Chapter I
Introduction and Review of Literature
1. **Introduction**

Hyperglycemia in patients with diabetes mellitus is always the result of a mismatch between the quantity of insulin necessary to regulate the person's metabolic processes and the amount of insulin being secreted by the person's β-cells. There is decreased or unregulated insulin secretion in type II diabetic patients. Effective glycemic control in diabetic patients requires appropriate regulation of both fasting and postprandial plasma glucose levels. Various drugs, each with special mechanism are used in the treatment of diabetes mellitus. The major therapeutic approaches such as insulin, insulin secretagogues, alpha-glucosidase inhibitors, insulin sensitizers etc. are used to control diabetes mellitus. Though several approaches are available in treatment of diabetes mellitus, the use of insulin or insulin secretagogues has no alternatives till date. The importance of these two is because no other therapy is able to combat the intrinsic need of insulin. While insulin is considered as the best therapy, being an injectable and control of dosage is tricky; insulin secretagogues are preferred for the ease of administration and are more cost effective. Sulfonylureas, a major class of insulin secretagogues, have been used in the treatment of type 2 diabetes mellitus for over 50 years. The sulfonylureas are popular as they are used orally, show rapid action and are cost effective. Though the sulfonylureas and other synthetic secretagogues are used extensively, they are associated with number of undesirable side effects. The major side effects of sulfonylureas are desensitization due to prolonged use, chance of hypoglycemic shock, problem of weight gain and coronary artery disease. Also long-term treatment with sulfonylureas over-stimulates β-cells, resulting in negative consequences such as apoptosis of β-cells and reduction in their mass. Given the possible deleterious effect of some sulfonylureas, alternatives to these as well as alternative insulin secretagogues may have to be considered.

Due to the side effects of synthetic remedies, there is growing interest in herbal remedies in recent years. The present study deals with evaluation of plants traditionally used for their antidiabetic and insulin secretagogue potential. It is essential to be able to isolate and identify plant components possessing such action. The insulin secretagogues are predominantly known to show their action through closure of K⁺ ATP channels or through opening of Ca²⁺ channels on the insulin secreting beta cells, or through
intracellular signaling leading to a similar effect. Evaluation of an insulin secretagogue for its precise mechanism is also very essential. Moreover, secretagogues are able to stimulate insulin secretion in a glucose independent or glucose dependent manner, the latter being a preferred mechanism as it can avoid hypoglycemic shocks.

The traditional Ayurvedic therapy suggests several plants in treatment of diabetes. It is suggested that these plants show a more holistic way of treatment with minimal side effects seen in synthetic drugs. One of the reasons is the presence of agonists or antagonists in the same herbal preparation that can augment or at times prevent the deleterious effects of the drug. The present study also delves into an enquiry of such mechanisms that can potentiate or minimize the hazardous side effects of insulin secretagogues. Such components that can prevent overstimulation of the beta cells of the pancreas eventually leading to their apoptosis, would be very desirable in long term treatment of diabetes.
2. **Review of literature**

Diabetes mellitus is a heterogenous group of metabolic disorders characterized by chronic hyperglycemia. Diabetes mellitus is defined as a metabolic disorder characterized by hyperglycemia with impaired metabolism of carbohydrate, fat and proteins as a result of defects in insulin secretion, insulin action or both (WHO 1999).

It has been centuries since this syndrome was first recognized. The two Indian physicians, “Charaka and Susruta” described the disease in 600 B.C. During the 18th and 19th centuries, a less clinically symptomatic variety of the disorder, identified by heavy glycosuria, often detected in later life and commonly associated with overweight rather than wasting, was noted, which today we recognize as type 2 diabetes mellitus. In the mid-1930s, Himsworth proposed that there were at least two clinical types of diabetes mellitus as insulin sensitive and insulin insensitive. The widespread acceptance of the terms “juvenile-onset diabetes mellitus” (today we recognize as type 1) and “maturity-onset diabetes mellitus (today we recognize as type 2)” at this time affirmed the concept that there were at least two major forms of the disease.

**Modern classification systems for diabetes mellitus**

Although there have been a number of sets of nomenclature, classification systems, and diagnostic criteria proposed for diabetes mellitus, no systematic categorization existed until the late 1970s. In 1979, the National Diabetes Data Group (NDDG) of the National Institute of Health, USA, proposed a classification for diabetes mellitus and other categories of glucose intolerance. The World Health Organization (WHO) Expert Committee on Diabetes in 1980 endorsed the substantive recommendations of the NDDG (WHO 1980). These groups distinguished two major forms of diabetes mellitus in Western countries, which they termed insulin-dependent diabetes mellitus (type 1 diabetes mellitus) and non-insulin-dependent diabetes mellitus (type 2 diabetes mellitus). The older terms juvenile-onset, maturity-onset, and adult-onset diabetes mellitus were recommended to be abolished.
The American Diabetes Association Classification System

In 1996 and 1997, an expert committee of the American Diabetes Association considered the research findings of the last 20 years and proposed some changes to the NDDG/WHO classification scheme (American Diabetes Association Expert Committee 2003). The main features of the changes in the classification are:

- Elimination of the terms insulin-dependent diabetes mellitus and non-insulin-dependent diabetes mellitus and their acronyms, IDDM and NIDDM. However, the terms type 1 and type 2 diabetes mellitus were proposed to be retained.
- Inclusion under type 1 diabetes mellitus of forms of diabetes mellitus involving pancreatic β-cell destruction, including those cases due to an autoimmune cause and those cases in which an etiology is not known.
- More precise definition under type 2 diabetes mellitus of the form of diabetes mellitus that is the most prevalent in the United States and is due to insulin resistance with insulin secretory defects.

2.1 Major types of diabetes:

i) Type 1 diabetes mellitus

The type 1 diabetes mellitus is caused by β-cell destruction. The major reason for type 1 diabetes mellitus is the autoimmune destruction of the pancreatic β-cells that leads to loss of insulin secretion and absolute insulin deficiency. The etiologic agents that cause the autoimmune process and β-cell destruction are not well established. It also includes cases in which causes of the β-cell destruction are not understood. This group comprises of approximately 5% to 10% of cases in the diabetes syndrome.

ii) Type 2 diabetes mellitus

The type 2 diabetes mellitus is caused by a combination of genetic and nongenetic factors that result in insulin resistance and insulin deficiency. Nongenetic factors include increasing age, high caloric intake, overweight, central adiposity, sedentary lifestyle, and low birth weight. This group comprises of approximately 90% to 95% of cases in the diabetes syndrome. The type 2 diabetes is discussed in more details further.
iii) Gestational diabetes mellitus

The gestational diabetes mellitus is caused by insulin resistance and relative insulin
deficiency associated with pregnancy. This occurs in approximately 3% to 5% of all
pregnancies.

iv) Other specific types of diabetes mellitus

These types comprise a heterogeneous etiologic group that includes those cases
of diabetes in which the causes are established or at least partially known. The causes
include known genetic defects affecting β-cell function or insulin action, diseases of the
exocrine pancreas, endocrinopathies, drug or chemical-induced pancreatic changes, and
diseases and conditions in which the incidence of diabetes is substantially elevated but a
precise etiology has not been established. This diverse group comprises of approximately
1% to 2% of cases in the diabetes syndrome.

Prevalence of diabetes

The prevalence of both type 1 and type 2 diabetes is increasing. Type 2 diabetes
is increasing far more rapidly, driven by increasing life expectancy and the epidemic of
obesity. It is believed that there will be as many as 300 million people with diabetes
worldwide by the year 2025. Most of this increase is predicted to occur in developing
countries.

Among the type 1 and type 2 diabetes mellitus, the type 2 comprises
approximately 90% of the diabetics and is the major type increasing at an alarming rate,
hence it needs to be understood in more details.

2.2 Type 2 diabetes mellitus

Type 2 diabetes mellitus may be unrecognized for years because of lack of
symptoms. Eventually, defects in insulin secretion leading to decompensate
hyperglycemia precipitate clinical onset of the disease. Type 2 diabetes mellitus is
characterized by insulin resistance in muscle, liver, and adipose tissue that probably
begins at a preclinical stage (possibly at the stage of impaired glucose tolerance). In
contrast to type 1 diabetes mellitus, patients with type 2 diabetes mellitus do not depend
on exogenous insulin for prevention of ketonuria and are not prone to ketosis. However, they may require insulin for correction of fasting hyperglycemia if this cannot be achieved with the control of diet or oral agents, ketosis may develop under special circumstances such as severe stress precipitated by infections or trauma.

Chronic hyperglycemia and development of diabetes-specific microvascular complications in the retina, renal glomerulus, and peripheral nerve are characteristic of all forms of diabetes. As a consequence of its microvascular pathology, diabetes is the leading cause of blindness, end-stage renal disease, and a variety of debilitating neuropathies.

Although the etiology of type 2 diabetes mellitus is unclear, the disease has a strong genetic basis as evidenced by the frequent familial pattern of occurrence, its high prevalence in certain ethnic groups, and genetic studies. (Harris 1995). Although type 2 diabetes mellitus is strongly associated with genetic factors, it is undoubtedly heterogeneous in its etiology because a variety of lifestyle and environmental factors have been identified as being risk factors for the condition (Haffner 1998). In all probability, the causes of type 2 diabetes mellitus lie in environmental and lifestyle factors superimposed on genetic susceptibility. Prominent among these factors is obesity, and approximately 50% to 90% of all patients with type 2 diabetes mellitus are obese (Harris 1995). A strong association between upper body obesity (central obesity) and type 2 diabetes mellitus prevalence and incidence has been demonstrated. Intra-abdominal fat deposition is the important sign conveying enhanced risk for type 2 diabetes mellitus (Bergstrom 1990). Other risk factors include increasing age, high caloric intake, sedentary lifestyle, and low birth weight.

Type 2 diabetes is characterized by hyperglycemia initially due to insulin resistance. However, slowly the production of insulin deteriorates leading to establishment of hyperglycemia. All further complications in diabetes are linked to the proper production and utilization of the insulin.
3. **Insulin, islets of Langerhans and regulation of insulin secretion**

3.1 **Insulin molecule**

The biologically active human insulin consists of two polypeptide chains, the A chain (21 amino acids) and B chain (30 amino acids), joined by two interchain disulfide-linked bridges at A-Cys\(^7\)/B-Cys\(^7\) and A-Cys\(^20\)/B-Cys\(^19\). There is also an intrachain disulfide bridge between A-Cys\(^6\)/A-Cys\(^11\). Insulin structure is highly conserved in higher vertebrate evolution showing several regions of invariability, including (a) the position of cysteines that form the disulfide bridges, (b) the N- and C-terminal regions of the A chain, and (c) hydrophobic residues at the C-terminus of the B chain. At an acidic pH in the presence of Zn\(^{2+}\), insulin forms a hexameric crystal unit, which consists of three insulin dimers arranged around an axis of two Zn\(^{2+}\) atoms interacting with B-His\(^{10}\) of the insulin molecules (Chothia *et al.* 1983). The association of Zn\(^{2+}\) with insulin was exploited in a technique for identification of insulin producing β-cells by using dithiozone. Diathiozone is a zinc chelating agent which rapidly and reversibly stains the islets into red on incubation with the dye. (Hansen *et al.* 1989).

![Insulin structure](http://www.uic.edu)

Figure 1: Insulin structure (Source of image- http://www.uic.edu)

The insulin molecules are synthesized, stored and secreted from a specialized cell known as ‘β-cells’ which are present in the islet of Langerhans of pancreas. The structural architect of Islets of Langerhans is discussed below.
3.2 Islets of Langerhans

The islets of Langerhans are clusters of endocrine tissue scattered throughout the exocrine pancreas. In adult mammals, the islets are 1% to 2% of the pancreatic mass. The single islet is a complex structure of cells. The general arrangement of major cell types in β-cell is shown in Figure 2.

![Pancreas](image)

Figure 2. The general endocrine system of the pancreas (Figure reference: Nelson and Cox, Lehninger Principles of Biochemistry, 4th edition).

Islets contain several different cell types including endocrine cells, endothelial cells, nerves, and fibroblasts. An incompletely defined and variable capsule encloses an islet and partially separates endocrine cells from exocrine cells. Pancreatic islets are highly vascularized mini organs that receive a disproportionately larger fraction of pancreatic blood flow (up to 5–10% of total pancreatic blood flow) than the exocrine portion of the pancreas. The average rat islet is 150 µm in diameter. Pancreatic islets were classically described to contain four different cell types: insulin-producing beta-cells (70 to 80%), glucagon producing alpha-cells (15 to 20%), somatostatin producing delta-cells (5%), and pancreatic polypeptide-producing PP cells. Recently, a fifth islet cell type, ghrelin-producing cells, was discovered and termed epsilon cells (Prado et al. 2004). In both mouse and human, numerous epsilon cells are present in islets during
pancreas development and at birth. However, it appears that their population declines postnatally (Heller et al 2005).

The primary function of pancreatic β-cells is the production, storage, and regulated secretion of insulin. Under normal circumstances, the β-cell maintains a condition where there is always a readily available pool of insulin that can be rapidly secreted in response to a stimulus, such as an increase in blood glucose. Any increase in insulin release is compensated for by a corresponding increase in insulin biosynthesis, so that β-cell insulin stores are constantly maintained. Thus, a biosynthesis and processing of the insulin molecule along the β-cells secretary pathway is a highly regulated and dynamic process.

3.3 Regulation of insulin secretion in pancreatic β-cell

Insulin secretion from the β-cell is regulated by the extracellular glucose concentration and is modulated by factors such as gastrointestinal hormones, other nutrients, and neural inputs. The general mechanism for insulin secretion is shown in Figure 3.

The β-cell contains an intricate glucose-sensing mechanism that consists of glucose transporter 2 (GLUT-2), glucokinase, and glycolytic and mitochondrial pathways that oxidize glucose to convert adenosine diphosphate (ADP) to adenosine triphosphate (ATP). The intracellular β-cell message that reflects the extracellular glucose concentration is the ATP:ADP ratio. This message controls an ATP-sensitive potassium ion channel (K⁺-ATP) located in the β-cell plasma membrane. The K⁺-ATP channels are open when the extracellular glucose concentration is low (fasting state with a low ATP:ADP ratio) and transport K⁺ from the intracellular to the extracellular compartment. As extracellular glucose concentration increases, the ATP:ADP ratio increases and an increasing number of K⁺-ATP channels close. This causes K⁺ to accumulate at the plasma membrane, which then depolarizes, causing voltage-dependent L-type calcium channels in the plasma membrane to open. Extracellular calcium ions enter the β-cell and increase the cytosolic calcium ion concentration. Increasing cytosolic Ca²⁺ stimulates the movement and secretion of insulin granules (Henquin 2000).
Under physiological conditions, the concentration of the blood glucose fluctuates only in a narrow range despite alternations in periods of food intake and fasting. Also there are several hormones that can prevent dangerous decline in blood glucose concentrations by stimulating glycogenolysis and gluconeogenesis. Insulin secretion by the β-cells of islets of Langerhans is the only efficient means by which the individual can decrease the blood glucose concentration. Any alteration in the β-cell functioning has a profound impact on the glucose homeostasis. The excessive secretion of insulin causes hypoglycemia while insufficient secretion leads to diabetes mellitus. Hence, the insulin secretion from the β-cells is subject to tight control. This control is ensured by glucose itself and by an array of metabolic, neural, hormonal and sometimes pharmacological agents.
The β-cells are equipped with specialized features which enables the tight regulation of their metabolism. The salient features of the β-cells and their role in metabolism is discussed in detail.

**Glucose transporter:** Glucose transport through specialized glucose transporters is very important step in glucose dependent insulin secretion. There are two broad categories of glucose transporters: Na\(^+\) dependent (SGLT) and Na\(^+\) independent (GLUT). There are six functional GLUT isoforms, of which five transport primarily D-glucose: GLUT-1, GLUT-2, GLUT-3, GLUT-4, and GLUT-7. All GLUTs have considerable homology but vary in their affinity for glucose. GLUT-1 is the most widely distributed, serving many cell types alone or in company with another isoform. In rodent β-cells, glucose entry is mediated by high capacity, low-affinity mM (K\(_m\) ~ 17 mM) glucose transporter GLUT-2 (Newgard and McGarry, 1995). The rate of glucose transport exceeds the rate of glucose utilization and is, therefore not limiting (De Vos et al., 1995). In human β-cells, GLUT-2 is less extensively expressed and GLUT-1 predominates (De Vos et al., 1995).

**Glucokinase:** After glucose has entered into β-cell, it is phosphorylated to glucose-6-phosphate by glucokinase (Hexokinase IV). The characteristics of glucokinase are (i) High K\(_m\) between 6 to 10 mM (ii) sigmoidal dependency on its substrate concentration and (iii) lack of inhibition by its product, glucose-6-phosphate. These characteristic features make it a suitable glucose sensor (Matschinsky, 1996) and this is the flux determining step of glycolysis. The activity of glucokinase is lower than that of other potentially regulatory enzymes of glycolysis and closely matches glucose usage (Iynedjian, 1993). Also mutations of the glucokinase gene result in patients with maturity-onset of the diabetes of the young (MODY-2) (Porter and Barrett, 2005) or hyperactive isoform in glucokinase linked hyperinsulinemia and hypoglycemia (Glaser et al., 1998).

The phosphorylated, glucose is metabolized by glycolysis to produce pyruvate, NADH and ATP. Both cytosolic pyruvate and NADH enter the mitochondria and are metabolized to produce ATP. The ATP produced is transported into the cytosol, increasing cytosolic ATP concentration and the ATP/ADP ratio (Detimary, 1996). The increased ATP/ADP ratio closes the K\(^+\)-ATP channels, which causes depolarization of plasma membrane, causing voltage-dependent L-type calcium channels in the plasma membrane to open. Extracellular calcium ions enter the β-cell and increase the cytosolic...
calcium ion concentration. Increasing cytosolic Ca$^{2+}$ stimulates the movement and secretion of insulin granules (Henquin 2000). The detailed structure of K$^+$-ATP channel and voltage-dependent L-type calcium channel is discussed later in detail.

In the absence of stimulatory glucose (generally less than 5 mM), rodent β-cells are electrically silent, with a resting membrane potential of approximately -70 mV due to a high resting K$^+$ conductance in these cells. Reduction of the resting K$^+$ conductance by stimulatory glucose leads to membrane depolarization. ATP-sensitive K$^+$ (K$^+$-ATP) channels set the β-cell membrane potential and closure of these leads to depolarization. Membrane depolarization triggers opening of voltage-dependent Ca$^{2+}$ channels (VDCCs), leading to Ca$^{2+}$ influx which triggers exocytosis. The membrane depolarization was diminished by the opening of voltage-dependent K$^+$ channels which limit Ca$^{2+}$ entry and thus prevents insulin release.

### 3.4 Biphasic insulin secretion

Over all five different modes (or phases) of insulin secretion can be identified as below (Caumo and Luzi 2004).

1) Basal phase insulin secretion - Basal insulin secretion is the way insulin is released in the post absorptive state

2) Cephalic phase insulin secretion - The cephalic phase of insulin secretion is evoked by the sight, smell, and taste of food (before any nutrient is absorbed by the gut) and is mediated by pancreatic innervations

3) First-phase insulin secretion - The first-phase insulin secretion is defined as the initial burst of insulin, which is released in the first 5–10 min after the β-cell is exposed to a rapid increase in glucose (or other secretagogues)

4) Second-phase insulin secretion - After the acute response, there is a second-phase insulin secretion, which rises more gradually and is directly related to the degree and duration of the stimulus

5) finally, a third phase of insulin secretion has been described, albeit only in vitro (Grodsky 1972).
The biphasic insulin release consisting of first phase and second phase by the islet β-cell upon glucose stimulation has long been recognized and accepted (Figure 4). After a change in glucose from a basal concentration of 2.8 mM to the stimulatory 16.7 mM, there is a short delay prior to the onset of the first phase of secretion. The rate of insulin secretion increases to a peak over the next 4 minutes and then declines over a further 4 minutes to a nadir that marks the end of the first phase. The second phase arising from the nadir is characterized by a steadily increasing rate until it reaches the plateau.

![Insulin concentration profiles](image)

**Figure 4.** Differences between the incremental (above basal) plasma insulin concentration profiles in response either to an intravenous glucose infusion producing a square wave of hyperglycemia (A) or to a meal (B). The response to the intravenous glucose challenge shows a biphasic profile, with distinct 1st (0–10 min) and 2nd phase (10–30 min) insulin-secretory responses. (Figure reference: Caumo and Luzi 2004).

While more information is available concerning the mechanisms and control of the first phase than that of the second phase, our knowledge of both is still rudimentary. Because the magnitude of the first phase response is reduced in people with type 2 diabetes, and can be reduced in people prior to the development of overt type 1 diabetes (Gerich 2002), the importance of understanding the biphasicity is obvious.
3.5 Insulin granule dynamics and exocytosis

The insulin molecules are stored in the β-cell’s secretory granule compartment in the resting state; it is not released from the β-cell unless there is a specific signal that stimulates exocytosis. The amount of insulin present in the endocrine pancreas is huge relative to the needs of the body at any particular time, and following a meal, only a small fraction of the granules in the pancreas will be released. Typically, only a small percentage of the granules, and therefore of the total insulin content of the β-cell, is secreted in response to a glucose stimulus. Insulin granules exist within the cell in various functional pools (Rorsman and Renstrom 2003). Shown in Figure 5, these include an intracellular reserve pool (90%), a morphologically docked pool (10%), and a readily releasable pool (RRP) that is chemically ‘primed’ for release (0.3–2.2%; Olofsson et al. 2002)

![Figure 5. Various pools of insulin granules and stimulus-secretion coupling in the pancreatic β-cell (Figure Reference: MacDonald et al., 2005).](image)

The size of the readily releasable pool (RRP) is a major determinant of the magnitude of the initial secretory response. While in the short-term, priming of granules
already docked at the membrane may account for refilling of the RRP, ultimately granules must be mobilized from intracellular reserve pools. The initial (or first phase) exocytotic response can be replicated by any stimuli that increases intracellular Ca\(^{2+}\), causing the release of already docked and primed vesicles. Sustained (or second phase) secretion on the other hand, which is dependent on vesicle mobilization and priming, can only be elicited by metabolizable fuel secretagogues (Gembal et al. 1992; Henquin 2000). Thus, glucose-derived signals are important for amplifying and maintaining secretion by promoting the mobilization and priming of insulin granules from reserve pools.

The insulin granules undergo sequential events like (a) Mobilization and docking at the plasma membrane (b) preparation for release (priming), and (c) exocytosis.

### 3.5.1 Mobilization of insulin granules

Insulin granules within the cell undergo extensive movement. There is a need to efficiently transport granules to the plasma membrane to replenish the ready releasable pool after an exocytotic event. Experimental evidence suggests that transport of β-granules to the plasma membrane is enhanced via a granule interaction with the β-cell cytoskeletal framework of microtubules and microfilaments. Studies demonstrated that second phase secretion results largely from the exocytosis of insulin granules newly recruited to the plasma membrane (Ohara-Imaizumi et al. 2004). In rat β-cells, glucose stimulates an increase in the number of insulin granules associated with the plasma membrane. Insulin granule movement can be stimulated by glucose or ATP. Agents that raise cAMP also increase insulin granule movement and PKC mobilizes catecholamine granules of chromaffin cells into the readily releasable pool (Gillis et al. 1996), although PKC may control secretion at a point between vesicle docking with the plasma membrane and exocytosis (Ammala et al. 1994). Within the cytoplasm vesicle movement likely occurs along microtubules, mediated by the ATP-dependent motor activity of the conventional kinesin KIF5B (Varadi et al. 2003). Inhibitors of 5’-AMP activated kinase (AMPK) enhance insulin secretion (Rutter et al. 2003) and a constitutively active AMPK mutant inhibited glucose stimulated granule movement (Tsuboi et al. 2003). AMPK is, therefore, suggested as a negative regulator of
granule recruitment and is inhibited by an increased ATP-to-ADP ratio (and reduced AMP). Thus, metabolically generated ATP (or reciprocal changes in ADP and AMP) may be a crucial factor in recruitment of insulin granules from intracellular reserve pools to the plasma membrane. There are conflicting reports as to the role of membrane depolarization and Ca\(^{2+}\) entry through VDCCs in regulating insulin granule traffic (Niwa et al. 1998).

3.5.2 Exocytosis of insulin granules

Fusion of an exocytotic vesicle with the plasma membrane is mediated by soluble NSF attachment protein receptor (SNARE) proteins. A docked insulin granule must first be primed for release. ATP is essential for granule priming prior to exocytosis (Eliasson et al. 1997; Bratanova-Tochkova et al. 2002).

Insulin secretagogues can be divided into two groups: the initiators and the potentiators. The former are capable of stimulating insulin secretion on their own and include nutrients, such as glucose, and drugs such as the sulfonylureas. All these substances act by inhibiting K\(^{+}\)-ATP channel activity but whereas the nutrients must be metabolised to effect channel closure, the drugs bind directly to the channel and block its activity. Potentiators of insulin secretion include a number of hormones [e.g., glucagon and glucagon- like peptide (GLP1)], transmitters (such as acetylcholine) and the amino acid arginine. These agents amplify or augment the insulin secretion induced by an initiator but cannot elicit insulin secretion by themselves because they do not close K\(^{+}\)-ATP channels and are only able to exert their effects after an initiator secretagogue has effected K\(^{+}\)-ATP channel inhibition. Inhibition of insulin release is produced by agents that open K\(^{+}\)-ATP channels such as the drug diazoxide. Thus, the K\(^{+}\)-ATP channel has a key role in the regulation of insulin secretion from the pancreatic beta cell.

4. The major pathways of β-cell stimulus-secretion coupling

4.1 K\(^{+}\)-ATP channel-dependent pathway. This is the triggering and most important pathway for insulin secretion. Increased concentrations of glucose and other nutrients cause depolarization via closure of the K\(^{+}\)-ATP channel, increased Ca\(^{2+}\) entry via voltage-dependent Ca\(^{2+}\) channels, increased [Ca\(^{2+}\)], and increased rates of exocytosis.
This is the most prominent way of insulin secretion. All the initiators such as glucose and drugs such as sulfonylurea stimulate the insulin secretion in this pathway. The sulfonylureas directly bind and closes the $K^+$-ATP channel while nutrients such as glucose must be metabolized to effect channel closer.

### 4.2 Increased concentrations of arginine

The amino acid arginine also stimulates the insulin secretion by means of depolarization of $\beta$-cell membrane. The depolarization in this case results from entry of the positively charged amino acid via CAT2A, a cationic amino acid transporter. Arginine is a potent potentiator of insulin release, stimulating secretion in the presence, but not absence of glucose (Smith et al., 1997).

### 4.3 $K^+$-ATP channel-independent $Ca^{2+}$-dependent pathway of glucose action

In 1992, the existence of a glucose signaling pathway that was “independent” of the $K^+$-ATP channel was reported (Gembal et al. 1992). This pathway does not increase $[Ca^{2+}]$, but strongly augments the secretory response when $[Ca^{2+}]_i$ is increased by other means. This pathway is usually described as the “$K^+$-ATP channel-independent” pathway and works in synergy with the $K^+$-ATP channel-dependent pathway (Aizawa et al. 1994). This pathway acts at a site distal to the elevation of $[Ca^{2+}]_i$. The mechanisms of action have not been defined, and several candidate mechanisms exist. Among these are a glucose-induced increase in the concentration of malonyl CoA, inhibition of carnitine palmitoyl transferase I, decreased fatty acid oxidation, and an increase in cytosolic long-chain fatty acids. The long-chain fatty acids have the potential to act as signal moieties (Deeney et al 2000) e.g., to activate PKC isoforms that can stimulate exocytosis or to act via palmitoylation or other acylation reactions.

The $[Ca^{2+}]_i$ requirement for both $K^+$-ATP channel-dependent and $K^+$-ATP channel-independent pathways supports with the long-held conviction that an elevation of $[Ca^{2+}]_i$ is critical to the stimulation of insulin secretion.
4.4 K\textsuperscript{+}-ATP channel-independent Ca\textsuperscript{2+}-independent pathway of glucose action

In latter studies, it was observed that glucose could augment insulin secretion in Ca\textsuperscript{2+}-depleted rat islets in the complete absence of extracellular Ca\textsuperscript{2+} and in the absence of any rise in [Ca\textsuperscript{2+}], (Komatsu et al 1995). This glucose augmentation effect was observed to operate best when β-cell protein kinase (PK) A and C are simultaneously maximally activated. Under these conditions, glucose augmentation can be demonstrated at very low [Ca\textsuperscript{2+}], (Komatsu et al. 1997). A physiological role for this novel pathway is possible because the combination of pituitary adenylyl cyclase activating peptide (PACAP) and carbachol, which activate PKA and PKC, respectively, promotes glucose augmentation of insulin release in the absence of any rise in [Ca\textsuperscript{2+}], in rat islets (Komatsu et al. 1997). The pancreatic islet in vivo is stimulated not by glucose alone or single agonists, but concurrently by multiple agonists, including amino acids, fatty acids, acetylcholine, PACAP, glucose-dependent insulinotropic polypeptide (GIP), glucagon-like peptide 1 (GLP-1), and several other agonists. Therefore, this pathway may have a
physiological role in the control of insulin secretion. The pathway can be described as “K⁺-ATP channel-independent, Ca²⁺-independent”. The concept of a G protein (Ge) controlling exocytosis, first postulated in 1986 and is well developed (Gomperts 1990), despite the fact that Ge has yet to be identified. Mastoparan, a tetradecapeptide purified from wasp venom with the ability to activate G proteins, stimulates insulin release in a K⁺-ATP channel-independent and Ca²⁺-independent manner and this stimulation is also augmented by glucose (Straub et al 1998).

4.5 Activation of phospholipases and PKC. These pathways are activated by hormones such as acetylcholine. Increased phosphoinositide turnover results in mobilization of stored calcium to increase [Ca²⁺], and increased production of diacylglycerol (DAG), which activates PKC isoforms. This pathway has important enhancing effects on stimulated release (Prentki 1987).

4.6 Stimulation of adenylyl cyclase activity and activation of PKA. These pathways are activated by hormones such as vasoactive intestinal peptide (VIP), PACAP, GLP-1, and GIP. These hormones, acting via Gs, stimulate adenylyl cyclase and cause a rise in cyclic AMP and activation of PKA. The increased activity of PKA potentiates insulin secretion (Sharp 1979). It should be noted, however, that there might be additional signaling pathways for agonists that activate Gs, as has been shown for VIP, PACAP, and GIP (Straub 1996) (A general scheme is shown in Figure 5).

5. Functional role of the K⁺-ATP channel in the pancreatic beta cell

In the pancreatic β-cell, the ATP-sensitive K⁺ channel (K⁺-ATP channel) plays an essential role in coupling membrane excitability with glucose-stimulated insulin secretion (GSIS) (Ashcroft and Rorsman 1990). K⁺-ATP channels are not only found in pancreatic beta-cells. They were first described in guinea pig cardiac muscle (Noma 1983) and then in beta cells (Cook 1984) and subsequently have been found in a wide variety of other tissues, including smooth and skeletal muscle, brain neurones, peripheral axons and epithelial cells. K⁺-ATP channels are membrane-spanning proteins that selectively conduct K⁺ ions across the cell membrane along its electrochemical gradient.
(Shieh 2000) and maintain the resting β-cell membrane potential of approximately −70 mV (Ashcroft 2000). The K⁺-ATP channel is formed by the unique combination of two types of subunit: an inwardly rectifying potassium channel subunit (Kir6.x) and a regulatory subunit called as the sulphonylurea receptor (SUR).

Two different Kir6.x genes have been described, Kir6.1 and Kir6.2. Likewise, two genes encodes for sulphonylurea receptors as SUR1 and SUR2. Further diversity is created by alternative splicing of SUR2 into SUR2A and SUR2B. The different combinations of Kir and SUR subunits account for the diverse properties of K⁺-ATP channels in different tissues. Kir6.2 is strongly expressed in beta cells, heart, brain and skeletal muscle. SUR1 serves as the regulatory subunit of the K⁺-ATP channel in beta cells and some types of neurones, SUR2A in cardiac and skeletal muscle and SUR2B in smooth muscle (Ashcroft and Gribble 1999).

The K⁺-ATP channel in the β-cell consists of four Kir6.2 subunits form the pore, and they are surrounded by four SUR1 subunits (Nichols 2006). The structure of the channel was shown in Figure 7. Kir6.2 is a member of the inwardly rectifying K⁺-channel family and has two transmembrane domains (TMs), linked by a pore loop and cytosolic amino and carboxy termini. The sulphonylurea receptors belong to the ABC-transporter superfamily. These proteins are characterised by multiple transmembrane domains and two intracellular nucleotide-binding domains (NBDs) which contain consensus sequences for nucleotide binding and hydrolysis. SUR is thought to possess 17 TMs arranged in three groups of 5 + 6 + 6 (Aguilar-Bryan and Bryan 1999). In contrast to most other members of the Kir channel family, Kir6.2 does not form functional channels in the absence of the sulphonylurea receptor. Hence both the subunits are necessary for function channel (Sakura et al 1995).
The ATP molecule (with or without Mg\(^{2+}\)) inhibits the K\(^+\)-ATP channels by directly binding to an intracellular site present in the proximal C-terminus of Kir6.2 (Tucker et al. 1997, Antcliff et al. 2005). It was observed that ATP can inhibit K\(^+\)-ATP channels at the Kir6.2 subunit in the absence of SUR1 (Tucker et al. 1997) and point mutations within Kir6.2 can attenuate the effect of ATP (Tucker et al. 1997; Drain et al. 1998). The SUR1 subunit also binds ATP (both with and without Mg\(^{2+}\)) and MgADP via the presence of two cytoplasmic nucleotide binding domains (NBDs) (Ashcroft 2000). It seems that both NBDs are involved because mutation of a single NBD is sufficient to abolish the stimulatory effect of MgADP. This is consistent with functional studies of bacterial and eukaryotic ABC transporters (Senior 1997). Nucleotide activation is dependent on the presence of Mg\(^{2+}\), does not discriminate between adenine, guanine or uridine nucleotides and MgADP is a more effective agonist than MgATP (Gribble et al. 1998; Shyng et al. 1997). In all these respects, the nucleotide-binding site of SUR differs from the ATP-binding site on Kir6.2. Binding of Mg-ADP to SUR1 is thought to stabilize the channel in the open configuration, and when the ATP:ADP ratio increases, Mg-ADP is displaced, and the Kir6.2 subunit becomes available for block by ATP.
Chapter I

Introduction & Review of Literature

(Ashcroft 2000) which leads to a insulin secretion as discussed earlier. Hence, overall the sulphonylurea receptor plays very important role in metabolic regulation of the K⁺-ATP channel. Mutations in the NBDs that abolish the stimulatory effects of Mg-nucleotides also prevent K⁺-ATP channel activation by metabolic inhibition in intact cells (Nichols 1996).

K⁺-ATP channels can also be activated (opened) by phosphatidylinositol phosphates, such as phosphatidylinositol (4, 5)-bisphosphate and long-chain fatty acyl CoA (LC-CoA). The binding site for phosphatidylinositol (4, 5)-bisphosphate is located on the C-terminus of Kir6.2 and overlaps with that for ATP. Accordingly, phosphatidylinositol (4, 5)-bisphosphate antagonises the inhibitory effect of ATP on Kir6.2 and stabilises the open state of the channel (Nichols 2006, Ribalet et al. 2005) and hence interfere with insulin secretion. The long-chain fatty acyl CoA also stimulates K⁺-ATP channel activity by reducing the sensitivity of the channel to ATP (Gribble et al. 1998, Branstrom et al. 1998). Type 2 diabetes is often accompanied by elevated levels of free fatty acids and long-chain fatty acyl CoA within the pancreatic β-cell. Indeed, mutations of Kir6.2 known to be associated with the onset of type 2 diabetes have been shown to hypersensitise channels to activation by LC-CoA (Riedel et al. 2003).

SUR1 also contains distinct binding sites for the potassium channel opener drug diazoxide, and the inhibitory sulphonylurea drugs tolbutamide and glibenclamide (Babenko et al 2000; Proks et al. 2002). Both types of drug are currently in clinically use: diazoxide is used to suppress insulin secretion in patients with congenital hyperinsulinism whereas the sulphonylurea drugs are used to stimulate insulin secretion in patients with type 2 diabetes.

5.1 K⁺-ATP channel openers

There are some drugs and chemicals which inhibits the secretion of insulin from islets. One of such drug is diazoxide. Diazoxide inhibits insulin secretion by opening K⁺-ATP channels (Henquin et al 1982). The diazoxides directly interacts with SUR1, keeps open the K⁺-ATP channels which leads to repolarization, closing of voltage-dependent Ca²⁺ channels (VDCCs), diminishes Ca²⁺ influx and ultimately inhibits the insulin
secretion (Gilon and Henquin 1992). Diazoxide thus counteract the generation of the triggering signal by glucose and other secretagogues that closes the K⁺-ATP channel. The risk that they inhibit insulin secretion is small because the selected drugs have a higher affinity for SUR 2A (the smooth muscle isoform) than the SUR 1 (the β-cell isoform).

6. Voltage-dependent calcium channels

The ability of pancreatic β-cells to respond to glucose has long been known to depend on extracellular Ca²⁺ (Hales and Milner 1968). Depolarization of insulinoma and rat pancreatic β-cells leads to opening of L-type VDCCs (Horvath et al. 1998). A role for other VDCCs in insulin secreting cells (P/Q-type, N-type and T-type) is controversial, although R-type channel have a recently demonstrated role in later stages (or second phase) of insulin secretion (Jing et al. 2005).

It has been generally accepted that [Ca²⁺], regulates both first and second phase insulin secretion and that L-, P/Q-, N- and R-type calcium channels are involved (Komatsu et al. 1989). The generation of various knockout mice helped to differentiate the roles of the different calcium channels in insulin secretion. Among the four genes that encode L-type calcium channels, CaV1.2 and CaV1.3 have been identified in β-cells of different species (Yang et al. 2005). In mice lacking the CaV1.3 subunit, neither the blood sugar level nor insulin secretion was affected (Platzer et al. 2000). Instead, deletion of CaV1.2 decreased the whole-cell calcium inward current (ICa) to the same extent as the calcium channel blocker isradipine, and almost abolished first phase of insulin secretion. Studies on CaV2.3−/− islets and mice suggested that CaV2.3 is responsible for the second phase of insulin secretion (Jing et al. 2005).

Release of intracellular Ca²⁺ stores is also thought to be involved in regulating insulin secretion. Intracellular Ca²⁺ stores may be released by the influx of extracellular Ca²⁺, called Ca²⁺-induced Ca²⁺ release (Graves and Hinkle 2003), or by other external signals in order to enhance or prolong insulin secretion rather than trigger it directly. Acetylcholine and cholecystokinin, for example, signal through inositol trisphosphate (IP3) to release internal Ca²⁺ stores and activate protein kinase C (PKC) (Lang 1999), thereby increasing insulin secretion.
6.1 Ca\textsuperscript{2+}-channel openers and blockers

Blockage of voltage-dependent Ca\textsuperscript{2+} channels by nifedipine and verapamil inhibits Ca\textsuperscript{2+} influx in β-cells (Ashcroft and Rorsman 1989) and therefore, antagonizes the ability of glucose and other depolarizing agents to increase [Ca\textsuperscript{2+}]. Hence, these drugs non-selectively inhibit the secretion of insulin induced by those agents whose effect depends on Ca\textsuperscript{2+} influx, regardless of the mechanism of depolarization. In the \textit{in vivo}, Ca\textsuperscript{2+}-channel blockers have no or little deleterious effect on insulin secretion and glucose homeostasis (Trost and Weidmann 1987).

Antagonism of L-type channels is sufficient to inhibit insulin secretion in humans, rodents, dogs, and from insulinoma cells (Giugliano \textit{et al.} 1980; Ohneda \textit{et al.} 1983; Kanatsuna \textit{et al.} 1985; Ohta \textit{et al.} 1993). Therefore, L-type Ca\textsuperscript{2+} channels are considered the effectors of insulin secretion.

7. Therapies used in diabetes mellitus

Hyperglycemia in patients with diabetes mellitus (DM) is always the result of a mismatch between the quantity of insulin necessary to regulate the person's metabolic processes and the amount of insulin being secreted by the person's β-cells. Effective glycemic control in diabetic patients requires appropriate regulation of both fasting and postprandial plasma glucose levels. Various drugs, each with special mechanism are used in the treatment of diabetes mellitus. The different major classes of antihyperglycemic agents in current use are as below:

7.1 Insulin therapy

Insulin therapy is a successful treatment strategy for patients with type 2 diabetes unable to achieve glycemic control through diet, exercise, and oral antidiabetic agents. The insulin is used alone or in combination with other oral antidiabetic drugs. The insulin therapy is associated with disadvantages such as weight gain, insulin resistance etc. The lowering of blood glucose concentrations to normal usually requires large doses of exogenous insulin, resulting in hyperinsulinemia with weight gain (Genuth 1990). Severe hypoglycemia (Casparie 1985) and insulin resistance in case of prolonged use.
7.2 Alpha-glucosidase inhibitors

Alpha-glucosidase inhibitors inhibit alpha-glucosidase enzymes such as maltase and sucrase in intestine consequently reducing postprandial hyperglycemia by delaying the absorption of carbohydrate from the small intestine. These agents reduce blood glucose without increasing insulin secretion and do not cause hypoglycemia or weight gain. In addition, treatment with an alpha-glucosidase inhibitor can improve lipid metabolism, reduce fasting plasma glucose levels, and improve insulin sensitivity in a non-invasive manner.

Alpha-glucosidase inhibitors (AGIs) act by inhibiting an enzyme on the enterocyte brush border that breaks down complex starches, delaying intestinal absorption of carbohydrate and particularly attenuating postprandial blood glucose elevations. Current members of this drug class include acarbose and miglitol. AGIs are approved for use as monotherapy and in combination with sulfonylureas and metformin. Side effects include abdominal bloating and cramping, frequently leading to cessation of drug use.

The adverse events regarding the alpha-glucosidase inhibitors are dose dependent, and are generally confined to the gastrointestinal system; they include flatulence, bloating, diarrhea, soft stools etc. All these side effects most likely to occur if initial doses are used that block all proximal alpha-glucosidase activity (Laar et al. 2005).

7.3 Insulin sensitizers

Insulin sensitizers improves the glycemic control by promoting insulin action. Rather than increasing the levels of circulating insulin, insulin sensitizers act to increase insulin sensitivity in key organs such as muscle and liver. The major class of insulin sensitizers are biguanides and thiazolidinediones (TZD’S)

7.3.1 Biguanides

Metformin, a biguanide, acts mainly by decreasing hepatic glucose production (Inzucchi et al 1998, Hundal et al 2000). The major target is inhibition of primarily
gluconeogenesis and probably through effects on AMP kinase. Circulating glucose levels are thereby reduced. Improved peripheral insulin resistance may also occur (Inzucchi et al 1998, Yu et al 1999). Other nonglycemic benefits have been reported, including modest lowering of lipid levels (DeFronzo and Goodman 1995). Metformin is the only oral antihyperglycemic agent shown to reduce macrovascular events in patients with type 2 diabetes. Gastrointestinal side effects of metformin are common. Because of the rare risk of lactic acidosis, several contraindications limit this drug’s use, including renal and liver dysfunction, heart failure, dehydration or hemodynamic compromise, and alcohol abuse. Several studies have described a surprising proportion of metformin-treated patients with active contraindications for its use (Masoudi 2003; Calabrese 2002).

7.3.2 Thiazolidinediones (TZD’s)

Thiazolidinediones act primarily on the peroxisome proliferator activated receptor-γ. They are used to treat insulin resistance; there is an indication of improvement of β-cell function and consequently insulin secretion following treatment with this class of drugs. Most notably, TZDs improve insulin sensitivity and enhance glucose utilization by adipocytes and skeletal muscle. PPAR-γ is most highly expressed in fat cells, and TZD therapy is associated with prominent effects on circulating fat derived factors that influence insulin sensitivity, such as free fatty acids, adiponectin, and tumor necrosis factor-α. Side effects of TZD’s include weight gain and edema, which have precluded their widespread use for patients with heart failure (Kimmel and Inzucchi 2005). A consensus statement from the American Diabetes Association and the American Heart Association addressed this issue and endorsed the FDA’s current recommendation that the drugs should not be used in patients with advanced heart failure symptoms (class III or IV New York Heart Association classification).

7.4 Glycogen phosphorylase inhibitors

Glycogen phosphorylase catalyzes the breakdown of glycogen to glucose-1-phosphate in liver and tissues with high and fluctuating energy demands. Inhibition of hepatic glycogen phosphorylase is a promising treatment strategy for attenuating hyperglycemia in type 2 diabetes. Furthermore, glycogen phosphorylase activity is
critical for normal skeletal muscle function, and thus fatigue may represent a major development hurdle for this therapeutic strategy (Baker et al. 2005).

Figure 8. Pharmacological approaches to the major metabolic defects of type 2 diabetes mellitus (Figure reference: Inzucchi 2002)

8. Insulin secretagogues

8.1 Sulfonylureas

Sulfonylureas have been used in the treatment of non-insulin-dependent diabetes mellitus (type 2 diabetes mellitus) for over 50 years. The discovery that sulfonylureas were effective oral hypoglycemic agents was accidental. In 1942 Janbon and colleagues studying the efficacy of a sulfonamide, 2254 RP, in the treatment of typhoid fever, observed hypoglycemia and seizures, particularly in undernourished patients. Janbon commented on this to Auguste Loubatieres, who was studying insulin-induced seizures. Loubatieres hypothesized a common cause and was able to show that 2254 RP caused hypoglycemia in dogs, an effect abolished by pancreatectomy. Subsequent work showed that blood from 2254 RP treated dogs lowered serum glucose levels and that perfusion of the pancreas of normal dogs with 2254 RP also reduced glucose levels. The initial
clinical trial on three diabetic patients showed that 2254 RP caused a decrease in glucose in the oldest patient, but was ineffective in the two youngest women. These studies showed that β-cells were the major target for sulfonylureas and led Loubatieres to propose that the concentration of the sulfonamide in contact with the islet cells was the factor responsible for the liberation of insulin and that 2254 RP was the agent exciting insulin secretion. Loubatieres also suggested that sulfonylureas might be used to treat a type of diabetes (now type 2) that arose as a result of a deficiency of insulin secretory mechanisms. In 1955, in Berlin, Franke and Fuchs reported that another antibacterial sulfonamide, carbutamide, also caused hypoglycemia. The drug was rapidly used to treat diabetic patients who did not require insulin, and was followed 1 year later by tolbutamide. Today more than half dozen sulfonylureas are available for the treatment of type 2 diabetes mellitus (Diabetes mellitus: a fundamental and clinical text, 2004).

Of all compounds known to directly positively modulate insulin release, the sulfonylureas are the most studied. As sulfonylureas require intact β-cells, they have no value in treating type 1 diabetes mellitus. They are named for their common core configuration, which consists of a sulfonylurea group attached via the sulfur to a benzene ring. In the case of first-generation sulfonylureas (chlorpropamide, tolbutamide, tolvazamide, and acetohexamide) the R1 substituents are small and polar, and therefore render the aryl-sulfonylurea more water-soluble. In the second-generation sulfonylureas (glyburide, glipizide, gliclazide, and glimepiride) the substituents are large, nonpolar, lipophilic groups that more readily penetrate cell membranes and are thus more potent.

8.1.1 Mechanism of action by sulfonylureas
The binding of sulfonylureas to SUR1 leads to closure of K⁺-ATP channels with subsequent depolarization of the plasma membrane, activation of Ca²⁺ influx, and rise of [Ca²⁺]. These drugs mimic the effect of glucose in the generation of the triggering signal, but do so independently of changes in β-cell metabolism. The different sites of sulfonylurea binding to SUR1 and the molecular mechanisms of its transduction into closure of $K^+_i,6.2$ have been reviewed recently (Proks et al 2002; Gribble 2003). In brief, SUR1 possesses an “A” site to which binds tolbutamide and the half of the glibenclamide molecule containing the sulfonylurea group, and a “B” site to which binds the nonsulfonylurea half of glibenclamide. Binding to one of these two sites is sufficient to produce the same final effects: closure of K⁺-ATP channels, membrane depolarization, and rise in [Ca²⁺].

8.1.2 Drugs interacting with K⁺,6.2

Many structurally different drugs used for the treatment of diseases other than diabetes occasionally shows hypoglycemia. In vitro studies have shown that they increase insulin secretion by closing β-cell K⁺-ATP channels through a direct interaction with K⁺,6.2.

Among these drugs are antimalarial quinolines such as quinine and mefloquine, antibacterial fluoroquinolones such as norfloxacin and lomefloxacin, antiarrhythmic agents such as disopyramide and cibenzoline. More interest has been paid to drugs with an imidazoline structure (phenitolamine, antazoline, midaglizole), which also inhibit K⁺-ATP channels in β-cells by interacting with K⁺,6.2. This effect largely explains their stimulation of insulin secretion. However, efaroxan and novel imidazoline compounds also or exclusively act on an amplifying pathway (Henquin 2004). From a mechanistic point of view, it is unimportant whether drugs close K⁺-ATP channels directly by an interaction with the pore formed by K⁺,6.2 or indirectly by an interaction with the regulatory subunit SUR1. The net result is the same: membrane depolarization, influx of Ca²⁺, and generation of the triggering signal. The major difference, however, is the distribution of the two targets. The much more restricted distribution of SUR1 than K⁺,6.2 considerably increases the tissue specificity of the drugs acting through it. Moreover, drugs closing K⁺,6.2 directly usually also affect other channels (Plant and Henquin 1990). All drugs that block K⁺-ATP channels stimulate insulin secretion, but
only those that interact with the SUR subunit are used therapeutically to treat type 2 diabetes.

Figure 9:- (A) Schematic representation of the membrane topology of Kir6.2 and sulphonylurea receptor subunits. (B) Dose response curve for gliclazide block of Kir6.2/SUR1 and Kir6.2/SUR2A currents in Xenopus oocytes. High affinity inhibition is mediated by the sulphonylurea receptor, whereas low affinity block may involve direct drug interaction with Kir6.2. (Figure reference: Gribble and Reimann 2003).

The sulfonylurea drugs do not appear to correct the defect in early insulin secretion characteristic of type 2 diabetes (Shapiro et al. 1989). Whereas it is widely accepted that, unlike glucose, sulfonylureas do not promote proinsulin biosynthesis, it has been suggested that, like glucose, they also stimulate insulin secretion through an amplifying pathway (Renstrom 2002). This is based on electrophysiological studies in which single β-cells are usually patch-clamped in the whole cell mode (permitting unrestricted exchange between cytoplasm and pipette milieu), and exocytosis of insulin granules is monitored as changes in membrane capacitance. In the presence of fixed
[Ca\(^{2+}\)], intracellularly applied sulfonylureas increase exocytosis (Renstrom 2002). It is therefore proposed that sulfonylureas penetrate β-cells and interact with SUR1 or a related protein in the membrane of the insulin granules to confer them release competence by facilitating their acidification. Surprisingly, this effect persists in SUR1 knock out β-cells, implying that the intracellular binding protein is not SUR1 (Eliasson 2003). It would be expected therefore that sulfonylureas retain an effect on insulin secretion—via the intracellular binding sites—in intact β-cells without K\(^{+}\)-ATP channels.

**8.1.3 Side effects associated with the use of sulfonylureas**

The major side effects associated with use of oral sulfonylureas are as shown below:

**8.1.3.1 The incidence of clinically significant hypoglycemia**

The major side effect of insulin secretagogues that limits their usefulness in treating patients with type 2 diabetes mellitus is hypoglycemia. (Henquin 2004; Philippe et al. 2005). Sulfonylurea elicits insulin release regardless of plasma glucose concentrations. In the presence of sulfonylureas, the K\(^{+}\)-ATP channel activity is disconnected from glucose sensing, so hypoglycemia resulting from hyperinsulinemia may occur in the fasting state (Ferner and Neil 1988). The longer the half-life of a particular analog the greater will be its probability of inducing hypoglycemia. There is evidence that some sulfonylureas actually enhance the usual inhibitory action that MgADP has on the nucleotide binding site of the Kir6.2 subunit and so this may increase insulin release even more under hypoglycemic conditions. MgADP effects on the K\(^{+}\)-ATP channel are 2-fold: it inhibits the Kir6.2 subunit and stimulates the SUR1 activity. There is therefore usually a balance between these effects. In the presence of some sulfonylureas (notably tolbutamide and glyburide), however, the interaction of MgADP with SUR1 is diminished, resulting in its unopposed inhibitory effect on Kir6.2 (Gribble et al., 1997).

**8.1.3.2 Desensitization of insulin secretion**

Prolonged stimulation of insulin secretion by depolarization and Ca\(^{2+}\) influx regularly leads to a reversible state of decreased secretory responsiveness to nutrient and
nonnutrient stimuli. This state is termed “desensitization.” The onset of desensitization may occur within 1 h of exposure to depolarizing stimuli. A desensitization produced by prolonged exposure to depolarizing insulin secretagogues is regularly accompanied by a marked reduction in the number of β-cell granules. It could be possible that in the *in vitro* conditions the reduced granule content may be due to a down regulation of granule formation rather than to an imbalance between a stimulated granule formation and an even more stimulated granule discharge (Rustenbeck *et al.* 2004)

8.1.3.3 Weight gain

Weight gain during insulin secretagogue or insulin therapy appears to be related to several factors. Improvement in glycemic control with a reduction in glycosuria results in some weight gain if caloric intake is not appropriately reduced. Hyperinsulinemia itself, however, appears to increase appetite and calorie intake. In any event, combinations of sulfonylureas with drugs such as acarbose or metformin achieve better glycemic control than sulfonylureas alone, yet weight gain is less and hyperinsulinemia is also less (Coniff *et al.* 1995). A frequently overlooked cause of weight gain is patients increasing their caloric intake in response to mild symptoms of hypoglycemia (hunger) or from fear that they may get hypoglycemia.

8.1.3.4 Effect on myocardium and vascular smooth muscle

It was found that K⁺-ATP channels in other tissues such as brain, myocardium, and vascular smooth muscle cells also can bind sulfonylureas, and that such binding results in their closure (Fosset *et al.* 1988). Sulfonylurea treatment therefore has the potential to diminish hypoxia-induced vasodilatation in the coronary arteries, reduce the myocardium's compensatory reductions in energy requirements, and abolish ischemic preconditioning (Betteridge and Close 2000). These observations have rekindled the controversy about potential cardiac toxicity of insulin secretagogues in type 2 diabetic patients who have a coronary ischemic attack while on treatment (Betteridge and Close 2000; Muhlhauser *et al.* 1997). *In vitro* and *in vivo* animal studies have shown that glyburide treatment prevents myocardial and coronary smooth muscle K⁺-ATP channels from opening under ischemic conditions and interferes with ischemic preconditioning.
(Cleveland et al. 1997). Initially the data in humans were unclear; however, several recent studies have shown that glyburide in doses that correspond to those used clinically prevents ischemic preconditioning in humans (Tomai et al. 1994). In those same studies, doses of glimepiride that cause equivalent blood glucose lowering had no effect on ischemic preconditioning.

Several large studies that have examined the clinical outcomes of patients with type 2 diabetes mellitus treated with sulfonylureas compared with other antihyperglycemic treatments have not shown any increase in cardiovascular events (Klamann et al.). At comparably effective antihyperglycemic doses of glibenclamide, glimepiride, and metformin, the vasodilatory response was the same, indicating that there is no difference in the effects of glimepiride and glibenclamide on the brachial artery smooth muscle K⁺-ATP channel (Spallarossa et al. 2001). The meglitinide, repaglinide and the phenylalanine derivative nateglinide have very little binding affinity for myocardial and vascular smooth muscle K⁺-ATP channels.

8.1.3.5 Effect of sulfonylureas on β-cells function

The closure of the K⁺-ATP channels by the sulfonylureas like tolbutamide and glibenclamide may induce Ca²⁺-dependent β-cell apoptosis in rodent and human islets (Efanova et al. 1998, Maedler et al. 2005). This effect was observed only in vitro and not consistently (Guerra et al. 2005). However, in an important recent clinical study comparing insulin and sulfonylurea treatment of type 2 diabetes, it was shown that treatment with insulin preserved β-cell function more effectively than glibenclamide (Alvarsson et al. 2003). It remains to be established whether it is the beneficial effects of insulin per se or the possible β-cell toxicity of glibenclamide that accounts for this observation. Given the possible deleterious effect of some sulfonylureas, alternatives to these as well as alternative insulin secretagogues may have to be considered. When applied for their respective circulating half-lives in vitro, repaglinide and nateglinide do not appear to have an apoptotic effect on human islets (Maedler et al. 2005).

In contrast to sulfonylureas, K⁺-ATP channels’ channel openers may exert protective effects on β-cells (Maedler et al. 2004, Ritzel et al. 2004). In 1976, Greenwood et al. were the first to report an improvement in insulin secretion after
administration of diazoxide to diabetic subjects for 7 days (Greenwood et al. 1976). Similar protective effects were observed more recently in patients classified with type 1 and type 2 diabetes (Guldstrand et al. 2002). Finally, other antidiabetic drugs that have emerged as protectors of β-cells from apoptotic stimuli include thiazolidinediones, glucagon-like peptide 1 analogs, and, last but not least, insulin (Maedler et al 2005).

8.2 Glinides

The nonsulfonylurea (benzamido) part of glibenclamide, known as meglitinide or HB-699, possesses blood glucose lowering properties that have been attributed to stimulation of insulin secretion. Twenty years ago it was shown that meglitinide mimics the sequence of events by which tolbutamide and glibenclamide trigger insulin secretion. A number of other non-sulfonylurea compounds have been developed more recently, some of which are already in clinical use (Dornhorst 2001). The best known are repaglinide (AGEE-623), nateglinide (A-4166), and mitiglinide (KAD-1229 or S-21403). They are functionally related but structurally different.

![Repaglinide and Nateglinide](Image)

The common family name of “glinides” derives from their trade name but does not refer to any specific chemical structure. Except for repaglinide, it is incorrect to call them “meglitinide analogs” or “benzamido compounds.” Both repaglinide and nateglinide are potent K⁺-ATP channel blockers, with repaglinide being 10-fold more potent than glibenclamide (Fuhlendorff et al. 1998). Binding studies on repaglinide and glibenclamide in an insulinoma cell line indicate that there are probably three distinct binding sites for these two compounds: a high-affinity repaglinide site and two lower-affinity sites for glibenclamide (Fuhlendorff et al. 1998). Meglitinide and repaglinide bind to the “B” site in SUR1, whereas nateglinide and mitiglinide bind to the “A” site.
Whatever the binding site, their eventual effect is similar to that of sulfonylureas: depolarization of β-cells, with subsequent rise in \([\text{Ca}^{2+}]\), and triggering of insulin secretion. Mitiglinide and nateglinide, both also display an advantageous greater selectivity for SUR1 than SUR2A or SUR2B (Proks et al. 2002) and, hence, for K⁺-ATP channels of the β-cell.

Repaglinide, in contrast to glibenclamide, did not stimulate insulin secretion in islets in the absence of glucose and is more effective than glibenclamide at higher glucose concentrations (16.7 mM; Fuhlendorff et al. 1998). However, nateglinide shows a glucose-induced insulin secretion profile similar to that of glibenclamide (Ikenoue et al. 1997). Nateglinide has the advantage in that it rapidly dissociates from the SUR1 and displays a more rapid onset of channel inhibition and faster reversal of same than rapaglinide (Hu et al., 2000). Because the effects of both drugs are rapid and short-lived they are used to curtail postprandial excursions in glucose (Kalbag et al. 2001). The plasma half-life of both compounds in healthy human volunteers is between 1 and 1.5 h, with nateglinide producing a much more rapid rise in insulin postprandially than does rapaglinide, when the agents are administered 30 min before eating (Kalbag et al., 2001). Thus the risk of hypoglycemia when treating with these drugs is lower than with traditional sulfonylureas. Thus, due its rapid action on the β-cell, administration of nateglinide to diabetic individuals preprandially produces a more physiologically normal insulin response than is seen with the sulfonylureas. There is a rapid 5-fold increase of insulin above basal values within 30 min of eating, which represents the prompt release of the insulin in the vesicles of the RRP followed by a decline within 120 min to values 2-fold above basal. However, this does not necessarily reflect a restoration of normal insulin secretory kinetics, as the early rise in insulin secretion was seen with nateglinide in the presence or absence of glucose, and it slowly decreased, most likely representing an exhaustion of the primed insulin secretory vesicles in the β-cell.

Side effects are otherwise similar to other secretagogues, including weight gain and hypoglycemia. These likely occur to a lesser degree than with sulfonylureas. Meglitinides must also be taken shortly before each meal and therefore have a more frequent dosing schedule than most other agents. Their cost is generally higher than that of sulfonylureas. Long-term outcomes data are still unavailable for this drug class. However, the effect on long-term complication rates is likely to be at least similar to that
observed with sulfonylureas. It is unlikely that such long-term outcomes studies will ever be conducted (Kimmel and Inzucchi 2005)

The normal regulation of insulin secretion is tightly coupled to the plasma glucose level. Increasing plasma glucose levels, such as those that occur following ingestion of food, results in an almost immediate increase in insulin secretion. Decreasing plasma glucose levels are associated with a rapid decline in the secretion and plasma levels of insulin. Fasting is accompanied by reductions in insulin secretion sufficient to increase hepatic production of glucose to maintain glucose homeostasis. The ideal insulin secretagogue, therefore, would be one that restores to normal the defective early meal-mediated insulin secretion of type 2 diabetes, increase insulin secretion to adequately overcome the insulin resistance, stimulates insulin release only in response to elevated plasma glucose levels, and has little or no lag time in its insulin secretory response to rapidly changing plasma glucose levels. None of the available insulin secretagogue fulfills all of these properties.

9. Emerging therapies

Additional pharmacological agents will soon become available for the management of patients with type 2 diabetes. These include other PPAR-agonists with additional effects on PPAR-\(\alpha\) and PPAR-\(\delta\), and consequently better lipid effects than current TZDs. Modulation of the incretin system is another area of active investigation by several pharmaceutical companies. Incretin mimetics include glucagon-like peptide-1 agonists and the dipeptidyl peptidase-IV inhibitors which augment endogenous incretin levels. These drugs improve glucose-dependent insulin secretion while simultaneously suppressing glucagon secretion, delaying gastric emptying, and decreasing appetite. Modest decreases in body weight are described with their use. GLP-1 is a 30-amino acid peptide synthesized in the intestine and secreted upon nutrient ingestion (Mojsov et al., 1986). It is effective in augmenting glucose-mediated insulin secretion in the diabetic state and in suppressing glucagon secretion. It acts through a specific G-protein-coupled receptor on the \(\beta\)-cell and ultimately increases both the number of cells secreting insulin and the amount secreted per cell by increasing insulin biosynthesis and recruiting more vesicles into the RRP (Drucker, 2002).
10. **Beneficial effects of antioxidants in diabetes**

In general, the development of type 2 diabetes is associated with pancreatic β-cell dysfunction occurring together with insulin resistance. Normal β-cells can compensate for insulin resistance by increasing insulin secretion, but insufficient compensation leads to the onset of glucose intolerance. Once hyperglycemia becomes apparent, β-cell function progressively deteriorates: glucose-induced insulin secretion becomes further impaired and degranulation of β-cells becomes evident, often accompanied by a decrease in the number of β-cells (Porte 1991; DeFronzo 1992; Yki-Jarvinen 1992). The significance of hyperglycemia as a direct cause of these phenomena, i.e., β-cell glucose toxicity, has been demonstrated by various studies (Robertson et al. 1992; Moran et al. 1997). Chronic hyperglycemia may impair β-cell function at the level of insulin synthesis as well as insulin secretion; when β-cell–derived cell lines are exposed to a high glucose concentration for a long period of time, insulin gene transcription and insulin content are dramatically reduced (Robertson et al. 1992; Moran et al. 1997). Under diabetic conditions, reactive oxygen species (ROS) are produced mainly through the glycation reaction (Hunt et al. 1991), which occurs in various tissues and may play a role in the development of complications in diabetes (Baynes 1991).

Although the neural cells and the lens crystalline are the most prone targets for the induction of the glycation reaction in diabetes, another target was recently shown to be the β-cell (Kaneto et al 1996; Tajiri et al. 1997). Indeed, advanced glycosylation end products (AGEs) were shown to be detectable in β-cells kept under high glucose concentrations (Tajiri et al. 1997). Also, the expression of antioxidant enzymes, such as superoxide dismutase, catalase, and glutathione peroxidase, is known to be very low in islet cells compared with other tissues and cells (Tiedge et al. 1997). Therefore, once β-cells face oxidative stress, they may be rather sensitive to it, suggesting that glycation and subsequent oxidative stress may in part mediate the toxic effect of hyperglycemia. Glycation mediated ROS production reduces insulin gene transcription and also causes apoptosis of β-cells (Kaneto et al 1996). Although animals have their own antioxidant defense systems, the defense can be externally strengthened. This might be especially true for the pancreas, since it has a relatively weak intrinsic defense system against oxidative stress (Tiedge et al. 1997). Thus, a sufficient supply of antioxidants may
prevent or delay \( \beta \)-cell dysfunction in diabetes by providing protection against glucose toxicity (Kaneto et al. 1999).

Understanding that decreased \( \beta \)-cell mass is an important factor in the pathogenesis of type 2 diabetes raises a concern regarding the application of drugs potentially harmful to the remaining \( \beta \)-cells. Given the possible deleterious effect of some sulfonylureas, alternatives to these as well as alternative insulin secretagogues may have to be considered. The ideal insulin secretagogue should have the following characteristics: it acts rapidly, so that insulin secretion is stimulated soon after meal ingestion; its effect is graded to increase as the plasma glucose increases from 60 to 180 mg/dl; it has little or no effect at plasma glucose levels of less than 60 mg/dl; and its duration of action is short so that is does not continue to stimulate insulin secretion beyond the postprandial period.

Approaches to the control of blood glucose and prevention of hyperglycaemia are central to the treatment of diabetes mellitus. At present, none of these therapies either alone or in combination can reinstate normal blood glucose homeostasis, and many limitations exist in the use of anti-diabetic drugs. New therapies are needed which reinstate a normal metabolic environment and prevent long-term complications. The development of new anti-diabetic drugs ideally requires new pharmacological treatments which stimulate both the secretion and the action of insulin (Bailey and Flatt 1995).

Due to these side effects of synthetic remedies, there is growing interest in natural remedies in recent years. More than 1200 species of organisms have been used ethnopharmacologically or experimentally to treat symptoms of diabetes mellitus. They represent more than 725 genera in 183 families, extending phylogenetically all the way from marine algae and fungi to advanced plants such as the composites (Marles and Farnsworth 1995). Among the various natural sources used in treatment of diabetes, plants and herbs are most frequently used in traditional remedies. Before the discovery of insulin in the early 1920s and the later development of oral hypoglycaemic agents, the major form of treatment of diabetes mellitus involved dietary manipulation and the use of plant therapies. The recommended use of plants dates back to the Eber’s papyrus of about 1550 BC. More than 400 plants worldwide have been documented for the treatment of diabetes and the majority awaits proper scientific and medical evaluation (Swanston-Flatt et al. 1991). The few examples of traditionally used plants are Cajanus...
cajan, Cicer arietinum, Phaseolus mungo, Phaseolus vulgaris, Aeglemarmelose, Mangifera indica, Morus alba, Musa sapientum, Psidium guajava, Punica granatum, Syzigium cumini, Vitis vinifera, Allium cepa, Annona squamosa, Beta vulgaris, Cucurbita pepo, Ipomoea batatus, Momordica charantia, Allium sativum, Brassica juncea, Cuminum cyminum, Curcuma, Murraya koeingii and Trigonella foenum graecum etc (Kaushik et al. 2010). Most of the traditional medicines are prepared from herbs, spices and plants which do not form part of the normal diet. However, several common components of the diet are traditionally recommended for regular consumption, and some are additionally taken as infusions, decoctions or alcoholic extracts (Gray and Flatt 1997).

Though there are many traditional plant remedies existing throughout the world from ancient years, only few have received scientific or medical scrutiny. The World Health Organization has also recommended further scientific evaluation of such plant remedies. (World Health Organization 1980). A balanced approach to traditional plant treatments for diabetes is required which allows for proper scientific and medical evaluation together with cautious optimism in the face of sometimes conflicting scientific evidence.

Several anti-diabetic plants have shown sufficient hypoglycaemic activity to warrant at least partial characterization of the active principle. However, for the majority of traditional plant treatments the active principle present together with their mode of action has yet to be characterized (Gray and Flatt 1997). Several plants that are used in traditional remedies are scientifically studied and number is increasing rapidly. Several plant remedies are reported with their mode of action e.g. the leaves of Ocimum sanctum are used traditionally to reduce blood glucose and shown to possess insulin stimulating activity. The closure of K⁺-ATP channels participates in the overall mechanism of action of O. sanctum. Also the omission of Ca²⁺ from extracellular medium did not completely abolish the insulin-secretory effects of extract and hence postulated that the extract can induce mobilization of intracellular Ca²⁺ as well as promoting Ca²⁺ entry (Hannan et al. 2006).

There is growing interest in finding the active principles responsible for antidiabetic activity. There are more than 200 pure compounds from plant sources reported to show blood glucose lowering activity (Marles and Farnsworth 1995). Several
examples of compounds isolated from plants showing antidiabetic activity are also identified:

Sodium salicylate (salt of 14) inhibits cyclooxygenase, thus preventing the metabolic cascade from arachidonic acid to the prostaglandins. Inhibition of β-cell PGE2 synthesis increases glucose-induced insulin secretion because this prostaglandin binds to specific β-cell receptors that are coupled to regulatory components that inhibit adenylate cyclase. Inhibition of this enzyme would lead to a decrease in intracellular cAMP (Robertson 1988). Additionally, arachidonic acid itself is an insulin secretagogue, acting to mobilize Ca^{2+}, increasing its free cytosolic concentration, and to activate protein kinase C (Metz 1988). Scoparia dulcis (Sweet Broomweed) has been documented as a traditional treatment of diabetes. The antihyperglycemic activity of the plant was scientifically proven and it was shown that glucose lowering effect of Scoparia dulcis ethanolic extract is associated with ability of extract to potentiate the insulin release from pancreatic islets (Latha et al. 2004). Further, scoparic acid D isolated from Scoparia dulcis is shown to be active constituent responsible for insulin stimulatory activity of Scoparia dulcis (Latha et al. 2009). The Southeast Asian herb, Gynostemma pentaphyllum Makino (Cucurbitaceae) has been reported to affect numerous activities including hypoglycemic effects. An active compound from group of saponins, named as Phanoside, was shown to possess insulin stimulating activity (Norberg et al. 2004). Aspalathin, a component isolated from Aspalathus linearis shows beneficial effects on glucose homeostasis in type 2 diabetes through stimulating glucose uptake in muscle tissues and insulin secretion from pancreatic β-cells (Kawano et al. 2009).

Though several pure components reported to be beneficial in treatment of diabetes were isolated, there is need for further studies because some of these compounds may have therapeutic potential, while others may produce hypoglycemia as a side-effect of their toxicity, especially hepatotoxicity. Some of the compounds reported to be active in vitro or at high doses in vivo, e.g., p-sitosterol-D-glucoside (daucosterol), occur so widely in nature that therapeutic activity seems unlikely. This could be due to their low concentrate ion in the plant or co-occurrence with complexing or counteracting constituents (Marles and Farnsworth 1995). Hence, further studies of such compounds regarding their mechanism of action, toxicity, effect on other tissue targets etc. are needed. There is, nevertheless, only one example of an approved antidiabetic drug that
was developed from an herb with a long history of use for diabetes: the biguanide metformin from French lilac (*Galega officinalis*) (Marles and Farnsworth 1995).

Several medicinal plants have been reported to possess potential hypoglycemic activity in Indian system of medicine. However, it is important to note that many of these are not edible and with an explosion in the number of diabetic patients especially in developing countries it is of importance to focus on plant food materials with hypoglycemic properties that are easily available, culturally acceptable, and economical and are absolutely free from side effects when compared with the drugs for managing diabetes (Kaushik *et al.* 2010). Numerous other herbs remain candidates for antidiabetic drug development. Clinical data are beginning to emerge, which support antidiabetic indications for several of these herbs. This viewpoint outlines the opportunity that exists for these herbs in the management of diabetes and the state of the evidence for their clinical antidiabetic efficacy (Vuksan and Sievenpiper 2005).

11. **Need for present study**

Approaches to the control of blood glucose and prevention of hyperglycaemia are central to the treatment of diabetes mellitus. At present, none of these therapies either alone or in combination can reinstate normal blood glucose homeostasis, and many limitations exist in the use of anti-diabetic drugs. New therapies are needed which reinstate a normal metabolic environment and prevent long-term complications. The development of new anti-diabetic drugs ideally requires new pharmacological treatments. (Bailey and Flatt, 1995). Understanding that decreased β-cell mass is an important factor in the pathogenesis of type 2 diabetes raises a concern regarding the application of drugs potentially harmful to the remaining β-cells. Given the possible deleterious effect of some sulfonylureas, alternatives to these as well as alternative insulin secretagogues may have to be considered.

In regarding all these factors, there is need to find an pure ideal insulin stimulating compound from plant source which possess following properties

i) The compound which stimulate both the secretion and the action of insulin
ii) Compound having glucose dependent action. Its effect should graded to increase as the plasma glucose increases from 60 to 180 mg/dl; it has little or no effect at plasma glucose levels of less than 60 mg/dl; and its duration of action is short so that is does not continue to stimulate insulin secretion beyond the postprandial period.

iii) Compound having rapid action and short-plasma half life.

iv) It should not over stimulate the β-cells and compound having β-cell protective action is desirable.

v) The source of compound should be easily available, cost effective.

vi) The components used in regular diet are desirable.

vii) The yield of compound from source plant must be high and the isolation process must be cheaper.

viii) The plant extract or isolated compound should not be toxic and are with no/less side effects when compared with the commercial drugs currently used for managing diabetes.

Hence, the current research aims to scientifically evaluate traditionally used plants in treatment of diabetes mellitus and their insulin secretagogue action. The present study aims further to find active component responsible for insulin secretion and elucidation of the pathway responsible for it.

Current research is designed on the basis of effectiveness of traditionally used antidiabetic plants in treatment of diabetes mellitus. The objectives of present work are rationalized on the basis of effectiveness as well as mechanism of action by which the antidiabetic activity of the plant is governed.

12. Objectives of the study

1. Induction of diabetes in experimental animals using alloxan/streptozotocin and standardization of the process for stable diabetes induction and assessment of the diabetic status through study of various biochemical parameters such as blood glucose, plasma insulin levels, glycated Hb, lipid profile, liver glycogen etc.
2. Standardization of experiments for in vitro studies such as isolation of pancreatic islets of Langerhans, estimation of insulin by ELISA, liver glycogen estimation, isolation of \( \alpha \)-glucosidase enzyme, antioxidant assay etc.

3. Evaluation of antidiabetic activity of some spices and evaluation of their insulin secretagogue, alpha-glucosidase inhibitory and antioxidant activity.

4. Study of antidiabetic activity of Kalanchoe pinnata through OGTT and long term treatment along with all the biochemical parameters mentioned above.

5. Evaluation of insulin secretagogue action of Kalanchoe pinnata, isolation of active component and prediction of probable pathway for the insulin secretion.

6. Study of antidiabetic potential of Cuminum cyminum through OGTT and long term treatment along with all the biochemical parameters.


8. Isolation of the active components from Cuminum cyminum extract by column chromatography and study of insulin secretagogue action of isolated compounds in vitro.

9. Identification of the active components of Cuminum cyminum through use of GC-MS, IR spectroscopy, NMR analysis.

10. Underlying the probable mechanism for insulin secretagogue action of the isolated compound from Cuminum cyminum.

11. Attempt to study beta cell protective action of Cuminum cyminum along with identification and probable mechanism of protection.

12. Study of antidiabetic activity of Cymbopogon citratus through OGTT. In vitro study of on insulin secretion, isolation of active component and prediction of probable pathway for the insulin secretion.

13. References


Chapter 1
Introduction & Review of Literature


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Chapter I

Introduction & Review of Literature


