ANTIDIABETIC ACTIVITY OF *NYPA FRUTICANS* WURMB. VINEGAR

by

NOR ADLIN BINTI MD YUSOFF

Thesis submitted in fulfilment of the requirements
for the degree of
Doctor of Philosophy

February 2017

ACKNOWLEDGEMENT

In the name of Allah, the Most gracious and the Most merciful

First and foremost, I would like to thank my main supervisor, Prof Dr Mohd Zaini Bin Asmawi whose expertise, knowledge, and patience, added considerably to my graduate experience. I sincerely appreciate his vast knowledge and his achievements are my inspiration. I would like to thank my co-supervisor, Dr Roziahanim Mahmud for her valuable time, thought, advice and guidance. I would also like to express my special gratitude Pn Mariam Ahmad, Dr Yam Mun Fei and Pn Khairul Niza Abd Razak for the assistance they provided at all levels of the research project.

Special thanks also go to the laboratory technicians, En. Roseli Bin Hasan, En. Jusfaridan Aizan and En. Yusuf for their advice and assistance during my work in the laboratories. Not forgotten, my colleagues, Tri Widyawati, Sook Yee, Navnett, Rabyah, Nasiba, Raghdaa, Lina, Farah, Fatim, Mir Reza, Faramarz, Idris, and Abdul Menem who made the weather at the workplace more cheerful. I appreciate the interesting discussions we had together and I wish you all the best!

My heartfelt thank you to my beloved husband, who is also my wonderful lab mate and my best friend, Bassel Al Hindi, my parents, En. Md Yusoff Bin Hassan and Pn Sepiah @ Razinah Mahmood, my sisters and brother for their unconditional love, endless motivation and continous support. Also thanks to my close friends, Nur Atiqah, Ernawita and Mardiaty Iryani. Even though we fight different battles, we share the same dream. Last but not least, to those who are not mentioned here, I thank you all.

Nor Adlin Md Yusoff.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF PLATES

LIST OF ABBREVIATIONS

^{o}C	Degree Celsius
α	Alpha
%	Percent
AE	Aqueous extract
AIP	Atherogenic index of plasma
ANOVA	Analysis of variance
AUC	Area under the curve
ATP	Adenosine triphosphate
B.W.	Body weight
DM	Diabetes mellitus
EE	Ethyl acetate extract
et al.	And others
EMA	European Medicines Agency
FDA	Food Drug Association
g	Gram
GLUT 2	Glucose transporter 2
GLUT 4	Glucose transporter 4
H_2O_2	Hydrogen peroxide
HPLC	High performance liquid chromatography
IDDM	Insulin dependent diabetes mellitus
IC_{50}	Half maximal inhibitory concentration
i.p.	Intraperitoneal
IPGTT	Intraperitoneal glucose tolerance test
kg	Kilogram
L	Liter
m	Meter
mg	Milligram
mM	Millimolar
NDR	National Diabetes Research
NIDDM	Non insulin dependent diabetes mellitus
NHMS	National Health and Morbidity Survey
NPV	Nipa palm vinegar
OGTT	Oral glucose tolerance test
OSTT	Oral starch tolerance test
OSucTT	Oral sucrose tolerance test
SEM	Standard error of mean
SD	Sprague Dawley
STZ	Streptozotocin
SSPS	Statistical Package for the Social Sciences
µg	Microgram
µL	Microliter
UKPDS	United Kingdom Prospective Diabetes Study
UV	Ultraviolet
WHO	World Health Organisation
w/v	Weight over volume
w/w	Weight over weight

AKTIVITI ANTIDIABETIK CUKA *NYPA FRUTICANS* WURMB.

ABSTRAK

Cuka *Nypa fruticans* Wurmb., secara tempatan dikenali sebagai cuka nipah (NPV) di Malaysia, dipercayai mempunyai ciri-ciri antidiabetik yang tinggi. Kajian ini dijalankan untuk mengkaji aktiviti antidiabetik dan mekanisma-mekanisma tindakan yang mungkin bagi NPV. Pengekstrakan cecair-cecair diguna untuk memisahkan komponen-komponen NPV berdasarkan kekutuban kepada ekstrak etil asetat (EE) dan ekstrak akues (AE). AE, seterusnya difraksinasi menggunakan kaedah asid hidrolisis kepada dua subfraksi: SFI dan SFII. NPV, EE, AE, SFI dan SFII diberikan kepada tikus non diabetik dan/atau tikus diabetik aruhan streptozotosin (STZ) menggunakan gavaj oral. Aras glukosa darah (BGLs), aras insulin dan perubahan histologi pada hati dan pankreas direkod. Kesan *in vitro* AE terhadap aktiviti enzim α–glukosidase and α–amilase, dan juga penyerapan/ pengambilan glukosa pada kantung jejuna tikus terisolasi dan otot rangka; turut dikaji. Tiada kesan hipoglisemik akut dilihat pada tikus non diabetik setelah diberikan NPV, EE dan AE pada dos 1 g/kg. Walaubagaimanapun, NPV dan AE merencat kenaikan BGLs secara signifikan dalam tikus terbeban glukosa pada minit ke 90. Namun, pemberian NPV, EE dan AE kepada tikus diabetik aruhan STZ menunjukkan tiada kesan antihiperglisemik direkodkan dalam pemerhatian selama 7 jam. Rawatan sub-kronik oral selama 12 hari pada tikus diabetik membuktikan bahawa AE mempunyai aktiviti antihiperglisemik yang paling tinggi, secara keseluruhannya. Penilaian imunohistokimia pada tisu pankreas menunjukkan,walaupun tiada kesan regenerasi, AE merangsang penghasilan insulin daripada sel-sel β yang masih berfungsi. AE juga memperbaiki struktur histologi pada hati. AE menurunkan

BGLs, memperbaiki aras insulin serum dan menormalkan profil lipid tikus diabetik tanpa menyebabkan ulser gastrik atau pertambahan/ penurunan berat badan. Justeru itu, AE dipilih bagi kajian mekanisma tindakan yang lebih mendalam. Ujian secara *in vitro* menunjukkan AE merencat penyerapan glukosa pada jejunum. Penemuan ini disokong dengan ujian toleransi karbohidrat oral apabila perencatan yang signifikan dilihat terhadap kenaikan aras glukosa darah. AE juga tidak merencat aktiviti α–glukosidase and α–amilase. Seterusnya, AE menunjukkan kesan rangsangan yang signifikan terhadap pengambilan glukosa oleh otot rangka dan kesan perlindungan terhadap hepar. Analisis fitokimia menunjukkan AE mempunyai jumlah fenolik dan kandungan antioksidan yang secara relatifnya adalah rendah berbanding dengan ekstrak atau fraksi yang lain. Analisis HPLC mencadangkan bahawa aktiviti antihiperglisemik AE adalah disebabkan oleh kehadiran asid asetik dalam kuantiti yang tinggi. Walaubagaimanapun, kajian yang lebih mendalam perlu dijalankan bagi mengenalpasti kandungan komponen bioaktif yang hadir dalam NPV.

ANTIDIABETIC ACTIVITY OF *NYPA FRUTICANS* WURMB. VINEGAR

ABSTRACT

Nypa fruticans Wurmb. vinegar, locally known as nipa palm vinegar (NPV) in Malaysia, is believed to have remarkable antidiabetic properties. This study sought to elucidate the vinegar's antidiabetic activity and possible mechanisms of action. Liquid–liquid extraction was used to separate the compounds in NPV based on polarity into an ethyl acetate extract (EE) and an aqueous extract (AE). AE was further fractionated using acid hydrolysis into two sub-fractions: SFI and SFII. NPV, EE, AE, SFI and SFII were administered using oral gavage in non diabetic and/or diabetic rats induced by streptozotocin (STZ). Blood glucose levels (BGLs), insulin levels, and liver and pancreatic histological changes were recorded. *In vitro* effects of AE on α–glucosidase and α–amylase activities, as well as on glucose absorption/ uptake in isolated rat jejunal sacs/ skeletal muscles; were observed. No acute hypoglycemic effect was shown in non diabetic rats upon administering up to 1 g/kg of NPV, EE or AE. Nevertheless, NPV and AE significantly ameliorated high BGLs at 90 minutes in glucose-loaded rats. Yet, administration of NPV, EE and AE to STZ-induced diabetic rats showed no antihyperglycemic effects for up to 7 h. Sub-chronic 12-day oral treatment in diabetic rats revealed that AE had a superior antihyperglycemic activity overall. Immunohistochemical assessment of the animals' pancreatic tissues showed that, albeit having no regenerative effect, AE stimulated the secretion of insulin from viable β–cells. It also precipitated structural recovery in the liver. It lowered BGLs, improved serum insulin levels and normalized the lipid profile of the animals, with no signs of gastric ulcer or abnormal body weight gain/loss. Consequently, AE was chosen for

elaborate antidiabetic mechanism studies. *In vitro* tests showed that AE inhibited the absorption of glucose from jejunum. This finding was further supported by significant suppression in the rise of blood glucose level seen in oral carbohydrate tolerance tests. AE also did not inhibit the activities of α-glucosidase and α-amylase. Furthermore, AE was shown to significantly enhance the uptake of glucose by skeletal muscles and exert hepatoprotective effect. Phytochemical analysis indicated that AE had relatively low total phenolic and antioxidant contents compared to the other tested extracts/fractions. HPLC analysis rather suggested that the antihyperglycemic activity was due to the presence of a major quantity of acetic acid. However, further investigation is needed to fully elucidate the nature of the bioactive compounds present in NPV.

CHAPTER ONE: INTRODUCTION

1.1 Background

Diabetes mellitus (DM) is a heterogenous metabolic disorder characterized by persistent hyperglycemia in fasting and/or postprandial states with abnormalities in the metabolism of carbohydrate, protein and fat due to the impairment of insulin secretion by pancreatic beta cells, insulin action at the targeted tissues or both (ADA, 2008; Adam, Ismail, Khamis, Mokhtar, & Hamid, 2011). Symptoms of marked hyperglycemia include polydipsia, polyuria, weight loss, extreme tiredness and blurred vision. Susceptibility to certain infections and impaired growth may also occur with chronic hyperglycemia. The chronic hyperglycemia will cause long-term damage and failure of specific organs, especially the kidneys, eyes, heart, nerves and blood vessels due to the complications of the macrovascular (peripheral vascular disease, ischemic heart disease and cerebrovascular disease) and microvascular (nephropathy, retinopathy and neuropathy) (Cade, 2008). Insulin therapy, oral antidiabetic drugs and lifestyle changes, for example exercise, weight control and diet management, are recommended for controlling and treating diabetes (Hui, Tang, & Go, 2009).

The global prevalence of DM is increasing at an alarming rate and has reached epidemic proportions. According to the International Diabetes Federation, approximately 382 million people, or 8.3% of adults worldwide living with diabetes in 2013 and the number has been forecasted to increase to 592 million by 2035. About 80% of the total diabetes cases reported in the low- and middle- income countries. Among the top 10 countries of the largest numbers of people with diabetes (20 to 79 years old), five are in the Asia region; China, India, Indonesia, Egypt and Japan.

According to the National Health and Morbidity Survey (NHMS III), Malaysia in particular has recorded a 31% increase in DM case among adults in the period of just 5 years, from 11.6% in 2006 to 15.2% in 2011 (Amal, Paramesarvathy, Tee, Gurpreet, & Karuthan, 2011). Furthermore, based on the latest result of NHMS and National Diabetes Registry (NDR) database, the prevalence of diabetes among adults age 18 years and above is predicted to rise to 21.6% by 2020.

Functional foods have captivated considerable interest as potential alternative therapies for the treatment of diabetes mellitus and its complications. Incorporating functional foods in the dietary regimen of diabetic patients proved to favourably influence blood glucose level. According to Functional Food Center USA, functional foods are defined as natural or processed foods that contain known or unknown biologically – active compounds; which, in defined, effect non – toxic amounts, provide a clinically proven and documented health benefit for the prevention, management, or treatment of chronic disease (Martirosyan & Singh, 2015). Besides of the minimal side effect in clinical practices and relatively low cost, the growing interest in functional foods is also because of the increase scientific studies regarding their effectiveness. Some of the extensively studied functional foods for the treatment of diabetes mellitus are *Allium sativum* L., *Momordica charantia* L., *Punica granatum*, *Curcuma longa L.*, and *Mangifera indica* (Perera & Li, 2012).

Vinegar is one of the widely consumed functional foods worldwide. The consumption of vinegar with meals was used as a home remedy for diabetes before the advent of pharmacologic glucose-lowering drugs (O' Keefe, Gheewala, & O' Keefe, 2008). Many recent scientific reports in both humans and animals have shown that vinegar consumption could reduce the postprandial blood glucose concentrations (Brighenti et al., 1995; Gu et al., 2012; Östman, Granfeldt, Persson, & Björck,

2005). The antiglycemic property of vinegar was also demonstrated to extend to individuals with type 1 diabetes mellitus and type 2 diabetes mellitus. Among the previously studied vinegars are apple cider vinegar (Abu-Zaiton, 2011; Hlebowicz, Darwiche, Björgell, & Almér, 2007; Johnston, 2009; Kondo, Kishi, Fushimi, Ugajin, & Kaga, 2009), grape vinegar (Kahraman et al., 2011), balsamic vinegar (Seok et al., 2012), wine vinegar (Liatis et al., 2010), and white rice vinegar (Gu et al., 2012).

Although different types of vinegars have been studied for decades for their medicinal properties, there is no scientific investigation on the effects of nipa palm vinegar (NPV) towards diabetes has been conducted. NPV has been widely consumed by the local community as part of the diet and also as a folk medicine to treat diabetes, high blood pressure, rheumatoid arthritis and insomnia (O. Salmiah, personal communication, October 27, 2014). It is made from a local alcoholic beverage called 'nira' which is produced from the flower cluster (inflorescence) of nipa palm (*Nypa fruticans*). Therefore, this study is designed to determine the effects of NPV prepared from Malaysian nipa palm plant on biochemical parameters of non diabetic and STZ-induced diabetic rat models. Mechanisms underlying the observed antidiabetic activities of NPV are further studied in an attempt to understand the possible role of NPV at the pancreatic, intestinal and peripheral levels.

1.2 Definition and classification of diabetes mellitus

The word 'diabetes' comes from a Greek word which means 'to pass through', was first used by Aretaeous of Capadocia in the 2nd century A.D. to refer to a condition of excessive production of urine or polyuria (Alexiou & Demopoulos, 2010). The adjective 'mellitus' is a Latin word that means 'sweet like honey' which refers to the high level of

sugar found in the urine (Merriam-Webmaster, 2008). In the early days, Greek physicians prescribed exercise, starvation diet, wine, and overfeeding to compensate fluid loss as ways to treat diabetes. In 600 B.C., well known ayurvedic physicians, Charaka and Susruta differentiated two types of diabetes, even though most of the described symptoms probably relate to currently used term of type 1 DM. Later, in the 18^{th} and 19^{th} centuries, another variety of DM which is less clinically symptomatic, characterized by glucosuria, commonly related with overweight, and often discovered in later life, was noted. This type is today recognized as type 2 DM. It has become understandable subsequently that the term DM covers a wide spectrum of disease, from acute and sometimes explosive onset to asymptomatic people whose disease is detected by screening (Sarmah & Sharma, 2014).

Nowadays, the American Diabetes Association has classified diabetes mellitus into four etiopathogenetic categories: type 1 diabetes, type 2 diabetes, gestational diabetes and diabetes due to multiple other specific causes, for example genetic defects, drug therapy, infection, etc (ADA, 2008). Type 1 diabetes mellitus, formerly known as juvenile diabetes or insulin-dependent diabetes, is characterized by an absolute deficiency of insulin secretion due to the progressive destruction of β-cells (Inzucchi & Sherwin, 2011). Insulin is a hormone crucially needed in the metabolism of carbohydrate, protein and fat (Figure 1.1). Loss of β-cells function is resulted from cellular-mediated autoimmune destruction of the pancreactic β-cells in which the body immune system is activated to destroy its own β-cells (Wang et al., 2013). Hyperglycemia condition happens when 80-90% of pancreatic β-cells have been destroyed. While this type commonly occurs in childhood and early adulthood, it can develop at any age. Patients with type 1 diabetes depend solely on the exogenous source

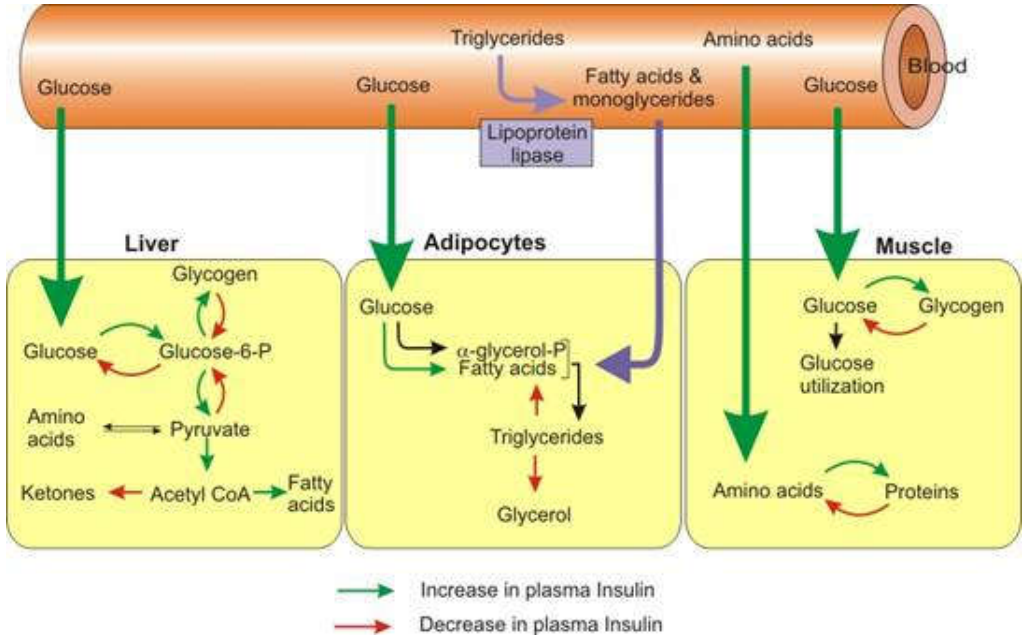

Figure 1.1: Diagram of the metabolic action of insulin on liver, adipocyte, and muscle cells. Green arrows represent the direction of processes enhanced by insulin whereas red arrows illustrate the direction of processes in the absence of insulin. Black arrows indicate non-insulin dependent processes. The lipoprotein lipase is located in the membranes of adipocyte capillaries (Adopted from Course D: Diabetes Lecture Notes by Dr Afia Ali In *e-Learning Pages*, Retrieved October 25, 2016, from http://elearn.pharmacy.ac.uk/diabetes/diabetes_07.htm. Copyright 2015 by Jason Cooper).

of insulin to control hyperglycemia as close as normal (Chiang, Kirkman, Laffel, & Peters, 2014). Type 1 DM accounts for only 5-10% of all diabetes cases.

Type 2 diabetes mellitus is the major form of diabetes, accounting for about 90–95% of those with diabetes. It previously referred to as adult-onset diabetes or non–insulin dependent diabetes. This form of diabetes is characterized by the presence of insulin resistance – a condition in which body does not respond properly to insulin, a relative lack of insulin secretion and increased hepatic glucose output (Matusda & De Fronzo, 1999). Majority of the type 2 DM patients exhibits abdominal obesity which itself is the main cause of insulin resistance. In addition, dyslipidemia, hypertension, and elevated inhibitor of plasminogen activator 1 level are often manifested in type 2 DM. The risk of developing this type of diabetes increases when diabetogenic lifestyle – inadequate calories expenditure, lack of exercise and obesity, is associated with having susceptible genetic predisposition (Wells et al., 2008). For some patients, insulin resistance may be improved with weight reduction through diet and exercise. Others might also need oral hypoglycemic drugs to manage the blood glucose level.

Gestational diabetes mellitus (GDM) is defined as glucose intolerance which is first diagnosed during the pregnancy. GDM is diagnosed in about 7% of all pregnancies. During pregnancy, the placenta and placental hormones develop an insulin insensitivity that becomes pronounced in the last trimester. Hence, it is recommendable to do risk assessment for GDM starting from the first prenatal visit. Clinical screening is important as management for GDM that will lower perinatal mortality and morbidity (ADA, 2010; Karagiannis, Bekiari, Manolopoulos, Paletas, & Tsapas, 2011).

The fourth type of diabetes is diabetes due to multiple other specific causes, including genetic defects of β-cells function and insulin actions, endocrinopathies,

diseases of the exocrine pancreas, endocrinopathies, nonpancreatic diseases, drug therapy, infection and non-pancreatic diseases (ADA, 2010).

1.3 Pathogenesis aspects of diabetes mellitus

DM is a heterogeneous disorder involving distinct pathogenic mechanisms in which hyperglycemia is the main symptom. Regardless of the cause, DM is closely related with a hormonal deficiency, namely, insulin, in either total, partial, or relative defects when discussed in the context of coexisting insulin resistance. Insulin defect is the main contributor to the metabolic derangement of carbohydrate, protein, and fat in DM and hyperglycemia, on the hand, plays a key role in the diabetes complications (ADA, 2012).

In type 1 DM, absolute insulin deficiency resulted mostly from the autoimmune – mediated destruction of β cells in the islet of Langerhans of pancreas. The pathogenesis of type 1 DM has been assumed to be an interaction between genetics and environment factors (for e.g. toxins, vaccines, diet, viruses, stress, and infectious agents). Patients with type 1 DM have strong genetic linkages with DQA, B genes and certain human leukocytes antigens (HLAs) such as DR3 and DR4. In a genetically susceptible individual, environmental factors stimulate an autoimmune process by activating macrophages and T lymphocytes to circulate autoantibodies against various β cell antigens that results in destruction of pancreatic β islet cells which leads to insulin deficiency and hyperglycemia (Pittas & Greenberg, 2003). Several antibodies that related to type 1 DM include islet cell antibody, insulin autoantibodies, insulin antibodies against islet tyrosine phosphatase, and antibodies directed against glutamic acid decarboxylase. These antibodies are usually considered as markers for detecting

type 1 DM, rather than mediator for β cell destruction (Pihoker, Gilliam, Hampe, & Lernmark, 2005).

Type 2 DM involves two primary pathogenic mechanisms which are, 1) impaired insulin secretion (Weyer, Bogardus, Mott, & Pratley, 1999) and 2) peripheral insulin resistance involving muscle, liver and adipose tissues (ADA, 2008; Ahrén & Pacini, 2005). Impaired insulin secretion occurred due to the progressive destruction in the pancreatic β cell function or β cells mass (Clark, Jones, de Koning, Hansen, & Matthews, 2001). Although the progressive destruction of β-cell does not happen rapidly, the decline in β-cell function is inevitable, progressive and central to the pathophysiology of type 2 DM (Tibaldi, 2008).

Insulin resistance happened when the body does not respond the secreted insulin effectively. Hence, glucose starts to accumulate in the blood circulation instead of being absorbed by the cells. The most common cause of insulin resistance is obesity (Bell & Polonsky, 2001) and the increasing prevalence of obesity is probably responsible for the increasing prevalence of T2DM globally. Insulin resistance occurs even in lean type 2 diabetic patients. With insulin resistance, liver, muscle, and adipose tissues do not response adequately to the insulin action (Wilcox, 2005). In mild type 2 DM individual, basal hepatic glucose production is elevated by approximately 0.5 mg/kg per minute. It is estimated that a diabetic individual of 80 kg with mild fasting hyperglycemia produces an additional 35 g of hepatic glucose during the overnight sleep hours. The additional fasting hepatic glucose production is the cause of fasting hyperglycemia (Cersosimo, Triplitt, Mandarino, & DeFronzo, 2015). During the fed state, insulin is released into the portal vein and carried to the liver. In healthy individual, hepatic glucose production will be suppressed. Due to the insulin resistance in type 2 DM individual, liver continues to produce glucose. At this point, there are two sources of

glucose in the systemic circulation; one from the gastrointestinal tract and second, from the liver. As a result, marked hyperglycemia ensued (Wilcox, 2005).

Skeletal muscle is the primary site of glucose uptake in the postprandial state. Approximately 80% of glucose is taken up by peripheral tissues is disposed of in skeletal muscles under euglycemic hyperinsulinemic condition (Thiebaud et al., 1982). In healthy individual, the glucose uptake by leg muscle increases linearly with time, reaching a plateau of about 10 mg/kg leg weight per minute after 60 minutes, in response to the increment in plasma insulin concentration. However, for mild type 2 DM individual, the ability of leg muscle to stimulate glucose uptake is decreased by 50% due to the 40 minutes delay in the onset of insulin action (DeFronzo & Tripathy, 2009).

In the postprandial state, the intracellular glucose transport into adipocytes is insulin-dependent via glucose transporter (GLUT) 4; it is estimated that adipose tissue accounts for about 10% of whole body glucose uptake (Smith, 2002). Insulin stimulates glucose uptake, promotes lipogenesis while suppressing lipolysis, which causes free fatty acid flux into the blood circulation. Adipose tissue depots differ in their response to insulin (Giorgino, Laviola, & Eriksson, 2005). Adipocytes in the type 2 DM and insulin resistant individuals have reduced GLUT 4 translocation, impaired intracellular signalling via reduced insulin receptor substrate – 1 gene and protein expression, impaired insulin-stimulated diphosphoinositide (PIP) – 3 kinase and protein kinase B (Shan, Chen, Zhu, Jiang, & Zhou, 2011).

As discussed above, with insulin resistance, muscle, liver, and adipocytes do not adequately respond to the insulin actions. Typically, β-cells compensate insulin resistance by secreting more insulin. Although insulin hypersecretion predisposes

patients to β-cell dysfunction, impaired glucose tolerance (IGT) and eventual type 2 DM (Rolla, 2004), many insulin-resistant patients do not develop diabetes as long as the islet β-cells continue to hypersecrete insulin (Boden & Shulman, 2002). However, when β – cells loss the ability to hypersecrete insulin, marks progression from IGT to the fasting hyperglycemia occurred, that further characterizes type 2 DM (Rolla, 2004).

1.4 Complications of diabetes

Diabetes is a metabolic disorder that is strongly associated with several microvascular and macrovascular complications, resulting in multiple tissues and organs dysfunction, especially the kidneys, eyes, heart, nerves and blood vessels (Cade, 2008). Microvascular complications refer to the long term complications of diabetes that affect small blood vessel such as capillaries, arterioles and venules whereas macrovascular complications involve large blood vessels such as arteries and veins (Zimmerman, 2016). According to UKPDS, both vascular complications are responsible for approximately one third to one half of morbidity and mortality in diabetes patients (Turner, Cull, & Holman, 1996). The severity and duration of hyperglycemia play the main role in initiating diabetic vascular complications through several metabolic and structural derangements (Cade, 2008).

Microvascular complications included diabetic retinopathy, neuropathy and nephropathy. Diabetic retinopathy (DR) is a microvascular complication that affects the peripheral retina, the macula, or both and is the main cause of visual impairment and blindness in diabetes patients (WHO, 2007). There are several histological changes that occur with DR which are loss of pericytes, formation of microaneurysm, thickening of capillary basement membranes and increased permeability of endothelial cells (Carlson

et al., 2003; Hammes, 2005). Development and progression of DR depend significantly on the severity and duration of hyperglycemia (Henricsson, Nilsson, Janzon, & Groop, 1997). Under hyperglycemia condition, retina blood flow impairment, capillary blockage and increased inflammatory cell adhesion to retinal blood vessels occur which can result in hypoxia and damage to the retina (Kohner, Patel, & Rassam, 1995). The severity of DR varies from nonproliferative and preproliferative to more chronic proliferative, characterized by the abnormal growth of new vessels on the surface of retina that can lead to vitreous hemorrhage (Harding, 2003). White areas on the retina can be a sign of impending proliferative retinopathy. If proliferation continues, blindness can occur through vitreous hemorrhage and traction retinal detachment. With no treatment, visual loss may occur. Laser photocoagulation can often prevent proliferative retinopathy from progressing to blindness (Watkins, 2003).

Diabetic neuropathy (DN) is defined by the American Diabetes Association as the existence of symptoms and/or signs of peripheral nerve failure in diabetes patients after the exclusion of other cause (ADA, 2012). Approximately half of the diabetic population have been affected by peripheral neuropathy (Dyck et al., 1993) and more than 80% of amputations that happen after foot injury are due to DN (Boulton, 1997). Peripheral neuropathy can be classified into several types depending on the number of affected nerves, their functions and locations. Mononeuropathy affects single peripheral nerve at a time. If multiple sensory and motor nerves in the peripheral nervous system are being affected simultaneously, it is known as polyneuropathy (Said, 2007). In addition, DN can also affect autonomic nerves and cause diabetic autonomic neuropathy, including cardiac autonomic neuropathy, gastrointestinal autonomic neuropathy, and genitourinary autonomic neuropathy (Duby, Campbell, Setter, & Rasmussen, 2004; Vinik, Maser, Mitchell, & Freeman, 2003). Even though the precise

nature of the nerves injury due to hyperglycemia is yet uncertain, it is likely associated with oxidative stress, accumulation of polyol and injury from advanced glycation end product (Hosseini & Abdollahi, 2013). Like those for other microvascular complications, duration and severity of hyperglycemia are the main risk factor for DN. In addition, some individuals may also possess genetic facets that affect their predisposition in developing DN (Fowler, 2008). The primary goal of DN treatment is to control and prevent the worsening of it symptoms by improving glycemic control. Even though there is no specific therapy for DN, many drugs have been prescribed to treat its symptoms, for example duloxetine and pregabalin (Boulton, 2007).

Diabetic nephropathy, a progressive complication in both type 1 and 2 diabetes patients, is the leading cause of renal failure in the US (Fowler, 2008). It is defined by the presence of proteinuria of more than 500 mg in a 24 h urine collection. This stage has been referred to as overt nephropathy, clinical nephropathy, macroalbuminuria or proteinuria (Gross et al., 2005). This condition is preceded by microalbuminuria - that is the presence of proteinuria of 30-299 mg/ 24 hours. Without any treatment, microalbuminuria typically progresses to macroalbuminuria and finally to renal failure (Drummond & Mauer, 2002). Pathological changes in kidney structure due to nephropathy include glomerulosclerosis, microaneurysm formation, hyalinosis, mesangial nodule formation and hyaline arteriosclerosis (Fowler, 2008; Raparia, Usman, & Kanwar, 2013). Approximately 15-40% of patients with type 1 DM and 5-20% of patients with type 2 DM have macroalbuminuria/ diabetic nephropathy. The prevalence of diabetic nephrophaty was higher in Asian, African Americans, and Native Americans populations than Caucasians (Gross et al., 2005). Like other diabetes microvascular complications, poor glycemic control remains as the major initiation and progression factors for diabetic nephropathy. Other putative risk factors include

glomerular hyperfiltration (Dahlquist, Stattin, & Rudberg, 2001), age of onset, smoking, hypertension, obesity and dyslipidemia (Kramer et al., 2005). Initial treatment of diabetic nephropathy, as of other diabetes microcascular complications, is prevention which is treating its known risk factors, namely hyperglycemia, hypertension, dyslipidemia and tobacco use. In addition, treatment with antihypertensive drugs, angiotensin-converting enzyme (ACE) inhibitors and angiotensin-II receptor blockers (ARBs) have shown to lower the risk of developing nephropathy and cardiovascular event in type 2 DM (Gross et al., 2005).

Macrovascular complications associated with diabetes include cerebrovascular, cardiovascular, and peripheral artery diseases. Cerebrovascular disease refers to many disorders involving blood vessels of the central nervous system (CNS). These disorders result from either inadequate blood flow to the brain (i.e., ischemic stroke) or from hemorrhages into the parenchyma or subarachnoid space of the CNS (i.e. cerebral hemorrhage) (King, Jones, & Warthen, 2005). Type 2 diabetes is an independent risk factor for cerebrovascular complications; diabetic patients are up to 2- 4 times more susceptible to a stroke event (Muntean, Mitrea, Mota, & Tudorica, 2012) and have poorer long-term prognosis, more severe neurological disability and higher incidence of stroke recurrence than non-diabetic patients (Elneihoum, Göransson, Falke, & Janzon, 1998; Sprafka, Virnig, Shahar, & McGovern, 1994). Hyperglycemia, hyperinsulinemia and excess free fatty acid cause deleterious effects on the cerebrovascular endothelial by several mechanisms namely oxidative stress, hypercoagulability, inflammation and artherosclerosis which lead to the initiation of cerebrovascular disorders (Patel, 2016). Sudden confusion, loss of coordination, unilateral weakness, and numbness are warning signs of a cerebrovascular event (King et al., 2005). The risk factors that may predispose a patient to a stroke include smoking, obesity, hypertension, dyslipidemia,

and transient ischemic attacks (Stegmayr & Asplund, 1995). The FDA has approved the use of an intravenous tissue plasminogen activator (tPA) for treatment of patients with acute ischemic stroke (King et al., 2005).

Cardiovascular diseases (CVDs) are a group of diseases of the heart and blood vessels which include coronary heart disease, myocardial infarction, heart attack, angina, stroke and congenital heart disease (WHO, 2007). Generally, individual with diabetes have 2 to 4 times greater risk to develop CVD and insulin resistance condition further worsen the long term prognosis of the CVDs (Buyken, von Eckardstein, Schulte, Cullen, & Assmann, 2007; Cade, 2008). Diabetic patients frequently have several traditional risk factors that contribute to CVDs namely lifestyle (tobacco use and diet), hypertension and dyslipidemia (Buyken et al., 2007). The combination of hyperglycaemia, insulin resistance, chronic inflammation and the aforementioned traditional risk factors for CVDs can cause injury to vascular endothelium, hypercoagulability, oxidative stress, impaired fibrinolysis, platelet hyperaggregability and glucose toxicity that lead to macrovasculopathy and CVDs in diabetic patients (Beckman, Creager, & Libby, 2002; Haffner & Steven, 2005). Like other diabetes complications, the primary goal of therapy of CVDs is prevention which could be achieved via several ways. For type 1 diabetes patients, maintaining good glycemic control helps to lower the risk of CVDs as several studies have shown that patients with higher degree of hyperglycemia tend to have higher heart rate which is related with higher risk of CVDs (Fowler, 2008). Good glycemic control can be achievable using insulin and/or any oral antidiabetic drugs (King et al., 2005). In type 2 diabetes patients, the risk of CVDs can be lowered by having ideal blood pressure which can be accomplished using antihypertensive agents namely ACE inhibitors, ARBs, thiazide diuretics and non-dihydropyridine calcium channel blockers (Chobanian, 2003;

Lindholm et al., 2002). Another available therapy for CVDs is maintaining blood lipid concentration namely LDL, triglycerides and HDL. Numerous studies have reported reduced risk of cardiovascular events in diabetic patients who are treated with lipid lowering agents such as statins (Bitzur, 2011).

Peripheral artery disease (PAD) is an atherosclerotic occlusive disease of the lower extremity arteries (Kullo et al., 2003). The fundamental symptoms of PAD are intermittent claudication during exercise in the affected leg and pain at rest which is experienced by patients with critical limb ischemia (Creager & Libby, 2001; Schainfeld, 2001). These conditions can cause functional disabilities and impairments of the lower extremities that eventually lead to amputations (McDermott et al., 2004). The strongest risk factor for PAD is diabetes mellitus and smoking (Fowkes et al., 2013). Diabetes patients have a threefold to fourfold greater risk of developing PAD, as well as accelerates its course, compared to patients without diabetes (Murabito, D'Agostino, Silbershatz, & Wilson, 1997). Insulin resistance, hyperglycemia and dyslipidemia promote the development and progression of PAD via several mechanisms include derangements of the vessel wall through stimulation of endothelial cell dysfunction, triggering vascular inflammation and causing abnormalities in muscle cells, blood cells and factor affecting homeostasis (Armstrong, Waltenberger, & Rogers, 2014). Other risk factors are hypertension, dyslipidemia, sedentary lifestyle, obesity and a history of cardiovascular disease (Wattanakit et al., 2005). Treatment of PAD focuses on lowering symptoms and hindering further development of PAD. In most cases, lifestyle changes including smoking cessation, regular exercise, healthy diet and controlled high blood pressure, diabetes and dyslipidemia are sufficient to delay the progression or even reverse the signs and symptoms of PAD (King et al., 2005). If needed, medications will be given according to the underlying causes of PAD for example statins

(antihyperlipidemic drug), ACE inhibitors (antihypertensive drugs), low dose aspirin and clopidogrel (antiplatelet drugs) and naftidrofuryl oxalate (Poredos & Jezovnik, 2015).

1.5 Oral diabetes medications

Diabetes mellitus is chronic metabolic disorder that requires continuous medical care with patient self-management education in order to achieve ideal blood glucose level, to avoid acute complications and to lower the risk of long term complications (Shafiee, Mohajeri-Tehrani, Pajouhi, & Larijani, 2012). Until the year of 1995, there are only two choices of therapy for managing diabetes; insulin for type 1 and type 2 diabetes and sulfonylureas for type 2 diabetes. After the year of 1995, there is a drastic change in the choice of therapy as number of insulin analogs and oral antidiabetic agents have been approved. Currently, eleven classes of oral antidiabetic drugs are available; sulfonylureas, meglitinides, thiazolidinediones, biguanides, α-glucosidase inhibitors, dipeptidyl peptidase IV inhibitors, glucagon-like peptide-1 receptor agonists, amylin agonists, bromocriptine, colesevelam and sodium glucose co-transporter 2 inhibitors (John R White, 2014). These drugs are classified according to their mechanism of glucose lowering action. Figure 1.2 shows schematic representation of potential new drug target areas. Sulfonylureas and meglitinides are known as insulin secretagogues due to their effects in triggering insulin release from the pancreatic β-cells. On the other hand, biguanides and thiazolidinediones are considered as insulin sensitizers because of their abilities to enhance insulin sensitivity of insulin-responsive tissues. American Diabetes Association recommends a trial of diet and exercise as the first line therapy for the management of type 2 DM (Pan et al., 1997). If the desired level of glycemic control

is not attained with diet and exercise within the period of three months, pharmacologic therapy should be introduced.

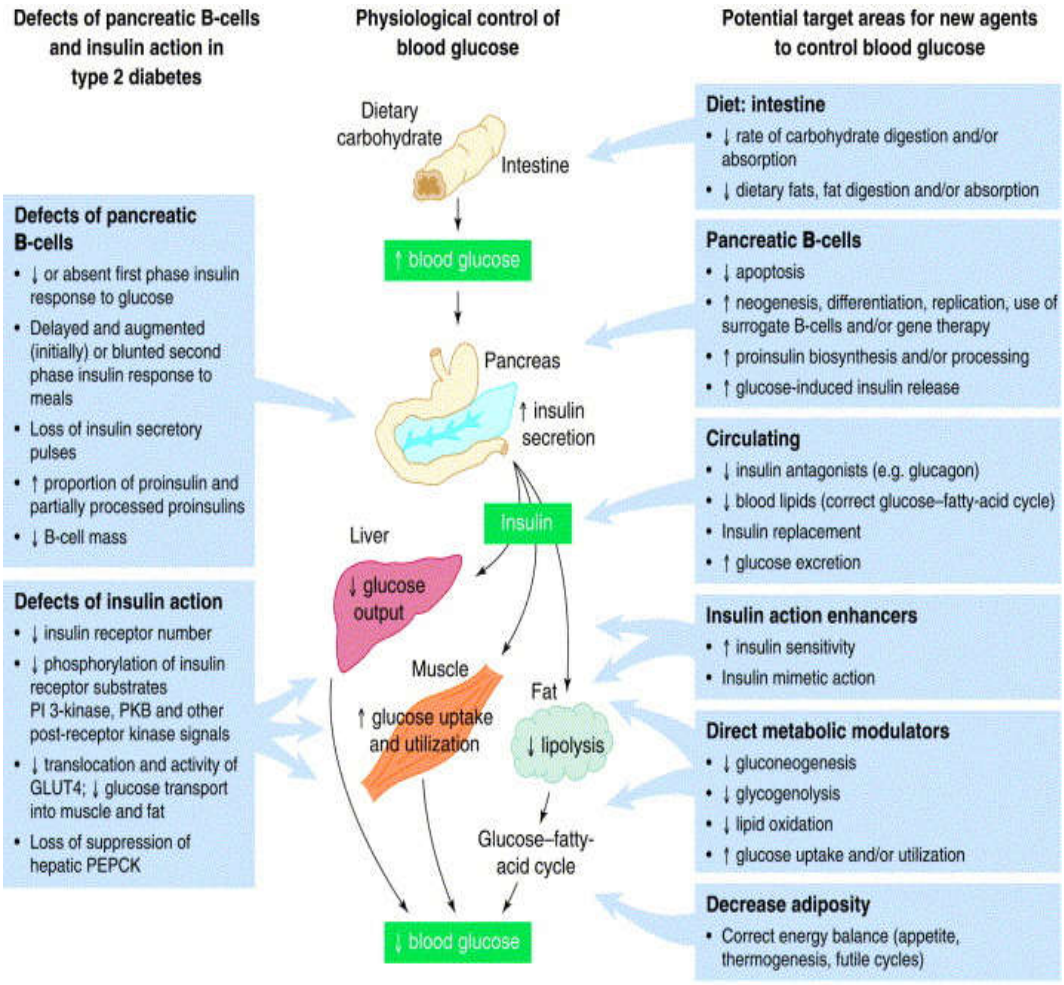

Figure 1.2: Schematic representation of potential new drug target areas. Abbreviations: ↑, increase; ↓, decrease; GLUT 4, insulin – stimulated GLUT 4; PEPCK, phospoenol pyruvate carboxykinase; PI 3 – kinase, phosphatidylinositol 3 – kinase; PKB, protein kinase B (Bailey, 2000).

1.5.1 Sulfonylureas

The primary action mechanism of sulfonylureas is to increase insulin release from pancreas. It binds to the sulphonylurea receptors on pancreatic β-cells that leads to the closure of adenosine triphosphate (ATP)-sensitive potassium channel, thus inhibit the efflux of potassium. The inhibition of potassium efflux results in depolarization of β-cells. Depolarization opens a voltage-gated calcium channel and allows an influx of Ca^{2+} into β-cells. The presence of intracellular Ca^{2+} caused translocation of insulin secretory granules to the cell surfaces. The insulin granules are subsequently released via exocytosis process (DiPiro et al., 2011). In addition, two other mechanisms of action of sulfonylureas have been suggested, which are reduction of serum glucagon concentration and potassium channels closure in extrapancreatic tissue (Katzung, Masters, & Trevor, 2004). The major adverse effect of sulfonylureas is hypoglycemia, which is more problematic with long-acting formulations, chronic liver disease or renal insufficiency. Elderly were the most at risk of hypoglycemia. Weight gain of approximately 2% is also observed following the initiation of sulfonylurea therapy.

1.5.2 Biguanide

Metformin, as the only clinically significant biguanide available, is the most widely 'insulin sensitizing agent' prescribed worldwide. It improves blood glucose level by increasing insulin-mediated suppression of hepatic glucose production, and by enhancing insulin sensitivity of peripheral tissues which optimize the uptake of glucose into these insulin – responsive tissues. Several mechanisms of how metformin improves insulin sensitivity have been proposed; by enhancing synthesis of glycogen and increasing the activity of insulin receptor tyrosine kinase and GLUT 4 (Giannarelli,

Aragona, Coppelli, & Del Prato, 2003). Metformin has no direct effect on the viable β-cells of pancreas, though insulin levels are lowered, corresponding to an increase in insulin sensitivity. Several non-glycemic benefits have been reported with the treatment of metformin which are decreasing incidence of myocardial infarction, lowering cardiovascular risk factors and reducing diabetes-related deaths. The occurrence of hypoglycemia with metformin monotherapy is minimal.

1.5.3 α-glucosidase inhibitors

α-glucosidase inhibitors (AGIs), also known as 'starch blockers' are a class of drugs that act by delaying the absorption of carbohydrate in the gastrointestinal tract. AGIs delay the absorption of carbohydrate by inhibiting several α-glucosidase enzymes (e.g. maltase sucrose, isomaltase, and glucoamylase) which are responsible for the breakdown of oligosaccharides and dissacharides into monosaccharides. This process will result in reduced postprandial glycemic levels, with a modest effect on fasting glucose (J. R. White, 2009). Acarbose is the most widely prescribed AGIs. AGIs might be a suitable candidate in the treatment of type 2 diabetes mellitus as they specifically use for marked postprandial hyperglycemia, a possible independent risk factor for the development of cardiovascular complications (Ceriello, 2005). Even though scarce cases of hepatic injury were reported, AGIs are expected to cause no hypoglycemic or other life-threatening events, even at overdoses (Chiasson et al., 2004).

1.5.4 Meglitinides

Meglitinides is a short acting insulin secretagogues drug, which in similar to sulfonylureas, lowers blood glucose level by stimulating pancreatic insulin release. The insulin release, however, is glucose dependent and becoming less at low blood glucose concentration. Hence, the risk of hypoglycemic event and weight gain with meglitinides therapy appears to be lesser than with sulfonylureas therapy. The rate of hypoglycemia of repaglinide and nateglinide (meglitinides) were recorded to be 3% and 15%, versus glipizide and glyburide (sulfonylureas) rates of 19% and 15%, respectively (DiPiro et al., 2011). In addition, these agents can be used to enhance insulin secretion during meals (when it is needed) in patients who are close to glycemic goals. They should be administered up to 30 minutes prior to each meal.

1.5.5 Thiazolidinediones

Thiazolidinediones, also referred to as TZDs or glitazone act by decreasing insulin resistance. They work by binding to the perixosome proliferator-activated receptor gamma (PPAR-γ), which located primarily on the adipose and vascular cells. The frequency of these receptors in the muscle is very low; hence muscle is not the main action site of TZDs. PPAR-γ is a nuclear transcription factor that modulate the expression of genes involved in glucose and lipid metabolism, transduction of insulin signal and differentiation of adipocytes. In diabetic patients, the major action site of TZDs is adipose tissue. These drugs promote the uptake and regulation of glucose, modulate the synthesis of lipid hormones, cytokines or other proteins that involved in energy regulation and control the apoptosis and differentiation of adipocytes. Pioglitazone and rosiglitazone are the two member of this class approved by FDA for

the treatment of type 2 DM. They have distinct side chains that produced differences in therapeutic action, metabolite profile, metabolism and side effects. Both of these agents have been related to fluid retention and must be prescribed with caution in congestive heart failure patients.

1.5.6 DPP-4 Inhibitors

Inhibitors of dipeptidyl peptidase (DPP) 4, also known as gliptins, are a group of oral hypoglycemic that block DPP-4, an enzyme that destroys incretin. The primary action of DPP-4 inhibitor is to prolong the half-life of endogenous incretin levels, which are glucagon-like protein 1 (GLP-1) and glucose-dependent insulinotopic hormone (GIP). Incretins help the body to release more insulin when it is required and lower the amount of glucose being produced by the liver when it is not needed. Incretins hormones are produced throughout the day and the levels go higher at meal times. In healthy person, incretins are removed from the body rapidly by the DPP-4. Thus, with the presence of DPP-4 inhibitors in diabetic patients, incretins could be retained in the body longer, resulting in decreasing glucagon level, which subsequently increasing insulin release, slowing gastric emptying and lowering blood glucose levels. The clinical data of DPP-4 inhibitor indicated there was no incidence of hypoglycemia compared with placebo or active-controlled anti-diabetic drugs after long term treatment (Li et al., 2016) and it could achieve a long-term effective and safe glycemic control for use as monotherapy or in combination with metformin (Liu et al., 2014).

1.5.7 Incretin mimetic

Incretin mimetics are a new category of pharmacological agents. Similar to the DPP-4 inhibitors discussed above, these agents employed the blood glucose lowering actions of the incretin hormone, GLP-1. Some of blood glucose lowering actions by GLP-1 are enhancing glucose – linked insulin secretion, inhibiting elevated glucagon secretion, and decreasing food absorption by slowing food digestion. However, due to the short half –life of native GLP-1 (1 to 2 minutes), the long – term use of this hormone in the treatment of chronic conditions, namely type 2 DM is limited. This led to the search for GLP-1 analogs which are less or more structurally similar to GLP-1 but are not degraded by DPP-4 enzyme and have much slower elimination rates, hence longer half-life. Exenatide is the first incretin mimetics approved by the US Food and Drug Administration in 1995, followed by liraglutide in 2010. Later, a long-acting form (once weekly) of exenatide became available for clinical use in 2012. Weight loss is one beneficial effect of incretin-based therapy. These agents, however, can cause significant adverse effects on gastrointestinal, particularly early in the therapy, with mild to moderate nausea becomes the most common observed effect.

1.5.8 Amylin analogue

Amylin, also known as islet amyloid polypeptide (IAP) is an endogenous neuroendocrine hormone which is co-secreted with insulin by the pancreatic β-cells. In glycemic regulation, amylin promotes satiety and delays gastric emptying, thereby preventing postprandial hyperglycemia spikes. Similar with insulin, patients with type 2 diabetes secreted less amylin, whereas patients with type 1 have relatively no amylin at all. Currently, the only available amylin agonist is pramlintide, approved by the FDA in

2005. It is administered subcutaneously via injection. This agent is usually reserved for use in type 1 diabetic patients that undergo intensive insulin therapy. The major side effect of pramlintide is nausea.

1.5.9 Sodium glucose co-transporter inhibitors

Studies on SGLTs started with the isolation of phlorizin, a glycoside from the bark of apple tree in the 19th century. Phlorizin was recognized as a non selective inhibitor of both SGLT 1 and SGLT 2 that are responsible for the reabsorption of glucose in the kidneys and small intestine (Chasis, Jolliffe, & Smith, 1933). Further development of phlorizin as an antidiabetic agent however, was limited by its poor oral bioavailability, lack of specificity and potential malabsorption with SGLT 1 inhibition (Mudaliar, Polidori, Zambrowicz, & Henry, 2015). This led to the pursuit of selective SGLT2 inhibitors with improved properties. The primary mechanism of action of SGLT 2 inhibitors is reducing plasma glucose concentration by enhancing renal excretion of glucose (Kalra, 2014). These drugs inhibit SGLT 2, a low-affinity glucose transporter that are expressed primarily in the proximal tubule of kidney and responsible for approximately 90% of renal glucose reabsorption. When competitive inhibition of SGLT 2 occurred, excess renal glucose is not reabsorbed, thus the glucose is excreted in the urine. These lead to a net loss of glucose and reduction in plasma glucose level (Poudel, 2013). Three SGLT2 inhibitors (dapagliflozin, canagliflozin, and empagliflozin) have been approved for use in the U.S. and Europe (Grempler et al., 2012). Currently, Japan has also approved luseogliflozin, topogliflozin, and ipragliflozin as antidiabetic drugs and several other compounds are in late-stage clinical trials (Mudaliar et al., 2015). The most common adverse effects of SGLT 2 inhibitors are

correlated to their mechanism of action, including uro-genital tract infections which occured due to the elevated glucose in urine. These effects are frequently noticed among the female patients and uncircumcised male patients (Sarnoski-Brocavich & Hilas, 2013). To date, there is no SGLT 1 inhibitor that has been approved by FDA or European Medicines Agency (EMA) as an antidiabetic agent. Few selective SGLT 1 inhibitors for example, GSK-1614235 and KGA-2727 were tested in nonclinical and clinical trials involving rat and human studies. The results showed that SGLT 1 inhibitors block intestinal glucose absorption, lower secretion of GIP, and increase GLP-1 and peptide YY secretion (Dobbins et al., 2015).

1.5.10 Bromocriptine

Bromocriptine is a sympatholytic D2 – dopamine agonist that has been approved for use as an antihyperlgycemic drug in 2009. The use of bromocriptine in treating diabetes has been recognized, based on its action in modulating the pathways of glucose and energy metabolism. These actions aid in resetting an abnormally high hypothalamic drive for elevated levels of plasma glucose, triglycerides, and free fatty acid in insulin – resistant patients (Shivaprasad & Kalra, 2011). Based on animal and clinical studies, a quick release bromocriptine formulation, given within two hours of awakening, showed to increase low hypothalamic dopamine levels and block excessive sympathetic tone of central nervous system, leading to enhanced suppression of glucose production by liver, thus resulting in a reduction in postprandial hyperglycemia. The doses used for diabetes therapy are far lower than those used in treating Parkinson's disease. Apart from nausea, this agent is well – tolerated.