carbohydrate and lipid metabolism abnormalities associated with the food of commerce and eventually results in a low prevalence of diabetes mellitus.

1.4.3 Exercise

Physical trained diabetics experience many benefits enhanced insulin sensitivity with a consequent diminished need for exogenous insulin, reduced total serum cholesterol and triglycerides within increased HDL levels that results in more anti-atherogenic state and in obese diabetics improved weight lose (Ranic and Berge,1979; Koivisto and Defronzo,1984; Selby et al., 1987; Pollack et al.,1984).

1.4.4 Insulin Therapy

For the treatment of type I diabetes and many patients with type II who are pregnant or severely hyperglycemic or symptomatic.

1.4.5 Oral Drug Therapy

Patients with type II diabetes may achieve adequate glycemic control using oral hypoglycemic agents like sulphonylureas (chlorpropamide, tolbutamide) biguanides (metformin), alpha-glucosidase inhibitors (acarbose) and thiazolidinediones (glitazones).

1.4.6 Potential new antidiabetic drugs

Inhibitors of fatty acid oxidation: Etoximer, Lisofyllin.

β_3 adrenergic receptor agonist: Lipolysis in fat cells.

Inhibitors of protein kinase C: Because of evidence implicating activation of this pathway in the development of vascular diabetic complications.

Insulinomimetic drugs: Vanadium salts, aminoguanidine, tenisetam.

1.4.7 Traditional Medicine (TM)

Traditional medicine refers to, for our purposes, the application, approach, knowledge, and belief in incorporating plant or animal based properties in remedies, singularly or in combination, for the purpose of treating or preventing disease as well as to maintain the well-being of an individual. As such herbal remedies have been used to cure a variety of disorders or conditions such as cardiovascular problems, cancer, weight control, dermal infirmities, sexual malfunction, and of course diabetes.. According to the World Health Organization, more than 70% of the world's population uses TM in order to fulfill their health necessities (E. Hernandez-Galicia, 2002). For example, in various oriental countries such as China, TM

account for about 30%-50% of total medicinal intake. Other countries like the United Kingdom have an annual expenditure on TM of US$230 million. In the United States, it is estimated that 158 million of the adult population use TM as alternative or complementary medicines and according to the USA Commission for Alternative and Complementary medicines, close to US$17 billion was spent on traditional remedies in 2000 (WHO, 2006). The practice of herbalism has been documented as far back as 13,000 - 25,000 BC (Wikipedia/Herbalism, 2007). Throughout history, the use of herbal medicine has played a role in alleviating those who are economically challenged or who could not afford the services of a physician; much as is the case today and thus must turn to alternative medicine for their health needs (Wikipedia/History of alternative medicine, 2007). Despite the large amount of individuals that have consumed alternative medicines over the years, many basic and fundamental questions have not been addressed, such as their mechanism of action.

1.4.8 Herbal Drugs in Treatment of Diabetes Mellitus

Herbal medicines have been used for thousands of years in many parts of the world. The attention paid by health authorities to the use of herbal medicines has increased considerably because they are often the lonely medicine available in less developed areas and they are popular as alternative medicine in more developed area. This switchover from modern system of medicine to traditional system demand for later scientific approaches to answer the questions of their safety and efficacy. However, the experiences from traditional use of herbal medicines over the years should not be ignored. A wide range of the plants and their constituents appears in literature as active hypoglycemic agents and many were found to have different sites of action within the body.

Site of action	Plants used
Drugs acting like insulin	*Momordica charantia, Panax ginseng*
Drugs increasing insulin secretion from β-cells of pancreas	*Azadirachta indiaca, Syzygium cumini, Eugenia jumbolana*
Drugs acting by regeneration of β-cells of islets of Langerhans	*Pterocarpus marsupium, Tinospora cordifolia, T.crispa Gymnema sylvestre*
Drugs inhibiting glucagons secretion from α-cells in pancreas	*Cyamopsis tetragonolobus, Oscimum sanctum*
Drug inhibiting aldose reductase activity	*Paeonia latiflora, Glycyrrhiza glabra, Atractyloide lancea, Cinnamomum zeylenicum, Aralia elata*

Drugs increasing glucose utilization	*Zingiber officinale, Nelumbo nucifera*
Drugs reducing lactic dehydrogenase and γ-glutamyl transpeptidase	*Lythrum salicaria*
Drugs inhibiting glycogen metabolizing enzymes	*Bryonia alba*
Drugs acting on liver glycogen	*Allium sativum, Allium cepa*
Drugs increasing glyoxalase 1 activity in liver	*Trigonella foenumgraecum*
Drugs increasing glucose uptake in adipocytes	*Swertia japonica*
Drugs inhibiting Glucose-6-phosphate system	*Bauhinia megalandra*

Many of the well-known pharmaceutical industries have also come up with anti diabetic herbal combinations. These preparations have been extensively studied clinically and scientifically at various centers and have shown their efficacy. They are claimed as easy to administer and also that their dose may be well regulated. Some of the combinations marketed are listed below.

BRAND NAME	MANUFACTURER	BRAND NAME	MANUFACTURER
BIO-GYMNEMA cap.	Ayush	GLUCOVA	Vasu
CARBOMET COMPOUND	Akshay	GLUSET	Vitalcare
COAGENT db + Tab.	Alkem	GLYCONTROL	Afdil
COSBEX	Cosmovision	GORANCHI	Sagar
DAIBINIL 16 Cap.	Jantayu	HERBO SAP	Fort Herbal
DEB-NIL Cap.	Four-S lab	HYPONIDD	Charak
DIABECON Tab.	Himalaya	INSUPRO Cap.	Pan Herbo
DIAMED	Bajarang	K-4	Zandu
DIABEGON Cap.	Dindayal	KARNIM Cap.	Univesral
DIABET Cap.	Medilinks	LIMIT Cap.	Ayulabs
DIABETAB-H	Vedlabs	MADHUCURE Cap.	Fizikem
DIABETEX STRONG Cap.	Multani	MADHUNA Churna	Ganga
DIACARE Cap.	Sanjeevani	MADHUMEHARI Granules	Baidyanath
DIAFORT Cap.	Fort Herbal	MADHUSUDAN	Pavaman
DIAKYUR	Amruntajan	NIRMADHU	Hemaadri
DIATONE Tab.	Manil	SHILA PRAMEHA VATI	Vyas
DYKURE Cap.	Herbal Body Cure	TRIBANGSHILA	Zandu
GLUCOLEV	Bajaj	X – DIABA Cap.	Suryaherbal
GLUCONIL Cap.	Herbochem	ZIABETICA	Lala's

Out of these marketed formulations, four formulations were selected which consists of herbs commonly employed in traditional system of medicine as a remedy for DM. Based on the traditional and scientific use of the herbs *Enicostemma hyssopifolium*, *Gymnema sylvestre*, *Tinospora cordifolia* and *Eclipta laba* were selected for the study. They have subjected for review of literature and scientific evidences which are reported in next chapter. The composition of selected formulation is given below.

Formulation	Content	
Formulation 1 (F-1)	*Gymnema sylvestre*----------	150mg
	Pterocarpus marsupium------	100 mg
	Osimum bassilicum----------	50 mg
	Momordica charantia--------	75 mg
	Azadirachta indica----------	25 mg
	Vinca rosea----------	25 mg
	Salacia chinensis----------	25 mg
	Aegle marmelose----------	25 mg
	Trigonella foenum graecum-	25 mg
	Syzigium cumini----------	25 mg
Formulation 2 (F-2)	*Pterocarpus marsupium*------	360 mg
	Enicostemma littorale--------	150 mg
	Eugenia jumbolana----------	150 mg
	Tinospora cordifolia----------	200 mg
	Pramehara Kvatha----------	40 mg
	Chandraprabha----------	40 mg
Formulation 3 (F-3)	*Curcuma longa*----------	100 mg
	Eugenia jumbolana----------	50 mg
	Swertia chirata----------	50 mg
	Shilajit----------	25 mg
	Trivanag bhasma----------	20 mg
	Cassia auriculata----------	100 mg
	Emblica officinalis----------	100 mg
	Enicostemma littorale--------	
	Gymnema sylvestre----------	
	Pterocarpus marsupium------	
	Tinospora cordifolia----------	
	Momordica charantia--------	
Formulation 4 (F-4)	Chandraprabha----------	120 mg
	Tinospora cordifolia----------	120 mg
	Curcuma longa----------	20 mg
	Kanya lauhadi----------	40 mg
	Medicated with the juice of Bilvapatra and karavellak	100 mg