CHAPTER- ONE
INTRODUCTION AND REVIEW OF LITERATURE
1. Introduction

This thesis describes the work towards understanding the mechanisms for anti-diabetic effects of *Cinnamomum zeylanicum* extract by studying its effects on key molecular targets, like PPAR (nuclear receptor), pancreatic β-cell GPCR, called GPR40 using CHO cells stably expressing hGPR40 receptor, enzymes like PTP1B, DPP-IV using various *in vitro* assays and its effect on expression level of key β-cell genes. Pancreatic GPCRs and other proteins, and some nuclear receptors are involved in the regulation of glucose homeostasis *in vivo*. The following pages will provide an overview of diabetes in general and key concepts of glucose homeostasis.

Diabetes is a chronic metabolic disease of carbohydrate and fat metabolism characterized by increased fasting and post prandial blood glucose levels. Type 2 Diabetes mellitus (T2DM) is a prevalent metabolic disease throughout the world and is characterized by hyperglycemia mainly due to the impaired insulin secretion or insulin resistance, impairment of GIP mediated insulinotropic effect and abnormalities in fat, carbohydrate, and protein metabolism in patients. It is a severe menace to the middle-aged and elderly people, and there is an unmet need for a safe drug for treatment. Products from natural herbs of known anti-diabetic potential would provide an inherent larger-scale structural diversity and a major resource of bioactive agents for discovery of novel drugs with potential for much reduced side effects. The anti-diabetic potential of cinnamon extract in animal models has been well established and it is being used as herbal medicine by many patients; however the mechanism of action by which it shows anti-diabetic action is not completely explored and only few reports describe the presence of compounds in aqueous or ethanolic extract for its anti-diabetic action. The global prevalence of diabetes is estimated to increase, from current to 7.8% by the year 2030. WHO has predicted that the major burden will occur in developing countries. According to certain studies conducted in the last decade in India, there is not only the prevalence of high diabetes but it also indicated that diabetes is increasing rapidly in the urban population. It is estimated that there are approximately 33 million adults (12% of overall diabetics in world) with diabetes in India. This number is likely to increase to 57.2 million in India by the year 2025. According to International diabetes federation (IDF) Atlas report released in Nov' 2011, number of people living with diabetes is expected to rise to 552 million worldwide by 2030 [1] and estimates that unless rapid action is taken, one person in
ten will have diabetes by 2030. The majority of people with diabetes are in the age range between 40-59 years. Some of the facts associated with diabetes demonstrate that cardiovascular disease is the major cause of death in diabetes, accounting for almost 50% of all diabetes fatalities. On average, people with T2DM die 5-10 years before as compared to non-diabetic people and most of this excess mortality is due to cardiovascular diseases arising or developed due to diabetes. People with T2DM are over twice as likely to have a heart attack or stroke as people who do not have diabetes. Part of the cardiovascular risk associated with impaired glucose tolerance (IGT) and diabetes is undoubtedly due to their association with other CV risk factors such as hypertension, high LDL-cholesterol and low HDL-cholesterol and smoking.

B. Aim

To study the mechanisms of anti-diabetic effects of Cinnamomum zeylanicum extract (CZE) with reference to glucose homeostasis.

Objectives

i. To evaluate the effects of CZE on PPARs (nuclear receptors) that are known to be involved in lipid and glucose metabolism using cell based PPAR reporter assays
   • To prepare and evaluate acetone (CZE-1) and hydroalcoholic {aqueous methanol (30:70, v/v; CZE-2) and aqueous ethanol (50:50, v/v; CZE-3} extract of C.zeylanicum for studying effects using various in vitro assays
   • To study the effects of CZE-3 on the PPAR transactivation potentiation in presence of key coactivators like PGC1α and SRC-1 that are known to be involved in PPAR mediated gene activation by TZD class of drugs
   • To study the effects of CZE-3 in HEK293/T cells expressing PPARγ, 3x PPRE luciferase reporter, but silenced for PGC-1α coactivator

ii. To study the effects of CZE-3 on key glucoregulatory enzymes like PTP1B and DPP-IV (involved in insulin signaling, glucose homeostasis) using in vitro assays
   • To evaluate PTP1B inhibitory potential of CZE-3 and isolated compounds
   • To evaluate DPP-IV inhibitory potential of CZE-3 and isolated compounds

iii. To investigate the effects of CZE-3 on pancreatic GPCRs (GPR40, endogenously expressed in β-cells) known to be activated by medium-long chain fatty acid,
involved in insulin signaling and glucose homeostasis role in glucose-stimulated insulin secretion (GSIS)

- To evaluate the effects of CZE-1, CZE-2 and CZE-3 for insulin secretion potential (insulin secretogogue activity) in HIT-T15 cell line
- To evaluate the effects on GSIS, using physiologic and supraphysiologic glucose concentrations
- To evaluate the effects on intracellular calcium responses in hGPR40-CHO stable cell line using FLIPR based 96-well plate assay

iv. To investigate the effects on expression levels of key pancreatic β-cell genes (insulin, Glut2, pdx-1 and glucokinase) involved in glucose homeostasis, using real-time qPCR studies

1. C. Review of Literature

1.1. Reported herbal plants with potential for anti-diabetic properties

There are many medicinal plants that have been demonstrated to possess anti-hyperglycemic activity in vivo and to play very important role in the treatment of diabetes. There is an increasing demand of natural product with anti-diabetic activity from patients. Many conventional drugs have been derived from prototypic molecules in medicinal plants. The old drug, Metformin has been shown to be very effective and efficacious oral glucose-lowering agent, but its activity is not glucose dependent and may cause hypoglycemia under some conditions. Herbal plants with known anti-diabetic activity can be used to isolate new potential anti-diabetic agents\(^2\). A large number of medicinal plants despite having anti-diabetic activity do possess some degree of toxicity. For example, it was reported that about one third of medicinal plants used for treatment of diabetes are considered to be toxic. The plant families, including the species (sp), most studied for their confirmed hypoglycaemic effects include: Leguminosae (11 sp), Lamiaceae (7 sp), Liliaceae (8 sp), Cucurbitaceae (7 sp), Asteraceae (6 sp), Moraceae (6 sp), Rosaceae (6 sp), Euphorbiaceae (5 sp), Amaranthaceae (1 sp), Anacardiaceae (1 sp) and Araliaceae (5 sp). The most studied species are:

- *Galega officinalis*
- *Citrullus colocynthis*
Herbal medicines with known anti-diabetic activity could be classified into four categories according to their mode of action; (i) Drugs acting like insulin or insulin mimics, (ii) Drugs acting on insulin secreting β-cells or insulin secretagogues, (iii) Drugs acting by maintaining glucose homeostasis or modulating utilization in tissues, (iv) Drugs acting by other miscellaneous mechanisms (either acting on pancreatic GPCRs or NRs or enzymes involved in glucose regulation).

*Galega officinalis*: This plant has been used to treat diabetes since many years. *Galega officinalis* is rich in guanidine, the hypoglycemic component. But, guanidine is known to be a toxic agent and thus may not be suitable for clinical use; alkyl biguanides like synthalin A and synthalin B were introduced as oral anti-diabetic agents in Europe in the 1920s but were discontinued after FDAs approval for clinical use of insulin injections. However, the experience with biguanides and guanidine triggered the development of metformin [3-4].

*Acacia arabica* (Babul): This plant is found all over India mainly in the wild habitat. The plant extracts works as an anti-diabetic agent by acting as insulin secretagogue to stimulate insulin release from pancreatic β-cells. The extracts from plant have been shown to reduce glucose in control rats but not in alloxan induced diabetic animals. Powdered seeds of *Acacia arabica*, when administered (2, 3 and 4 g/kg body weight) to normal rabbits caused hypoglycemic effects by initiating release of insulin from pancreatic β-cells [5].

*Aegle marmelos* (Bengal Quince, Bel or Bilva): Administration of aqueous extract of leaves improves digestion and reduces blood sugar and urea, serum cholesterol in
alloxanized rats as compared to control. Along with exhibiting hypoglycemic activity, the extract also prevented peak rise in blood sugar at 1h in OGTT [6].

**Allium cepa (onion):**
Ether soluble fractions as well as insoluble fractions of dried onion powder have demonstrated anti-hyperglycemic activity in diabetic rabbits. Allium cepa is also known to have antioxidant and hypolipidaemic activity. Administration of a sulfur containing amino acid from Allium cepa, S-methyl cysteine sulphoxide (SMCS) (200 mg/kg for 45 days) to alloxan induced diabetic rats significantly reduced blood glucose as well as plasma lipid, normalized liver hexokinase activity, glucose 6-phosphatase and HMG Co A reductase [7-8]. Studies in streptozotocin induced (STZ) diabetic rats with diet containing 15 mg % capsaicin or 3% freeze-dried onion powder produced significant reduction in hyperglycaemia. Study revealed the beneficial effects of onion feeding on metabolic status of diabetic individuals due to its hypocholesterolemic and hypoglycaemic effects [9].

**Allium sativum (garlic):** Allium sativum is a perennial herb cultivated throughout India. This plant has a pungent odour due to presence of Allicin, a sulfur-containing compound. Allicin has been reported to have a significant glucose lowering activity in animal models [10]. The anti-diabetic effect of allicin is probably assumed to be due to reduced hepatic glucose production (HGP) and enhanced insulin secretion from pancreatic β-cells [11]. Aqueous garlic extract (10 ml/kg/day; p.o) when administered to sucrose fed rabbits (10 g/kg/day in water for two months), caused significant increase in hepatic glycogen and free amino acid content, decreased FBG and plasma TG levels as compared to sucrose controls [12]. S-allyl cystein sulfoxide (SACS) is sulfur containing amino acid and a precursor of allicin is involved in lipid peroxidation and reported to be more efficacious than glibenclamide and insulin. SACS is also reported to stimulate in vitro insulin secretion from isolated islet β-cells from normal rats [13].

**Aloe vera and Aloe barbadensis:** Aloe is very popular plant and being cultivated throughout India. It has a long history of being used as multipurpose folk remedy. The plant gives two basic products: gel and latex. Aloe vera gel is derived from leaf pulp or mucilage while aloe latex ("aloe juice") is a bitter yellow exudate derived from the pericyclic tubules just beneath the outer skin of the leaves. Extracts from aloe gum
has been reported to be effective in improving glucose tolerance in both normal and diabetic rats \[14\]. Chronic treatment of Aloe barbadensis leave exudates reduced hyperglycemia in alloxan induced diabetic rats. Acute as well as chronic doses of bitter principle ingredient from same showed reduction of blood glucose in diabetic rats likely via β-cell stimulation leading insulin biosynthesis and/or secretion \[15\].

**Azadirachta indica (Neem):** Hydroalcoholic extract of this plant has been reported to have anti-hyperglycemic activity in STZ treated rats. The effect is due to increased glucose uptake and glycogen synthesis \[16\].

**Mangifera indica (Anacardiaceae, Mango):** Mango leaves are routinely used as an anti-diabetic agent in Nigerian folk medicine. OGTT studies in normal SD rat demonstrated significant anti-diabetic activity of plant extract when it was co-administered along with glucose and also when the extract was administered to rats 60 min prior to glucose load. This effect is thought to be due to a reduced glucose absorption in intestine \[17\].

**Caesalpinia bonducella:** Caesalpinia bonducella is widely distributed throughout the coastal region of India and is used ethnically by the Indian tribal’s for controlling blood glucose within normal range. Both, aqueous and ethanolic extracts have been shown to possess potent anti-hyperglycemic activity in diabetic rodant models. These extracts have been reported to cause increased glycogenesis leading to increased hepatic glycogen content \[18\]. Two fractions, BM169 and BM170 B have been reported to cause enhanced insulin secretion from isolated islets. Aqueous and 50% ethanolic extracts (180 mg/kg) of C. bonducella seeds have demonstrated anti-hyperglycemic and hypolipidemic activity in STZ induced diabetic SD rats \[19\]. The anti-hyperglycemic action of the seed extracts has been attributed to be due to the reduced or decreased glucose absorption in intestine.

**Capparis decidua:** This plant is found allover India and is reported to have anti-hyperglycemic effect in alloxan induced SD rats when fed with 30% extracts of Capparis decidua (C. decidua) fruit powder for 3 weeks. The extract also exhibited significant reduction in alloxan induced lipid peroxidation in erythrocytes, kidney and heart. C. decidua has been found to reduce oxidative stress by modulating superoxide dismutase (SOD) and catalase enzyme activity \[20\].
**Coccinia indica**: Dried extracts of *Coccinia indica* (500 mg/kg body weight) has potential to modulate certain enzymes involved in glucose regulation. Diabetic patients administered with 500 mg/kg body weight of *Coccinia indica* extracts for 6 weeks, restored lipoprotein lipase (LPL) enzyme levels which were otherwise reduced and also restored glucose-6-phosphatase (G-6-Pase) and lactate dehydrogenase (LDH) activities, which were significantly elevated in untreated diabetic patients. A study with 500 mg/kg (p.o) of *C. indica* leaves showed significant reduction of glucose levels in alloxan induced diabetic dogs and improved glucose tolerance in normal and diabetic dogs.

**Eugenia jambolana (Indian gooseberry, Jamun)**: Jamun seed extracts as well as pulp has been traditionally used as household remedy for diabetes in India. The plant seed extract also forms a major constituent of many herbal formulations for diabetes. Anti-hyperglycemic effect of aqueous, alcoholic extract as well as lyophilized powder has been well documented. The extract shows 73.51% improvement in glucose levels in patients with impaired glucose levels (having plasma glucose >180 mg/dl). However, in patients with moderate (>280 mg/dl) and severe plasma glucose (>400 mg/dl), it showed 55.62% and 17.72% improvement respectively. It has been shown that, pulp extract exhibited anti-hyperglycemic activity in STZ induced diabetic mice within 30 min of administration. Oral administration of extract caused elevated plasma insulin in diabetic rodent models. Plant extract showed significant GSIS effect in isolated islet β-cells from normal as well as diabetic SD rats.

**Momordica charantia (bitter gourd)**: *Momordica charantia* is widely used as an anti-diabetic and anti-hyperglycemic agent in India as well as in other Asian countries. Extracts from fruit pulp, seed, leaves and whole plant has been reported to have anti-hyperglycemic effect in various animal models mainly via activation of PPARs. Polypeptide p, the ingredient isolated from fruit, seeds and tissues of *M. charantia* exhibits significant glucose lowering effect when administered subcutaneously to langurs and humans. Ethanolic extracts of *M. charantia* (200 mg/kg) has been shown to exhibit an anti-hyperglycemic effect in normal and STZ diabetic SD rats. The main mechanism has been attributed to be due to inhibition of glucose-6-phosphatase, stimulation of hepatic glucose-6-phosphate dehydrogenase activities and activation of PPAR receptor in liver and adipose tissues.
**Ocimum sanctum (Tulsi):** Tulsi plant has been known for its medicinal properties since ancient times. The aqueous extract of *Ocimum sanctum* leaf has been reported to exhibit significant improvement in overall blood glucose level in normal and alloxan induced diabetic SD rats \(^{[25]}\). The plant extract has been shown to exhibit significant reduction in FBG, uronic acid, total amino acid, total cholesterol, triglyceride and total lipid levels in diabetic SD rats \(^{[26]}\). The plant also exhibits anti-asthmatic, anti-stress, anti-bacterial, anti-fungal, antiviral, antitumor, antiulcer activity, antioxidant, and anti-mutagenic activities.

**Phyllanthus amarus (bhuiamala):** An herb from family Euphorbiaceae and commonly known as Bhuiamala. The plant is found throughout India, mainly Deccan, Konkan and south Indian states and has been used traditionally for the treatment of diabetes. The methanolic extract from this plant has been demonstrated to exhibit improvement in blood glucose in alloxan induced diabetic SD rats \(^{[27]}\). Besides having anti-diabetic activity, the plant also exhibits anti-inflammatory, anti-mutagenic and anti-carcinogenic activity.

**Pterocarpus marsupium:** *Pterocarpus m* is a moderate to large tree found mainly in the hilly regions of India. *Pterostilbene*, a major constituent derived from the wood of this plant has been shown to exhibit anti-hyperglycemic activity in dogs \(^{[28-29]}\). Flavonoid fraction from *Pterocarpus marsupium* has been shown to stimulate regranulation of pancreatic β-cell \(^{[30]}\). Marsupin, pterosupin and liquiritigenin from this plant have been shown to exhibit anti-hyperlipidemic activity. Epicatechin (-), the active principle is reported to be insulinotropic in action and demonstrated to enhance insulin release in cell lines \(^{[31]}\).

**Trigonella foenum graecum (fenugreek):** The plant is found all over India and fenugreek seeds are widely used as one of the major constituents of Indian spices. 4-hydroxyleucine, a novel amino acid from fenugreek seeds has been shown to stimulate GSIS in isolated islet cells from both rats and humans \(^{[32]}\). A dose-dependent reduction of blood glucose in both normal as well as diabetic rats has been observed with oral administration of 2 and 8 g/kg of plant extract \(^{[33]}\). Administration of fenugreek seeds has been reported to improve glucose metabolism and normalize creatinine kinase activity in heart, skeletal muscle and liver of diabetic rats along with reduction of hepatic and renal G-6-Pase and fructose 1,6-biphosphatase activity.
**Tinospora cordifolia (Guduchi):** It is a large, glabrous, climbing shrub belonging to the family Menispermaceae. The plant is widely distributed all over India and commonly known as Guduchi. *T. cordifolia* has been widely used for treatment of T2DM as an Indian ayurvedic medicine. Chronic treatment in alloxan induced diabetic rats were shown to exhibit significant reduction in blood glucose and total plasma lipids when orally administered with *Tinospora cordifolia* root extract for 6 weeks. The extract also caused a decrease in body weight. Though the aqueous extract at a dose of 400 mg/kg has been effective and could elicit significant anti-hyperglycemic effect in various animal models, its effect was equivalent to only one unit/kg of insulin. A, once a day oral administration of either alcoholic or aqueous extract of *T. cordifolia* has been reported to cause decrease in the blood glucose level and also increases glucose tolerance in rodents.

**Achyranthes aspera L. (Amaranthaceae):** The ethanolic extract of *Achyranthes aspera* exhibited significant dose-related anti-hyperglycemic effect in normoglycaemic and alloxan-induced diabetic rabbits orally administered with 2, 3, and 4 g/kg. In these animals, water and methanol extracts also decreased blood glucose levels. A

**Nelumbo nucifera (Nymphaeaceae, Lotus):** Methanol extract of *Nelumbo nucifera* Gaerth (East Indian Lotus) obtained from finely pulverized rhizomes has been shown to exhibit anti-diabetic activity in diabetic rodent models. Oral administration of methanol extract (300 mg/kg and 600 mg/kg) in STZ induced diabetic rats showed a 53 % and 55 % decrease of blood glucose at 12 h. Oral administration of the ethanolic extract of rhizomes of *N. nucifera* resulted in marked reduction of blood glucose in normal, glucose-fed hyperglycaemic and streptozotocin-induced diabetic rats as compared to vehicle group. The extract improved glucose tolerance and potentiated the action of exogenously injected insulin in normal rats.

### 1.2 Type 2 Diabetes

Type 2 Diabetes mellitus (T2DM) is a prevalent metabolic disease throughout the world and is comprised by an array of dysfunction characterized by hyperglycemia mainly due to the impaired insulin secretion or insulin resistance (insulin insensitivity), impaired regulation of hepatic glucose production, impairment of GIP
mediated insulinotropic effect and abnormalities in fat, carbohydrate, and protein metabolism in patients. T2DM is associated with microvascular (i.e., retinal, renal, possibly neuropathic), macrovascular (i.e., coronary, peripheral vascular), and neuropathic (i.e., autonomic and peripheral) complications. It is a severe menace to the middle-aged and elderly people, and there is a huge unmet need for drugs having superior safety profile. Unlike patients with type 1 diabetes mellitus (T1DM), patients with T2DM are not completely dependent upon insulin for life. This distinction was the basis for the older terms for type 1 (insulin dependent) and type 2 (non-insulin dependent) diabetes. However, many patients with T2DM are ultimately treated with insulin at later stage of disease wherein the β-cells are completely destroyed along with damage in muscle, liver and fat cell. They are considered to require insulin during progression of disease, as they retain the ability to secrete some endogenous insulin, but not to completely depend upon exogenous recombinant insulin injections. Nevertheless, given the potential for confusion due to classification based on the treatment rather than etiology, these terms have now been abandoned. Currently, because of the prevalence, epidemic of obesity and reduced physical activity in children, the onset of T2DM begins in children aged as low as 2 yrs. T2DM is a chronic disease that needs a long-term medical attention to limit the progression of its devastating complications and to manage them life long. Presumably, T2DM develops due to diabetogenic lifestyle (i.e., excessive caloric intake, inadequate caloric expenditure, obesity) is superimposed upon a susceptible genotype. The body mass index at which excess weight increases risk for diabetes varies with different racial groups. About 90% of patients who develop T2DM are obese. However, diabetes mellitus may also be caused by environmental pollutants that may play a role in the development and progression of disease. Products from natural herbs of known anti-diabetic potential would provide an inherent larger-scale structural diversity and a major resource of bioactive agents for discovery of novel drugs with potential for much reduced side effects. The anti-diabetic potential of cinnamon extract in animal models has been well established and it is being used as herbal medicine by many patients; however the mechanism of action by which it shows anti-diabetic action is not explored and studied in detail. There are many drugs available for the treatment of T2DM like, insulin sensitizers TZDs (Glitazones), recombinant human insulin, increasing endogenous insulin production with sulfonylureas and meglitinides, biguanides, alpha-glucosidase inhibitors, and the recently approved ones, like GLP-1
mimetics \[^{[41]}\] , DPP-IV inhibitors (Gliptins like, Vildagliptin, Sitagliptin, Saxagliptin and Allogliptin) \[^{[42-43]}\] and DPP IV resistant GLP-1 analogs such as Exendin-4 \[^{[44]}\] , Liraglutide and Lixisenatide which is undoubtedly a major advance in such a direction. However, there are associated side-effects with most of these drugs and there is also a black-box warning from food and drug administration (FDA), USA, for the potential risk of pancreatitis and cancer. New generations of small molecule agonists for various GPCR targets and improved DPP-IV inhibitors are being investigated for improved efficacy and superior safety profiles. This includes (a) Insulin sensitizers including protein tyrosine phosphatase-1B inhibitors (PTP1B), glycogen synthase kinase 3 (GSK3), (b) Inhibitors of gluconeogenesis like pyruvate dehydrogenase kinase (PDH) inhibitors, (c) Fat oxidation including carnitine palmitoyltransferase (CPT) I, II inhibitors, (d) PPAR\(\alpha/\gamma\) dual agonists and (e) Specific DPP-IV inhibitors, (f) GPR40 agonists, and (g) GPR120 agonists. With many new opportunities for drug discovery, the prospects are excellent for development of innovative therapies to effectively manage diabetes and prevent its long term complications.

\[\text{Figure 1.1. Progression of Diabetes}\]

\[\text{Epidemiology}\]

\[\text{1.2.1 Prevalence and pathogenesis of T2DM}\]

Although T2DM typically affects individuals older than 40 years, it has been diagnosed in children as young as 2 years of age who have a family history of
diabetes and the prevalence of the disease increases with advancing age. T2DM is the most common form of diabetes comprising 90-95% of overall diabetic population and approximately 5-10% have type 1 diabetes and 1-5% has other types. With the increasing number of obese people, the elderly and members of higher-risk minority groups in the population, prevalence is increasing. Prevalences have been calculated for each country and region in two ways: (a) National or regional prevalence and (b) Comparative prevalence. T2DM is a heterogeneous disorder having varying prevalence among different ethnic groups. In the United States the populations most affected are Native Americans, particularly in the desert Southwest, Hispanic-Americans, African-Americans, and Asian-Americans. However, Caucasian-Americans are also affected, but not at the same disproportionate percentage levels. IDF (International Diabetes Federation) Atlas, 2009 reported global prevalence of T2DM (in age group: 20-79) as 6.6% (285 million) in 2010, which is projected to increase up to 7.8% (close to 552 million) by 2030. More worrying is 7.9% prevalence of patients with impaired glucose tolerance (IGT), a pre-diabetic stage which is projected to increase to 8.4% by 2030. T2DM, once thought to be diseases of rich (developed) countries is more prevalent in developing countries like India, China. India is the diabetes capital of the world with 12% prevalence of T2DM.

Figure 1.2. Prevalence (%) of people with diabetes by age and sex.

According to Centers for Disease Control and Prevention (CDC) report in 2011, nearly 26 million Americans are estimated to have diabetes \(^{[45]}\). Additionally, an estimated 79 million Americans have prediabetes (IGT). CDC projected that as many
as 1 in 3 U.S. adults could have diabetes by 2050 if current trends continue. According to the National Diabetes Fact Sheet for 2011, diabetes affects around 8.3% of Americans of all ages and 11.3% of adults aged 20 years and older \cite{45}. Prediabetes affects 35% of adults aged 20 years and older. Pre-diabetes, as defined by the American diabetes association (ADA), is that state wherein blood glucose levels are elevated than the normal but not high enough to be diagnosed as diabetes. It is presumed that most people with elevated glucose levels approaching the level required for diagnosis of diabetes would subsequently progress to diabetes. The disease is increasing and more people are developing diabetes due to following reasons: a) changed life style, but people with diabetes are living longer (due to improved disease management), b) glycated hemoglobin A1c [HbA1c] diagnostic tests are now frequently used and c) Increased awareness in people. Indeed, the aging of the population is one reason that T2DM is becoming increasingly common. Virtually all cases of diabetes mellitus in older individuals are of type 2. In addition, however, the incidence of T2DM is increasing more rapidly in adolescents and young adults than in other age groups.

![Pathophysiologica Changes in Diabetes](image)

**Figure 1.3.** Pathophysiologica changes in body during diabetes.

The disease is being recognized increasingly in younger persons, particularly in highly susceptible racial and ethnic groups and the obese. Insulin resistance in muscle
and liver and failure of pancreatic β-cell represents the core pathophysiologic defects in T2DM. De Fronzo in his Banting Memorial Lecture considers eight body organs viz, muscle, liver, β-cell, adipose tissue (accelerated lipolysis), gastrointestinal tract (incretin deficiency/resistance), α-cell (hyperglucagonemia), kidney (increased glucose reabsorption), and brain (insulin resistance) responsible in the development of glucose intolerance in T2DM. Insulin resistance has been linked to increased adiposity primarily believed to be due to initial insensitivity to insulin resulting in peripheral insulin resistance and later on to insulin deficiency. So, basically the key cellular dysfunction in T2DM is not due to the β-cells (as in Type-1 diabetes), but rather due to the defects in muscle, liver, and fat cells, and also the damage to the red blood cells (due to glycation).

### 1.2.2 International statistics

Incidence of diabetes is increasing worldwide. At least 366 million people currently have diabetes, and this figure is likely to more than double to 552 million by 2030. Of this, India alone (2012) has around 53.8 million diabetics which is going to increase up to 87 million by 2030. The prevalence of T2DM in urban Indian adults has increased from < 3% in the 1970s to > 12% in 2012. The top 10 countries (with respect to numbers of people with diabetics) are currently India, China, the United States, Indonesia, Japan, Pakistan, Russia, Brazil, Italy, and Bangladesh [46]. The greatest increase in percentage with diabetes will occur in Africa in over the next 20 years. However, at least 80% of people in Africa with diabetes are undiagnosed, and many in their 30s to 60s will die from diabetes being undiagnosed. The global population is getting progressively older and more obese in most of the countries.

### 1.2.3 Risk factors for T2DM

The genetics of T2DM is complex and poorly understood. However, there are evidence that support the involvement of multiple genes in pancreatic β-cell dysfunction and insulin resistance as the major driving cause. Following are the major risk factors for T2DM:

- Age ≥ 45 years (though, as noted above, type 2 diabetes mellitus is occurring with increasing frequency in young individuals)
- Weight greater than 120% of desirable body weight
• Family history of type 2 diabetes in a first-degree relative (eg, parent or sibling)
• Hispanic, Native American, African American, or Asian American,
• History of previous impaired glucose tolerance (IGT) or impaired fasting glucose (IFG)
• Hypertension (>140/90 mm Hg) or dyslipidemia (high-density lipoprotein [HDL] cholesterol level < 40 mg/dL or triglyceride level >150 mg/dL)
• History of gestational diabetes mellitus or of delivering a baby with a birth weight of >9 lb
• Polycystic ovarian syndrome (which results in insulin resistance)

Some forms of diabetes however, have a clear association with genetic defects. The syndrome previously known as maturity onset diabetes of youth (MODY) has now been reclassified as a variety of defects in β-cell function. To date, 6 mutations have been identified in various genes {HNF-1-alpha (named as MODY3), HNF-4-alpha, glucokinase gene (GCK and named as MODY2), IPF-1/PDX-1, HNF-1β and NUROD1}{\cite{47-48}}. All of these genes are expressed in the insulin-producing pancreatic β-cell, and mutations in the heterozygous state lead to β-cell dysfunction and diabetes mellitus. These genes are also expressed in other tissues, and altered liver function and kidney and genital abnormalities may be evident in some forms of MODY especially HNF-1β–related MODY (MODY5). A primary defect in insulin secretion was coined in original definition of MODY due to decreased insulin responses to oral or intravenously administered glucose, or defective insulin secretion, or mutations in the glucokinase gene leading to decreased enzymatic activity, including lower affinity for glucose and ATP and catalytic activity {\cite{48-51}}.

1.2.4 Diabetes, associated mortality and morbidity

Diabetes mellitus is one of the leading causes of morbidity and mortality in the United States and in India because of its role in the development of cardiovascular, renal, neuropathic, and retinal disease{\cite{52}}. These complications, particularly cardiovascular disease are the major source of expenses for patients with T2DM. Around, 4.6 million people between the age group of 20-79 years died from diabetes in 2011, accounting for 8.2% of global all-cause mortality of people in this age group. This estimated number of deaths is similar in magnitude to the combined deaths from several infectious diseases. Forty-eight percent of deaths due to diabetes are in people under
the age of 60 and these are mainly in countries with the largest numbers of diabetic patients: India, China, United States of America, and the Russian Federation. T2DM is also the leading contributor of end-stage renal disease (ESRD) in the United States. Intensive glycemic control (HbA1c < 6.5%) may be deleterious in patients with chronic kidney disease (CKD) and higher HbA1c (≥ 9.0%) is associated with adverse outcome in stages III to IV CKD patients. Generally, patients with diabetes have a higher risk of developing bladder cancer and coronary heart disease (CHD) than normal. Cardiovascular disease is the major source of mortality in patients with T2DM. Nearly, two-thirds of people with diabetes die because of heart attack or stroke. In T2DM, the early onset type group (duration >10 y) appears to be at higher risk of major CHD. Studies by Kengne et al in 2012 shows linkage of C-reactive protein (CRP) with cardiovascular disease and associated risk factors.

![Figure 1.4. Deaths attributable due to diabetes in age group (20-79) by IDF 2011 report. (Source: IDF reports, 2011).](image)

1.2.5 **Insulin resistance and β-cell dysfunction in diabetes**

Insulin resistance simply means insensitivity to insulin. The fat, liver, and muscle cells of patients with T2DM do not respond correctly to insulin and this is broadly termed as “insulin resistance”. As a result of this physiologic condition, blood glucose does not get into the major cells for storing energy. When glucose does not enter cells, it starts building up in the blood leading to hyperglycemia. T2DM usually occurs slowly over time. Most people with the disease are overweight when they are diagnosed. Insulin resistance is a metabolic hallmark of T2DM, wherein pancreatic β-
cell dysfunction is common and represents a diagnostic determinant of the disease, resulting in the loss of GSIS. Researchers have identified the molecular pathways involved in the progression of T2DM from mouse model [56]. These mice lacked mgat4a gene that codes for GnT-4a glycosyltransferase. GnT-4a is involved in the positioning of the slc2a2 that code for glucose transporter-2 (GLUT-2) glycoprotein on the cell surface. Protection from disease can be conferred by enhanced β-cell-specific GnT-4a protein glycosylation which eventually leads to GLUT surface expression and the preservation of glucose transport [57]. The major question that still remains unanswered in insulin resistance is to how does excess body-fat content culminates into this condition? It is generally believed (Lipid ectopia hypothesis) that the initial culprit is intra-abdominal fat/visceral fat in abdominal viscera [57] and not the subcutaneous fat. Adipose tissues can hold only a certain amount of fat and excess load results into redistribution of lipid to ectopic sites like, liver and skeletal muscle. Hepatic steatosis is commonly observed in individuals with the metabolic disorders. Non-alcoholic fatty liver disease has a prevalence of 57% to 74% in obese individuals. The ectopic triglyceride (TG) deposition in liver and skeletal muscle has deleterious effects. There is both tissue damage (lipotoxicity) and progression of insulin resistance.

![Type 2 Diabetes: Insulin Resistance](image-url)

**Figure 1.5.** Insulin resistance and downregulation of GLUT4 in Diabetes. (Source: diabetes.org)

Another aspect of the lipid ectopia hypothesis suggests that β- cells that are damaged by excess fat deposition culminate into a gradual failure to produce sufficient insulin. Evidence for validity of this hypothesis comes from rare cases of lipodystrophic
diabetes. Circulating FFAs, resistin, and TNFα potentially worsens the insulin resistance and leads to decreased insulin sensitivity and reduced glucose disposal in muscle, fat, liver etc. The use of Thiazolidinedione (TZD) class of drugs that activate PPARγ receptor may stimulate the development of new adipose tissues, allowing the redistribution of stored fat. TZDs are known to be effective in treating T2DM; however their use is limited due to their potential to cause side effects like edema, weight gain, cardiovascular risk etc.

**Beta-cell dysfunction**

T2DM is characterized by defects in insulin action and insulin secretion and both are believed to be genetically predetermined. In diabetic patients, during the absence of a defect in β-cell function, individuals can compensate indefinitely for insulin resistance with hyperinsulinemia, as observed in obese people like the Pima Indians of Arizona. However, loss of β-cell function eventually results in fasting and postprandial hyperglycemia, majorly observed in T2DM. β-cell dysfunction leads to impaired glucose tolerance (IGT), failure of GSIS leading to hyperglycemia, hepatic steatosis, systemic insulin resistance and diminished insulin action in muscle and adipose tissues. Preservation of β-cell GnT-4a glycosylation and GLUT-1, GLUT-2 expressions may break this pathogenic cycle and its link to diet and obesity. Mouse and human studies by researchers in the U.S. and Japan suggests that elevated levels of FFAs leads to both reduced expression of the transcription factors; FOXA2 and HNF1A in pancreatic β-cells and their exclusion from the cell nuclei resulting into a decreased expression of GnT-4A glycosyltransferase in β-cells \(^{[57, 59]}\). Studies by Kazuaki et al in 2011 demonstrate that wild-type mice fed with high-fat diet (HFD) became deficient in pancreatic MGAT4A and SLC2A2 RNA, as compared to mice fed with a regular diet \(^{[58]}\), due to a reduced binding of FOXA2 and HNF1A in the promoter region of these genes. This reduced binding coincides with decreased abundance of the FOXA2 and HNF1A proteins, a marked reduction in their nuclear localization and increased cytoplasmic localization \(^{[58]}\). Additional evidence came from studies wherein palmitic acid (PA) treatment to cultured islet cells from mice fed with normal diet caused a nuclear exclusion of FOXA2 and HNF1A resulting into downregulation of MGAT4A and SLC2A2 (GLUT2) gene expression. Similar studies in human islets from healthy non-diabetic donors showed that addition of palmitic
acid resulted in reduced nuclear localization of FOXA2 and HNF1A and diminished binding to the promotor regions of MGAT4A, SLC2A1, and SLC2A2 genes, resulting in attenuation of mRNA expression of three genes, which coincided with the loss of GSIS suggesting that islets from human T2DM patients (donors) may show defects comparable to those observed in mice fed with HFD \[^{58}\]. Some external factors, like consumption of high-calorie diet, lack of exercise, weight gain etc. poses increased insulin requirements in insulin resistant conditions. Glucotoxicity and lipotoxicity induced \(\beta\)-cell dysfunction could be reversed with appropriate restoration of metabolic control. Therefore, attention to these toxicities can delay the onset of \(\beta\)-cell deterioration to major extent \[^{59}\].

1.2.6 Role of incretins in insulin signaling and glucose homeostasis

These incretin hormones are also reported to be associated with insulin biosynthesis, proliferation of pancreatic \(\beta\)-cells and inhibition of food intake \[^{60, 41-43, 61-62}\]. Incretin hormones modify the activity of both cell types as appropriate. In diabetes, however, the normal incretin response is lost, GLP-1 secretion is reduced by around 25 per cent, \(\beta\)-cells are reduced in number and are under-active; insulin feedback to alpha cells is diminished so these are persistently overactive \[^{42, 62}\].

![Figure 1.6. Role of incretin hormone in glucose homeostasis.](image)
The healthy glucagon-insulin balance is lost resulting in both fasting and postprandial hyperglycaemia. There are two ways of attempting to restore the correct balance via the incretin response. Incretin mimetics or GLP-1 analogs boost GLP-1 levels artificially to supra-physiological levels while incretin-enhancers inhibit the DPP4 enzyme to sustain GLP-1 levels and prolong its activity as shown in Figure 1.6. Both drug types are effective in reducing HbA1c \[42-44\]. Incretin mimetic- Byetta (Exenatide, Lilly), Victoza (Liraglutide, Novo Nordisk) and Bydureon (Exenatide-LAR, Lilly) delays gastric emptying and increases satiety leading to weight loss of around 4 to 5 kgs over two years. It reduces HbA1c by around 1%. "Incretin enhancers (DPP-IV inhibitors), administered orally, do not delay gastric emptying, but they boost insulin sensitivity and may preserve β-cell function and increase β-cell mass, according to animal studies. There are currently three approved GLP-1 mimetic drugs for clinical use, Exenatide (Byetta), Liraglutide (Victoza) and Lixisenatide (Lyxumia) for treatment of T2DM. Byetta is a twice-daily injection and recently an extended release, once weekly formulation of same (Bydureon) is launched (approved in early 2012) and achieves satisfactory reductions in HbA1c.

### 1.2.7 Current drugs for the treatment of T2DM

T2DM is a complex disease and requires a lifelong commitment to following:

- Regular blood sugar monitoring
- Healthy eating with balanced calories
- Regular exercise, with diabetes medication or insulin therapy

Taking adequate measures would help keep the blood sugar level within normal range and delay the further progression. There are many drugs currently available for the treatment of T2DM and efficacious in lowering blood glucose levels to a recommended HbA1c level of < 7% (8.5 mmol/l, whole blood glucose). Healthy individuals have HbA1c <5.7% (6.2 mmol/l, whole blood glucose) and the range in pre-diabetic individuals varies from 5.7-6.4%. Diabetic individuals have HbA1c levels ≥ 6.5% (7.7 mmol/l, whole blood glucose). Most of the currently available medications falls into two categories: (i) those that increase insulin synthesis and secretion in pancreatic β- cells like sulfonylureas, other secretagogues, insulin analogs, GLP-1 mimetics etc.; (ii) those that help to reduce insulin resistance like
biguanides, thiazolidinediones, DPP-IV inhibitors. Although these drugs are efficacious, there are certain limitations with respect to long term safety, occurrence of hypoglycemia or common side effects like nausea, dizziness etc. Following drugs/class of drugs shown in table are available for clinical use.

Table 1.1. Current class of drugs for treatment of diabetes.

<table>
<thead>
<tr>
<th>Drug Class</th>
<th>Molecular target</th>
<th>Site of action</th>
<th>Adverse events</th>
</tr>
</thead>
<tbody>
<tr>
<td>Insulin</td>
<td>Insulin receptor</td>
<td>Liver, muscle</td>
<td>Hypoglycemia, wt gain</td>
</tr>
<tr>
<td>Sulphonylurea</td>
<td>SU receptors</td>
<td>Beta cells</td>
<td>Hypoglycemia, wt gain</td>
</tr>
<tr>
<td>biguanides</td>
<td>Unknown</td>
<td>Liver</td>
<td>GI problems, lactic acidosis</td>
</tr>
<tr>
<td>Acarbose</td>
<td>Alpha glucosidase</td>
<td>Intestine</td>
<td>GI problems</td>
</tr>
<tr>
<td>Tiazolidinediones</td>
<td>PPAR-gamma</td>
<td>Fat, liver, muscles</td>
<td>Wt gain, oedema, anaemia</td>
</tr>
<tr>
<td>Oral DPP-IV</td>
<td>DPP-IV enzyme</td>
<td>Intestine, in blood circulation</td>
<td>Thrombocytopenia, alopecia</td>
</tr>
<tr>
<td>inhibitors</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>GLP-1 mimetics</td>
<td>GLP-1 R</td>
<td>Pancreas, CNS</td>
<td>Pancreatitis, nausea, risk for thyroid cancer</td>
</tr>
</tbody>
</table>

a) Medicines that mimic natural insulin or insulin therapy

Some individuals with T2DM can manage their blood sugar with diet and exercise alone, but others need oral medications or insulin therapy. Some studies indicate that early intervention with medication, even before the HbA1c is significantly elevated, may improve control of blood sugar levels over time. Diabetic patients who are unable to control their blood glucose levels in normal range with available oral anti-diabetic medicines are usually administered insulin injections. Most commonly available are a combination of short-acting and long-acting insulin. These insulin analogs help to maintain blood glucose within target range. Insulin pumps that are designed and programmed to dispense specific amounts of insulin automatically are also advised to some patients. It can also be adjusted to deliver more or less insulin depending on meals, activity level and blood glucose level. Currently, there are many available brands of such insulin injections including the rapid-acting insulin or long-acting insulin generic versions like Lispro insulin (Humalog), Insulin aspart (NovoLog), Insulin glargine (Lantus), Insulin Detemir (Levemir) and Humulin.
Patients, who use glargine as basal insulin, are reported to have lower incidence of hypoglycemia, lower fasting blood sugars and less weight gain than with intermediate-acting insulin preparations (e.g. NPH insulin) [63]. However, insulin analogs can cause hypoglycemia and mild pain at the injection site.

a) Medicines that helps β-cells to produce and secrete more insulin

*Sulphonylurea class of drugs:

When metformin as monotherapy is not sufficient to control glucose levels, other oral or injected medications are required to lower the blood glucose in T2DM. Sulfonylureas class of drugs helps the body to produce and release more insulin from β-cells. While 1st generation medicines like Diabinese and Tolinase are less efficacious, 2nd generation drugs are more potent, efficacious, and differ in dosage and duration of action. These are Glipizide (Glucotrol, Glucotrol XL), Glyburide (DiaBeta, Glynase, and Micronase), glimepiride, and other medicines that work in combination (Glucovance, Metaglip). Meglitinides like Repaglinide (Prandin) and Nateglinide (Starlix) increase the insulin secretion from pancreas. Meglitinides and a combination medicine (Prandimet) are efficacious in lowering postprandial blood glucose in T2DM [64]. These drugs interact with ATP-sensitive potassium channels in the β-cell, increases the intracellular calcium levels and insulin secretion. The adverse reactions to these medications include weight gain and hypoglycemia.

*DPP-IV Inhibitors:

This class is well known as “Gliptins” and helps to maintain sustained levels of endogenous incretin hormones like GLP-1 and GIP. These drugs inhibit the circulating plasma DPP-IV enzyme that usually cleaves the incretins and makes them biologically inactive. Thus, the patients are advised to take this medicine before meal (once a day or twice a day depending on dosage and duration of action; short acting or long acting). They are usually known as “incretin enhancers” as they enhance the endogenous levels of incretins [65-67]. DPP-IV inhibitors lower HbA1c values by 0.74%, comparable to other antidiabetic drugs. Various drugs of this class are approved by U.S. FDA and are available for clinical use and treatment of T2DM like Sitagliptin (Januvia, approved by U.S. FDA in Oct’ 2006), Saxagliptins (Onglyza, approved by U.S. FDA in July’ 2009), Vildagliptin (Galvus, approved by EU in 2008)
Linagliptin (approved by U.S. FDA in May’ 2011 and Alogliptin (approved by U.S. FDA in early 2012). There are many more drugs of this class in various phases of clinical trial and would likely to be available for use upon approval.

**Incretin Mimetics:**

These classes of drugs unlike others are injectable. Pharmacological actions of these drugs mimic the endogenous incretins like GLP-1(7-36) which is secreted by the gut cells post meal, circulated in blood and act via GLP-1 receptor in pancreas. They have 52% (Exenatide) to 97% (Liraglutide) homology with endogenous GLP-1(7-36) peptide. The therapeutic use of both, GLP-1 and GIP was limited owing to their shorter half life ($t_{1/2} \approx 1$ min) due to cleavage by circulating DPP-IV. The second generation mimics such as Exenatide (Byetta TM, available in 5-10 µg dosage, twice daily), Bydureon (approved in Jan 2012; once weekly extended release exenatide formulation), Amylinomimetics, such as pramlintide (Symlin), Liraglutide (Victoza, approved in 2010 and available as once-daily injection) became available for clinical use in 2005. Few other drugs of this class like Taspoglutide and Albiglutide are currently in Phase-III clinical trials. These next generation mimics are DPP-IV resistant and thus are more effective and efficacious pharmacological agents for enhancing the insulin release from β-cells via activating GLP-1R. Taspoglutide, Liraglutide and others have amino acid site for fatty acid acylation wherein albumin binds thereby increases their *in vivo* half life. Because of the mechanism, these drugs also cause moderate weight reduction (1.5 to 3.5%). However, these agents also cause common side-effects like nausea, vomiting and decrease in gastric motility. However, there exists a blackbox warning from U.S. FDA with both available drugs viz: Byetta and Victoza which cautions towards developing increased risk for pancreatic and thyroid cancer and pancreatitis in patients taking these medications. These peptidic drugs (GLP-1 R-agonists) bind to GLP-1 receptor and activate them for longer duration to cause sustained increase in insulin secretion from pancreas.

**b) Medicines that helps body to reduce insulin resistance**

**Biguanide class of drugs:** Commonly used biguanides, like metformin (Glucophage, Glumetza) and other oral medicines that are usually combined with metformin (Prandimet, Avandamet) are used for the treatment of T2DM. These medicines help
body to reduce hepatic glucose production (HGP) and increase peripheral glucose utilization. However they may cause side effects such as nausea and lactic acidosis which is rare but potentially fatal complication associated with these drugs. The Glucophage XR with once a day dosage form is as effective as sulfonylurea in lowering HbA1c. They do not cause hypoglycemia or weight gain on their own, perhaps they may cause weight loss due to gastrointestinal effects.

**Thiazolidinediones (TZDs):** TZD class of drug like, pioglitazone (Actos) is currently used for the clinical treatment of patients with T2DM. They block the action of enzymes involved in breakdown of carbohydrates or make tissues more sensitive to insulin (Insulin sensitization). These drugs are reported to activate the NRs known as PPARs. The activated PPAR upregulate the genes involved in glucose regulation. It is reported that the ligand and co-activator type determines the requirement of charge clamp for coactivation of PPARγ receptor[^70^]. The main sites of action for these drugs are muscle, fat and liver. However these medicines are associated with side effects and they may cause nausea, weight gain or edema (fluid retention). The first TZD drug-Troglitazone has been withdrawn from the market due to its fatal effects on hepatocytes. Rosiglitazone (Avandia) has been banned and only pioglitazone is approved for clinical use.

c) Medicines that slows down the absorption of carbohydrate: Alpha-glucosidase inhibitors

These drugs usually slow the absorption of carbohydrate in the intestine and thus prevent the rapid surge in blood glucose. These medications inhibit the alpha-glucosidase enzymes that line the brush border of the small intestines, interfering with hydrolysis of carbohydrates and delaying absorption of glucose and other monosaccharide. They are usually taken with meal and reported to cause 0.5 to 1% reduction in HbA1c levels. These include: Alpha-glucosidase inhibitors, such as acarbose (Precose) and miglitol (Glyset). The adverse reactions include abdominal pain, bloating and flatulence due to unabsorbed carbohydrates. Because of the severity of their side-effects, these medications are rarely used in the United States, however commonly prescribed in Europe. They do have the potential to cause weight loss by lowering the amount of sugar metabolized.
1.2.8 *Cinnamomum zeylanicum* as anti-diabetic herb

**Nomenclature:**

Botanical Species: *Cinnamomum zeylanicum* blume

Genus: Cinnamon, Family : Lauraceae

Parts Used: Bark (inner bark), leaves, flowers, buds and fruits.

**Common name and Synonyms:** Commonly known as Cinnamon, dalchini (in hindi) and native to Bangladesh, srilanka, india, china and other countries. It is represented by 250 species of which 26 species are commonly found in India. It is also known as: Ceylon Cinnamon (derived from the Srilanka’s old name Ceylon) and *Cinnamomum verum*.

**Distribution:** *C.zeylanicum* is a perineal shrub indigenous to many regions of India (mainly Himanchal pradesh, Rajasthan, South India, Rajputana Dessert, and also in other countries like Sri lanks, China, United states etc.

**Commercial source:** Bark was obtained from local source, Manakarnika Aushadhalaya, Chinchawad, Pune, Maharashtra.

**Cultivation and harvesting techniques:** Light porous soil is preferred for cultivation. The tannin content of bark usually increases with the plant age.

**Handling, drying, packing, and storage:** Bark, flowers, and seeds after sorting are shade dried and stored in gunny bags in dark.

**Traditional use for medical indications:** Cinnamon has been traditionally used as a culinary spice and medicinal herb for many years. This popular aromatic spice was used to support healthy microbial balance and gastrointestinal function. However, research on cinnamon has led to exciting discoveries with respect to its beneficial glucose lowering effects, effects on body composition and cardiovascular health, and its role as an antioxidant. True Cinnamon, is *Cinnamomum verum* (synonym: *Cinnamomum zeylanicum*), native to Bangladesh, India and Nepal. The genus *Cinnamon* belongs to Lauraceae family and is represented by 250 species of which 26 species are commonly found in India. It is the inner bark (and sometimes essential oil) that is referred to as the cinnamon spice in Latin America and Europe. Beneficial use of *C. zeylanicum* is as old as civilization. All parts of *C. zeylanicum* are used extensively in Traditional systems of medicine like Ayurveda and the Traditional
Chinese medicinal system. Cinnamon is also one of the oldest remedies used in traditional Chinese herbalism. In Indian Traditional system of medicine, it is used for treating diabetes and sinuses. The 12th century German abbess and herbalist, Hildegard of Bingen, recommended *C. zeylanicum* as "the universal spice for sinuses" and to treat cold, flu, cancer and "inner decay and slime." In American continent, it is used orally for the treatment of diarrhea, stomach upset, against respiratory ailments and externally as a skin antiseptic and rubefacient. *C. zeylanicum* also finds important place in modern Herbal medicine. Some of them are discussed in brief. Pre-diabetes is a condition in which blood glucose levels are higher than normal, and the cells that would normally accept insulin from the pancreas in order to lower these levels begin to reject it. According to studies, cinnamon (*Cinnamomum aromaticum*) may support healthy blood sugar levels when used as part of diet, by activating insulin secretion, glucose transport and improving glucose metabolism.

**In vitro and in vivo studies:** The anti-diabetic activity of cinnamon extracts has been attributed to a variety of compounds including polyphenols [71, 72-74], hydroxychalcones [75], and cinnamaldehyde [76-77]. Polyphenols from cinnamon has been shown to improve insulin sensitivity and glucose uptake *in vitro* in pre-adipocyte cells and in animal studies [78, 123]. *Cinnamom cassiae* contains cinnamaldehyde, cinamonic acid, tannin and methyl-hydroxychalcone polymer (MHCP) as main components. Cinnamon Extract (CE) is reported to exhibit insulino-mimetic properties and improve glucose tolerance in *db/db* mice [79]. *In vitro* and *in vivo* studies have demonstrated that cinnamon enhances glucose uptake by activating insulin receptor kinase activity, autophosphorylation of insulin receptor and glycogen synthase activity [80]. Some studies also reported the hepatoprotective effects of aqueous and ethanolic cinnamon extracts against carbon tetrachloride CCl4 induced lipid peroxidation and hepatic injury in rats. In these studies the elevated serum AST and ALT enzymatic activities induced by CCl4 were significantly restored to near normal by oral administration of 200 mg/kg of either extracts (oral, once daily for 7 days), as compared to untreated rats [81]. Anderson et.al in 2004 demonstrated that cinnamon extract, mainly the water-soluble polyphenolic polymers like procyanidin oligomers of catechins and/or epicatechins enhances the activity of insulin [72].
Desoky et.al in 2012 demonstrated the hypoglycemic and hypolipidemic effects of cinnamon extracts when administered in alloxan induced diabetic rats with significant improvements in body weight gain, FI and FER \(^{[82]}\).

**Pre-clinical and Clinical studies:** The positive effects of cinnamon on glucose metabolism were discovered by accident at a U.S. FDA testing center when scientists were assessing the effects of various foods on blood sugar levels. According to their studies, cinnamon lowered blood sugar levels by almost 30%, increased insulin production and lowered total cholesterol. A water-soluble cinnamon extract \(^{[71]}\), which is high in antioxidant, has been reported to reduce blood glucose levels and oxidative stress in the body. Other studies have demonstrated that aqueous cinnamon extract (500 mg/day) plays a vital role in maintaining healthy circulation and energy levels in the body and reduce risk factors associated with diabetes. It decreased oxidative stress and improved impaired fasting glucose \(^{[83]}\). Both *Cinnamomum zeylanicum* and *Cinnamomum cassia* appears to have some beneficial role in lowering blood glucose levels although study results have not always been consistent.

Aqueous Cinnamon extract is reported to lower glucose, total lipid levels and cholesterol in diabetic patients \(^{[84-86]}\). In a randomized, controlled trial study that included 109 pediatric, adult and geriatric patients with type 2 diabetes, administered with 1gm cinnamon capsules daily for 90 days. HbA1c was drawn at baseline and after 90 days. Cinnamon lowered HbA1c by 0.83% as compared to usual care alone, which lowered HbA1c by 0.37%. These results indicate that taking cinnamon in addition to usual care could be useful for reducing HbA1c in patients with T2DM. \(^{[87]}\). Naturally occurring chromium and polyphenols found in Cinnamon (*Cinnamomum cassia*) have been reported to have similar effects on insulin signalling and glucose control. A double-blind placebo-controlled study in T2DM patients who took Chromium supplementation showed an improvement in glucose levels, insulin, cholesterol and had lower HbA1c. Cinnamon polyphenol has been shown to improve insulin sensitivity in human studies \(^{[71]}\) as well. Studies in healthy volunteers who were administered 300 gm of rice pudding alone or 300 gm rice pudding along with 1-3 g cinnamon (oral) and aimed at evaluating the effect of cinnamon on the rate of gastric emptying, postprandial blood glucose response and satiety showed significantly delayed gastric emptying and lowered postprandial glucose without
affecting satiety [88-89]. Reports from yet another study wherein, seven lean young healthy male volunteers underwent three oral glucose tolerance tests (OGTT) supplemented with either 5 g placebo {OGTT (control), 5 g of cinnamon, OGTT (cin), or 5 g of cinnamon taken 12 h before (OGTT (cin12 h pre))} in a randomized-crossover design showed that Cinnamon ingestion reduced total plasma glucose as well as improved insulin sensitivity [90]. Mang et al in 2006 demonstrated that aqueous extract (amount corresponded to 3 gm/day; 4 months) shows moderate effect in reducing FBG in T2DM patients with poor glycaemic control (n=79) [91]. Cinnulin PF rich in polyphenolic compounds made from aqueous cinnamon extract is being used by many diabetic patients and claimed to be safe and effective nutrional solution to many patients suffering from insulin resistance. A Sino-american study showed glucose lowering effect of another patented aqueous Cinnamon extract (CinSulin) from Chinese company, Tang An Medical. The study findings were published in FASEB annual meeting in Anaheim and showed that diabetic subjects receiving CinSulin (500 mg orally, once a day for two months), had 7.5 % reduction in fasting blood glucose as compared to 1.5% reduction in placebo group [92]. Cinnamon bark contains antioxidants that may have significant inhibitory effects on AGES (advanced glycation endproducts). Cinnamon inhibits an alpha-glucosidase enzyme that helps metabolize carbohydrates into simple sugars, which would help to maintain glucose within normal range [93]. A meta-analysis study by Davis and Yokoyama in 2011 showed that cinnamon intake (either as whole cinnamon or as cinnamon extract) improves the FBG of patients with type 2 diabetes or pre-diabetes [231]. Cinnamon and its components have been shown to possess beneficial effects and improvement in insulin sensitivity, glucose, total lipid, antioxidants, blood pressure, and body weight in patients suffering from metabolic disorders. Different parts of C.zeylanicum have been studied to isolate a number of chemical constituents [94-95]. A brief review of this has been given along with structures and molecular formulae in Table 1.2.

**Isolation of components from different parts of Cinnamomum zeylanicum:**

**Leaves:** Leaves are good source of mannitol (7). Also (E)-Cinnamaldehyde and camphor (15%) has been reported from leaves.
**Flowers:** Analysis of steam-distilled oil of flowers by GC and GC-MS showed presence of 23% hydrocarbons and 74% oxygenated compounds. Major constituents are cinnamyl acetate (41.98%), trans-α-bergamotene (7.97%), and caryophyllene oxide (7.2%).

**Buds:** The cinnamon oil mainly contains; terpene hydrocarbons (78%) and oxygenated terpenoids (9%), α-Bergamotene (27.38%) and α-copaene (23.05%) (31).

**Fruits:** Five major purified compounds have been identified from fruit extract; 3,4-dihydroxybenzoic acid (protocatechuic acid), epicatechin-(2β-O-7,4→8)-epicatechin-(4β→8)-epicatechin (cinnamtannin B-1), 4-[2, 3-dihydro-3-(hydroxymethyl)-5-(3-hydroxypropyl)-7-methoxybenzofuranyl]-2-methoxyphenyl β-D-glucopyranoside (urolignoside), quercetin 3-O-(6-O-α-L-rhamnopyranosyl)-β-D-glucopyranoside (rutin), and quercetin 3-O-α-L-rhamnopyranoside by using extensive spectral studies.

**Stem bark:** Fractionation of the alkali-extractable polysaccharide, isolated from the stem bark of *C. zeylanicum*, gave α-glucan as the major component, with a (1→4)-linked α-D-glucan back bone. Other compounds that have been reported from stem bark are cinnamyl alcohol, coumarins, cinnamic acid and cinnamaldehyde.

**Essential oil (bark):** This has been extensively studied. Analysis of one such oil sample is given here - α -Pinene, α -phellandrene, p-cymene, linalool, β-caryophylene, α -terpineol, benzyl acetate, cinnamic aldehyde, eugenol, cinnamyl acetate, eugenyl acetate and benzyl benzoate, were main constituents as detected by gas chromatography and IR spectroscopy.

**Branches:** Major components identified from the branch oil are monoterpenes α-pinene (9.9%), β-pinene (3.5%), α-phellandrene (9.2%), p-cymene (6.2%), limonene (7.9%) and linalool (10.6%), followed by the sesquiterpenes α-copaene (3.3%), β-caryophyllene (6.7%), caryophyllene oxide (3.1%) and the allylbenzenes (E)-cinnamaldehyde (7.80%), (E)-cinnamyl acetate (9.7%).

**Table 1.2.** Major constituents (compounds) isolated and reported from *C. zeylanicum* stem bark extract.

<table>
<thead>
<tr>
<th>Sr.No.</th>
<th>Compound</th>
<th>Structure</th>
<th>Molecular formula</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Benzyl benzoate</td>
<td><img src="image" alt="Structure" /></td>
<td>C14H12O2</td>
</tr>
<tr>
<td></td>
<td>2. Borneol (Monoterpene)</td>
<td><strong>C_{10}H_{18}O</strong></td>
<td></td>
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<tr>
<td>---</td>
<td>--------------------------</td>
<td>-----------------</td>
<td></td>
</tr>
<tr>
<td></td>
<td>3. Isoborneol (Monoterpene)</td>
<td><strong>C_{10}H_{18}O</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>4. α-bergamotene (Sesquiterpene)</td>
<td><strong>C_{15}H_{24}</strong></td>
<td></td>
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<tr>
<td></td>
<td>5. Cinnamaldehyde (Phenylpropanoid)</td>
<td><strong>C_{9}H_{8}O</strong></td>
<td></td>
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<tr>
<td></td>
<td>6. Cinnamic acid (Phenylpropanoid)</td>
<td><strong>C_{9}H_{8}O_{2}</strong></td>
<td></td>
</tr>
<tr>
<td></td>
<td>7. Cinnamyl acetate (Phenylpropanoid)</td>
<td><strong>C_{11}H_{12}O_{2}</strong></td>
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<tr>
<td></td>
<td>8. Cinnamyl alcohol (Phenylpropanoid)</td>
<td><strong>C_{9}H_{10}O</strong></td>
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<tr>
<td></td>
<td>9. Cinnamyl butyrate (Phenylpropanoid)</td>
<td><strong>C_{13}H_{16}O_{2}</strong></td>
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<tr>
<td></td>
<td>10. Caryophyllene (Sesquiterpene)</td>
<td><strong>C_{15}H_{24}</strong></td>
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<td></td>
<td>11. Camphene (Monoterpene)</td>
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<td>12. Coumarin</td>
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<td>13. Copaene (Sesquiterpene)</td>
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<td>14. Ethyl cinnamate (Phenylpropanoid)</td>
<td><strong>C_{11}H_{12}O_{2}</strong></td>
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1.3 Target Proteins and Receptors Involved in Glucose Homeostasis

1.3.1 Dipeptidyl peptidase IV

Dipeptidyl Peptidase IV (DPP-IV or DPP4, EC3.4.14.5) is a tetrameric serine protease present in blood circulation and is involved in the cleavage of biologically active incretin hormones like glucagon like peptide (GLP-1,7-36, 7-37) , glucose-dependent insulino tropic polypeptide (GIP) and other secretory hormones that are physiological substrate, making them biologically inactive. DPP IV exists on the surface of various cell types, such as kidney, liver, small intestine, pancreas, and in a soluble form in the plasma [96]. This serine protease cleaves the penultimate L-proline or L-alanine at the N-terminus of several polypeptides [97]. GLP-1 and GIP are gastrointestinal peptides involved in the regulation of postprandial glucose homeostasis [98-99]. The hormones they target are secreted naturally in the gut in response to food intake to regulate the activity of both, alpha and beta islet cells of the pancreas. An endogenous physiological DPP-IV substrate is defined as a peptide whose endogenous circulating levels of intact versus NH2-terminally cleaved forms are altered following reduction or elimination of DPP-IV activity in vivo. Inhibition of DPP-IV is associated with increased plasma circulating levels of active GIP and GLP-1(7-36, 7-37) [65-66]. These peptides augment glucose-induced insulin release (GSIS)
from pancreatic β-cells \cite{98-99}. Abundant studies in both human and animal models have established the therapeutic importance of DPP-IV inhibition that results in GLP-1 elevation, as a promising strategy for the treatment of T2DM \cite{67-69,100-101}. Many, DPP-IV class of drugs are currently available for the treatment of T2DM, like Vildagliptin (Galvus, Novartis), Sitagliptin (Januvia, Merck Co.), Alogliptin (Takeda Pharmaceuticals), Saxagliptin (Bristol-Myers Squibb) etc. The Food and Drug Administration and the European Medicines Agency have approved few more DPP-IV inhibitor for clinical use. Researchers concluded from an \textit{in vitro} study, that DPP-IV activity and mRNA levels were enhanced by exposure of human glomerular endothelial cells to high glucose \cite{102}. However, there are contradicting clinical reports published in various journals claiming either increased circulating levels of DPP-IV \cite{103-104} or decreased \cite{105-106} in T2DM. Moreover, several reports, including clinical studies, have confirmed that widely used anti-diabetic agents, metformin \cite{98,107-108} and pioglitazone \cite{107} reduced the levels of circulating DPP-IV activity \textit{in vivo}. Drugs that work via the manipulation of incretins (GLP-1 and GIP) and play an important role in diabetes are already blockbusters in clinic. Pharmaceutical industries show more interest in developing orally-administered small molecule DPP-IV inhibitors. There are around 30 companies who have products in their pipeline and in various phases and many others who have completed P III and looking forward for an FDA approval.

\textbf{1.3.2 Alpha-glucosidase}

The inhibitors of alpha-glucosidase are known as Acarbose (Precose), miglitol, volix (Voglibose) class drugs. Acarbose (Precose, available as 25, 50 & 100 mg dose) helps to keep blood glucose levels within a target range by lowering the rate at which the intestines absorb glucose from food. These medicines neither induce pancreas to produce more insulin nor they cause low blood sugar (hypoglycemia) unless they are used in combination with other oral medicines for diabetes or with insulin. These classes of diabetes drugs are sometimes called “starch blockers” because they block the action of enzymes involved in breakdown of certain carbohydrates in the upper part of the small intestine. Because the drugs prevent the immediate breakdown of starches into monosaccharides, or simple sugars, that are absorbed into the
bloodstream quickly, more of the carbohydrate consumed at a meal gets absorbed further “downstream” in the gastrointestinal tract, toward the end of the small intestine or the colon. Slowed absorption of carbohydrate gives the β-cells more time to secrete adequate insulin to cover the meal. The first alpha-glucosidase inhibitor to become available in the United States was acarbose (Precose), which was approved by the U.S. FDA in 1995. A second alpha-glucosidase inhibitor, miglitol (Glyset) became available in 1999. Precose® as monotherapy is indicated as an adjunct to diet to reduce blood glucose in T2DM patients when hyperglycemia cannot be managed by diet alone. They can be used by people with either T1DM or T2DM. However, there are certain drawbacks like reduced absorption of carbohydrate in small intestine; belly discomfort with bloating, flatulence, diarrhea and nausea associated with these inhibitors. Because of their novel mechanism of action, they can be combined with other oral diabetes drugs. Although, alpha-glucosidase inhibitors do not show good efficacy alone, they show enhanced efficacy when combined with other diabetes medicines (sulfonylurea medicines and insulin). These inhibitors are beneficial for people with T2DM who are unable to keep their blood glucose levels within a safe range by taking a balanced diet, losing weight and exercise.

1.3.3 Nuclear receptors and peroxisome proliferator activated receptor

Nuclear receptors (NRs) are super-family of ligand-activated transcription factors (LATF) involved in diverse cellular and biological processes, such as cell proliferation, differentiation, and intracellular signaling. They are abundant in various organisms, from worms to humans, and are involved in the regulation of key physiological genes \[\text{[109-110]}\]. Currently, there are more than 66 NRs that have been identified and reported e.g. estrogen receptors for steroid receptors, to non-steroidal receptors, such as the thyroid hormone receptor, retinoic acid receptor (RXRs) and peroxisome-proliferator activated receptors (PPAR) \[\text{[111-112]}\]. Most of these nuclear receptors contain various isotypes (such as $\alpha$, $\beta$, $\gamma$) and even further isoforms (like, $\alpha1$ or $\gamma2$), which are all involved in specific regulatory biological pathways. The distinguishing feature of these nuclear receptors in comparison to other transcription factors is that these proteins are ligand-dependent transcription factors. Binding of ligand induces a conformational change that is unique for recruitment of a set of co-activators or co-repressors. One such NR family is a ligand-activated transcription
factor called PPAR, which plays important role in glucose metabolism and lipid homeostasis [67, 70, 113-115]. There are three subtypes of PPAR viz; PPARγ, PPARα and PPARβ. PPARγ forms a heterodimeric complex with the retinoid X receptor (RXR). These NRs consist of several functional domains. These domains are classified as A-F domain, which includes a variable N-terminal A/B domain (activation), a DNA binding domain (DBD) referred to as the C domain, a flexible hinge region/D domain, and a C-terminal ligand-binding domain (LBD) referred to as the E domain, and the F domain [111-112, 115]. NRs may either homodimerize or heterodimerize with self or other and control various physiologic processes. The PPAR-RXR activated complex recognizes a specific DNA sequence in the promoter region of target genes referred as PPREs [116] and transactivates target genes involved in insulin signaling, lipid/glucose metabolism, immune response, cell cycle, and differentiation of epithelial or mesenchimal cells. As a part of the insulin signaling cascade, PPARγ up-regulate proteins involved in glucose and lipid metabolism. PPARγ receptor is selectively activated by endogenous fatty acid derivatives, such as 15-deoxy-delta 12, 14-prostaglandin J2, and by a panel of chemically diverse full agonists such as glitazars and TZDs [114]. Synthetic agonists that activate PPARγ such as the thiazolidinediones (TZDs-Pioglitazone) are efficacious glucose lowering drugs being used as “insulin sensitizers” for the treatment of T2DM. PPARγ plays a major role in the efficient storage of energy and has emerged as a central mediator connecting energy reserves and systemic homeostasis. PPARγ is expressed in appreciable amounts in the adipose tissue, colon, retina, and to a lesser extent in muscle and liver [114-115]. PPARγ ligands also activate glucose transporter gene leading to increased production of glucose transporters (e.g., GLUT4) in muscle, liver and fat [114-117]. PPARγ has been reported to have an effect on leptin production in adipose tissues which is supportive of its role in promoting lipogenesis [118]. Interestingly, both PPARγ and leptin gene expressions are increased upon feeding, thereby leading to a decreased food intake. The activity of PPARγ or PPARα or PPARδ depends on the degree to which these receptors are phosphorylated and/or upon its ligand induced conformational change. The regulation of gene expression by PPARs is largely dependent on the recruitment of accessory proteins known as co-activators or co-repressors to the transcriptional heterodimer complex (PPAR-RXR)
PPARγ co-activator 1alpha (PGC1α) is one such crucial co-activator which is expressed in all the tissues where PPARγ is expressed including muscle. Different PPARγ ligands have differential coactivator recruitment/involvement capability (this is indeed ligand driven due to differential receptor conformation induced by ligand) and thus this is indeed responsible for differential regulation of set of genes in different tissues in body. Many pharmaceutical companies were working on PPARγ or α or dual activators for treatment of diabetes and dyslipidaemia, but eventually due to certain toxicities and side effects (mainly in liver, heart, fluid retention etc.) observed with PPAR based drugs, further development with these drugs took a halt.

1.3.4 Protein tyrosine phosphatase 1B

Protein-tyrosine phosphatases (PTPs) play a major role in regulating insulin signaling in cells expressing insulin receptor. PTPs catalyze the dephosphorylation of tyrosine-phosphorylated proteins and are negative regulators of tyrosine kinase receptor mediated signaling. Among the PTPs that regulate this signaling pathway, PTP1B plays a crucial role. PTP1B inhibits insulin signaling and has previously been shown to bind to the activated insulin receptor (IR). PTP1B has been implicated as a negative regulator of IR signaling and a potential drug target for the treatment of T2DM. Pharmacological inhibitors that block the activity of PTP1B augment insulin action in vitro and in vivo and the region reported to be involved in binding IR lies in the N-terminal, catalytic half of PTP1B. Point mutations within this region of PTP1B disrupts its binding to IR but do not affect the catalytic activity of this phosphatase suggesting that binding is required for the physiological effects of PTP1B on IR signal transduction. PTP1B directly interacts with both the IR and IGF-1R. The importance of PTP1B in hepatic metabolism has been demonstrated in vivo and in various cellular models. Mice lacking ptpn1 gene exhibits increased insulin sensitivity due to enhanced phosphorylation of IR in liver and skeletal muscle, resistance to weight gain on a high-fat diet, and an increased basal metabolic rate. It has been demonstrated that the ability of insulin to suppress hepatic glucose production (HGP) is enhanced in PTP1B−/− mice demonstrating that sensitivity to insulin in liver as a result of PTP1B deficiency. It has been demonstrated that lack of PTP1B also promotes insulin sensitivity in the liver of IRS2−/− mice through the
restoration of IRS1-mediated Akt/Foxo1 phosphorylation (pAkt and pFoxo1) and the inhibition of gluconeogenic enzymes. This molecular mechanism has also been implicated as the mechanism by which resveratrol inhibits or downregulates PTP1B expression \cite{125}. Rodriguez et al in 2010 also demonstrated that expression of PTP1B is upregulated in the liver of IRS2-/- mice and the absence of this phosphatase enables activation of IRS1-mediated Akt/ Foxo1/signaling, thereby restoring hepatic insulin sensitivity. Thus, pharmacological inhibition of PTP1B is an excellent therapeutic intervention as they may act as insulin mimetics or insulin sensitizers. However, it was realized later that though an excellent target, the synthetic PTP1B inhibitors possess poor solubility and ADME properties limiting its further development. Treatment of cells with PTP1B inhibitors, both in the presence and in the absence of insulin, markedly enhances phosphorylation of IRbeta and IRS-1, activation of Akt and ERK1/2, GLUT4 translocation and glucose uptake.

1.3.5 GPR40 and GPR120

There are seemingly infinite numbers of receptors on a cell and they can be classified by different ways depending on factors like: their function, molecular structure or nature of the activating agent, receptor ligand (agonist, antagonist, inverse agonist, positive allosteric modulators or negative allosteric modulators etc.). A complete overview of all known receptors involved in glucose homeostasis is beyond the scope of this work and thus certain key receptors are described here. Glucagon like peptide receptor (GLP-1R), gastric inhibitory peptide (GIP) and glucagon (GCGR) receptor belong to a class B GPCR family and their ligands are peptidic in nature and will not be discussed. GPR40 and GPR120 belong to a family of class A GPCRs and coupled to G\textsubscript{aq} leading to activation of phospholipase C (PLC), downstream signaling and an increase in calcium concentration \cite{126}. GPCRs including free fatty acid receptors FFAR-1, 2 and 3 play an important role in the regulation of physiological functions. GPR40 (also known as FFAR-1) and GPR120 have been identified as key receptors for activation by medium and long chain fatty acids. FFAR-2 and FFAR-3 (previously named as GPR41 and GPR43 respectively) are activated by short-chain free fatty acids. Apart from glucose, the major stimulator of insulin secretion from pancreatic \(\beta\)-cells, other stimuli like amino acids, hormones and free fatty acids (FFAs) also influence insulin
secretion. FFAs serve not only as nutrients but also as cell signaling mediators in vivo. The pleiotropic effects of FFAs on pancreatic β-cells are well known. FFAs act as ligand for GPR40 and there are ample reported evidences that support the beneficial role of GPR40 in FFA-induced insulin secretion. Overexpression of GPR40 in β-cells under the control of insulin promoter factor-1/pancreatic duodenal homeobox factor-1 (IPF-1/PDX-1) has been reported to result in β-cell dysfunction, hypoinsulinemia and diabetes in rodents. Steneberg et al, have reported that GPR40 deficient β-cells secrete less insulin in response to FFAs, demonstrating receptors crucial role in FFA-stimulated insulin secretion. Edfalk et al, reported that secretion of GLP-1 and GIP is diminished in GPR40 null mutant mice. RT-PCR studies have revealed that it is preferentially expressed in pancreatic β-cells and enteroendocrine cells in gut. Activation of receptor upon binding of FFAs results in glucose-stimulated insulin secretion (GSIS). Pancreatic islets expresses lipoprotein lipase, therefore can access plasma TG as a source of FFAs. Pancreatic β-cells continuously sense the levels of blood glucose, lipids, FFAs and other fuels and respond to such stimuli by secreting insulin to maintain normal fuel homeostasis. Coupling of glucose sensing to insulin secretion by pancreatic β-cell is crucial part of glucose metabolism leading to the formation of acetyl-CoA via pyruvate and then further increase in ATP/ADP ratio by subsequent mitochondrial oxidation results in closure of ATP-sensitive K+ (KATP) channels, depolarization of the plasma membrane, opening of voltage-dependent Ca2+ channels and finally insulin granule exocytosis. Lipids and FFAs also stimulate the secretion of several gut “satiety” hormones known as incretins; GLP-1, GIP, CCK, PYY via GPR40 and GPR120 in the enteroeendocrine cells in gut. Other GPCRs like, GPR119 which is also expressed in β-cells and enteroeendocrine L-cells, also contribute for ligand-induced incretin release in gut and insulin secretion in pancreas. Phospholipids, oleylethanolamide (OEA) and polyylethanolamide (PEA) are known ligands of GPR119.