ANTIHYPERGLYCEMIC ACTIVITY OF *TINOSPORA CRISPA* EXTRACTS AND FRACTIONS IN RAT

MIR MOHAMMAD REZA SEYED HASSANI

UNIVERSITI SAINS MALAYSIA
2015

ANTIHYPERGLYCEMIC ACTIVITY OF *TINOSPORA CRISPA* EXTRACTS AND FRACTIONS IN RAT

By

MIR MOHAMMAD REZA SEYED HASSANI

Thesis submitted in fulfillment of the requirements for the degree of Master of Science

August 2015

ACKNOWLEDGEMENTS

All praises to Allah the Almighty, without whom will everything would cease to be, who gave me the strength, inspiration and patience to continue this research. First of all Special thanks to my parents Mir Jafar, Mahin and my brother Mir Morteza for their supports all this years. I would like to express my deep gratitude and sincere appreciation to my supervisor Prof. Dr. Mohd Zaini Asmawi who provided me the germ of the idea for this research. Over the months of research and analysis, his deep and broad knowledge, constructive suggestions, stimulating discussion and comments on the preparation of this thesis as well as his wisdom and wit helped that germ to sprout/germinate and grow. He had always open door for me to see him for whatever duration or reason although he was busy with his other students. I also wish to thank my co-supervisor Dr. Roziahanim Binti Mahmud for her guidance and assistance throughout the chemical aspect of this work. I would like to express my gratitude to the Dean of School of Pharmaceutical Sciences Dr. Munavvar Zubaid Abdul Sattar, for giving me the chance to pursue my higher education in this school. Also, my thanks go to the Institute of Postgraduate Studies and Universiti Sains Malaysia for their kind cooperation and assistance rendered in the preparation of this thesis and a warm thanks to director of International office, Assoc. Prof. Dr. Anees Janee Ali for his helps, kindness and guides. A number of other academic and non-academic staff at the Universiti Sains Malaysia also gave me their support and assistance, including Mr. Rosli, the laboratory assistant in the pharmacology research laboratory and Mr. jasmie for his helps. Also, I am very grateful to all my colleagues who were always my real friends Abdulmenem, Adlin, Bassel, Faramarz, Idris, Item, Michael, Nasiba, Raghdaa and Sook yee for their helps, kindness and supports.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

^{0}C	Degree Celsius
%	Percent
AF	Aqueous fraction
ANOVA	Analysis of variance
BF	n-butanol fraction
b.w.	Body weight
et al.	And others
g	Gram
GC-MS	Gas chromatography–mass spectrometry
HF	n-hexane fraction
HPLC	High-performance liquid chromatography
IDDM	Insulin dependent diabetes mellitus
i.p.	Intraperitoneal
IPGTT	Intraperitoneal glucose tolerance test
IU	International units
Kg	Kilogram
KRB	Krebs-Ringer bicarbonate
OGTT	Oral glucose tolerance test
STZ	Streptozotocin
WF	Water fraction

LIST OF PUBLICATION ASSOCIATED WITH THIS THESIS

Jornal Pablication

Accepted

Mir Mohammad Reza Seyed Hassani, Ashfaq Ahmad, Mohd. Zaini Asmawi, Roziahanim Mahmud. Preliminary investigation of normoglycemic, anti-hyperglycemic and dyslipidemic activities of different extracts of *Tinospora crispa* on diabetic rat (2015).

On Review

Mir Mohammad Reza Seyed Hassani, Item Justin Atangwho and Mohd. Zain Asmawi. Antihyperglycemic activity of extract and fractions of *Tinospora crispa* in diabetic rats and intestine (2015).

Poster Presentation

Mir Mohammad Reza Seyed Hasani,Pros Dr. Mohd. Zaini Asmawi, Parastoo Zarghami Moghaddam, Helen Hemati. Evaluation of anti-hyperglycemic effect of *Tinospora crispa* in traditional medicine in *in vivo* model. University of Medical Sciences, Bojnurd, Iran (2013).

AKTIVITI ANTIHIPERGLISEMIK EKSTRAK DAN FRAKSI *TINOSPORA CRISPA* PADA TIKUS

ABSTRAK

Tinospora crispa (Menispermaceae) yang nama tempatanya dikenali sebagai "akar patawali", telah lama digunakan dalam perubatan tradisional Malaysia untuk rawatan kencing manis. Tujuan kajian ini adalah untuk mengesahkan kesan hipoglisemik dan antihiperglisemik ekstrak *Tinospora crispa* yang berbeza beza dalam tikus normal dan tikus diabetik aruhan streptozotocin (STZ). Serbuk batang *Tinospora crispa* kering diekstraksi secara berturut-turut scara maserasi dengan eter petroleum, kloroform, metanol dan air untuk menghasilkan empat ekstrak. Tidak satu pun daripada keempat-empat ekstrak (1.0 g / kg) yang diberikan secara oral dapat menurunkan paras glukosa darah tikus normal yang menunjukkan bahawa ekstrak-ekstak tersebut tidak mempunyai sebarang kesan hipoglisemik. Keempat-empat ekstrak juga tidak menghalang kenaikan paras glukosa darah tikus yang dimuatkan dengan glukosa secara intraperitoneal. Pemberian ekstrak secara oral 1 g / kg setiap hari selama 9 hari pada tikus diabetik aruhan STZ mendapati hanya ekstrak air dapat mengurangkan paras glukosa darah secara signifikan ($P < 0.05$) berbanding dengan kumpulan kawalan yang menunjukkan bahawa ekstrak air mempunyai kesan antihyperglisemik. Ekstrak air kemudiannya difraksikan untuk mendapatkan fraksi n-butanol dan fraksi air. Walau bagaimanapun, rawatan setiap hari untuk 12 hari mendapati hanya fraksi air (0.5 g / kg) menurunkan paras glukosa darah tikus diabetic aruhan-STZ. Penyaringan fitokimia ekstrak air *Tinospora crispa* menunjukkan kehadiran alkaloid, saponin dan glikosida. GC-MS fraksi air menunjukkan kehadiran dodesil akrilat, asid pentadekanoik dan asid propanoik

manakala analisis HPLC menunjukkan kehadiran asid galik dan salsolinol. Dodesil akrilat, asid pentadekanoik, asid propanoik dan asid galik telah dilaporkan sebelum ini memiliki kesan antidiabetik. Secara keseluruhan, hasil kajian ini menunjukkan *Tinospora crispa* mempunyai kesan antidiabetik yang mungkin menyokong penggunaannya di kalangan pesakit kencing manis. Beberapa sebatian yang dikenalpasti diatas mungkin telah menyumbang secara individu atau dalam gabungan kepada aktiviti antidiabetik tersebut.

ANTIHYPERGLCEMIC ACTIVITY OF *TINOSPORA CRISPA* EXTRACTS AND FRACTIONS IN RATS.

ABSTRACT

Tinospora crispa (Menispermaceae), locally known as "akar patawali", has long been used in Malaysian traditional medicine for treatment of diabetes. The aim of this study was to verify the hypoglycemic and antihyperglycemic effects of different *Tinospora crispa* extracts in normal and streptozotocin (STZ)-induced diabetic rats. Pulverized *Tinospora crispa* dried stems were extracted successively by maceration with petroleum ether, chloroform, methanol and water to yield the four extracts. None of the four extracts (1.0 g/kg) administered orally lowered the blood glucose levels of normal rats which suggest that they have no hypoglycemic effect. The four extracts also did not inhibit the rise of blood glucose level of glucose intraperitoneally loaded rats. Daily oral administration of the extract 1 g/kg for 9 days in STZ-induced diabetic rats found that only the water extract managed to lower the blood glucose levels significantly ($P<0.05$), as compared with the control group which suggests that it has antihyperglycaemic effect. The water extract was then fractionated to obtain n-butanol and aqueous fractions. However, daily treatment for 12 days found that only water fraction (0.5 g/kg) lowered the blood glucose level of STZ-induced diabetic rats. Phytochemical screening of the water extracts of *Tinospora crispa* indicated the presence of alkaloids, glycosides and saponins. GC-MS of water fraction showed the presence of dodecyl acrylate, pentadecanoic acid and propanoic acid whereas HPLC analysis showed the presence of gallic acid and salsolinol. Dodecyl acrylate, pentadecanoic acid, propanoic acid and gallic acid have been previously reported to possess antidiabetic effect. Overall,

the findings of this study showed *Tinospora crispa* possesses antidiabetic effect which may justified its use in diabetic patients. Some of the identified compounds above might have contributed individually or in combination to its antidiabetic activity.

CHAPTER ONE : INTRODUCTION

1.1 Introduction

The use of herbal remedies, in different cultures and traditional medicines, have been practiced for centuries because of traditional beliefs and out of habits. In majority of the world population, including those of Asian and western countries, the modern medicine has prominently taken over the life style, practice and use of herbal remedies to meet health needs. Surprisingly, in this modern era, people are still using herbal medicines in conjunction with modern conventional methods. Worldwide, especially in developed countries, a substantial amount of money is being annually spent on research aimed at identifying and assessing herbal products. A number of pure isolated herbal compounds, such as taxol and artemisinin, have been derived from plants and have already been through clinical development. In general, herbal products are widely believed to cause less adverse side effects, and are less potent compared to synthetic and modern pharmaceuticals. However, their use can play a key role in the reduction of the national health expenditure (Holt & Chandra, 2002).

These studies investigating the biologically active constituents of medicinal plants, have made it possible for the development of new drugs for clinical use (Kamboj & Dhawan,. 1982). Natural products serve as a source of lead compounds for drug development. In some Asian countries such as China, India, Japan and Korea, herbal remedies have been officially recognized (World Health Organization., 2003).

The reawakening, obviously if any real improvement in healthcare systems and research especially in Asia and the developing countries were to be made, there should be an optimal use of all their natural resources and potential, and of course traditional herbal medicines are undoubtedly amongst the best options. International organizations, like the World Health Organization (WHO), have showed enthusiasm regarding to the use of herbal medicine, and it suggests reinforcing the use of traditional medicines which may spark globally. In some countries, traditional medicinal plants have come to be placed on the same footing as modern medicines and sometimes even more popular. This is the time to accept the importance of medicinal plants such as *Tinospora crispa* as an alternatives for health care (Farnsworth & Bingel,1977)

1.2 Diabetes Mellitus

Diabetes mellitus (DM) as a chronic metabolic disorder, has long been a major public health problem and its prevalence, in the modern world, is increasing. The World Health Organization estimated that in 2000, diabetic mellitus affected 171 million individuals globally, and moreover it has been projected that, by 2030, this number would increase to 366 million patients (Whiting et al., 2011). It is a group of metabolic diseases characterized by chronic high blood glucose levels, caused either by the inability of the β-cells of pancreas to produce adequate amount of insulin, or failure of the target cells (particularly the skeletal muscles and adipose tissues) to respond to secreted insulin (Shoback, 2008). Food digestion always commences with amylase in the mouth terminating in the small intestines. During the digestion process in the intestines, food is converted to glucose, a universal source of energy. As more foods carbohydrate change to glucose and the blood glucose level, this main source of energy in body reaches the postprandial level a signal given to the pancreas causes it to release the hormone insulin into the blood stream, enabling the cells to use the readily available glucose (Holt et al.,2003).

The chronic high blood glucose level (hyperglycemia), produces classical symptoms, such as polyuria (frequent urination), polydipsia (increased thirst) and polyphagia (increased hunger). The treatment focus therefore, is to maintain steady blood glucose within physiological limits (euglycemia). However, this is often scarcely achieved in practice, hence there are reported occasional incidences of hypoglycemia, besides the grave side effects of some of the conventional drugs in use. Moreover, along with pharmacological treatment, non-pharmacological treatment measures have also become essential for the prevention and management of diabetes mellitus, due to the variety of

complications accompanying DM, such as malnutrition and cardiovascular diseases. For instance, dietary advice given to older people with diabetes would be intended to maintain adequate nutrition, thereby complementing observed tissue wasting, as diabetes also make damage on nerve supply and further muscle wasting which referred as diabetic amyotrophy and mostly effect on hips and shoulders muscles. Furthermore, besides their nutritional complementary role, most leafy vegetables also exert therapeutic functions, for which reason they are referred to as functional foods. Natural products including medicinal plants have equally been used as source of drugs in traditional management of diabetes with the aim to circumvent the drawbacks of conventional medication, and in most instances to complement their therapeutic effect (Nascimento et al., 2006).

At the moment, an estimated 285 million individuals suffer from Type II diabetes which correspond to 90% of all diabetic cases worldwide. In Malaysia alone 1.6 million individuals are affected by Type II diabetes (Saleh et al., 2013), thereby suggesting that this is no longer a disease associated with middle and old age individual. Recent evidence suggests that children as young as 10 years old may also be affected, indicating that, a holistic approach in sourcing a cure is urgently needed. Diabetic is also at high risk of macrovascular complications, such as ischemic heart disease and strokes. Some risk factors in diabetes include having a family history of heart disease and high cholesterol levels. Actually high level of some cholesterols, for instance low density lipoprotein (LDL) as one of the five major groups of lipoproteins and also triglycerides, can increase the risk of heart disease. They can build up inside of blood vessels, leading to the narrowing and hardening of arteries, blocking the arteries and raising the risk of getting heart disease and strokes **(**Almdal et al., 2004).This may be attributed in part to the late diagnosis and poor glycemic control. However, being highly associated with components of the metabolic syndrome,

such as obesity, hypertension (10-37%) and hyperlipidemia (63-76%), diabetes actively predisposes DM patients to such complications (Funk et al., 2004). More worrisome is the fact that many of those affected by DM may do not even know that they suffer from the disease. Hence, it is really necessary to discover new and better methods for treating and controlling DM, such as the utilization of herbs.

1.3 Classification of Diabetes Mellitus

Diabetes is generally classified into insulin dependent diabetes mellitus (IDDM), which is commonly known as Type -I diabetes, and non-insulin dependent diabetes mellitus (NIDDM) known as Type -II diabetes. Presently, as many types of anti-diabetic drugs are available, they are typically classified according to various criteria, such as the type of diabetes they are used for, patients age, or other factors like history of any particular disease, inherited sickness or even gender and physical factors which is contributing to the disease manifestation. Type I diabetes mostly affects children with an onset before the 15^{th} birthday (Harjutsalo et al., 2008) and since insulin is destroyed when ingested orally, the treatment have to be administered through subcutaneous injections. On the other hand, Type II diabetes usually affects adults, especially the obese. These patients may not require insulin treatment and sufficient to be given with oral antidiabetic agent. Some agents induce greater insulin production, sensitize the tissues to insulin, and prevent excessive glucose absorption from gastrointestinal tract are available for non-insulin dependent diabetes mellitus patients. Among the five classes of anti-diabetic drugs the two main classes widely used are sulfonylureas (e.g. glibenclamide) and biguanides (e.g.metformin), which can be taken separately or in combination (Phung et al., 2010)

1.4 . Pancreas

The pancreas is an integral part of the digestive system. Due to its exocrine and endocrine secretions, the pancreas as an exocrine gland always considered to be a mixed gland. Pancreas secretes digestive enzymes and alkaline substances into the small intestines. It acts as an endocrine gland as well as secreting certain hormones into the blood stream (Gradwohl et al., 2000).

Hormones from clusters of cells, referred to as Islets of Langerhans or the pancreatic islets, are stored and secreted in the human body. Those clusters contain four special groups of cells: alpha (α), beta (β), delta (δ) and F cells. Insulin and glucagon are the main hormones which help regulate glucose metabolism. Alpha cells, responsible for glucagon secretion, are effective in raising blood glucose levels. Conversely, beta cells are specialized in producing insulin to lower blood glucose levels. Delta cells secrete the hypothalamic growth inhibiting hormone, while the F cells regulate the release of pancreatic digestive enzymes, such as the pancreatic polypeptide (Pritchett et al., 1995).

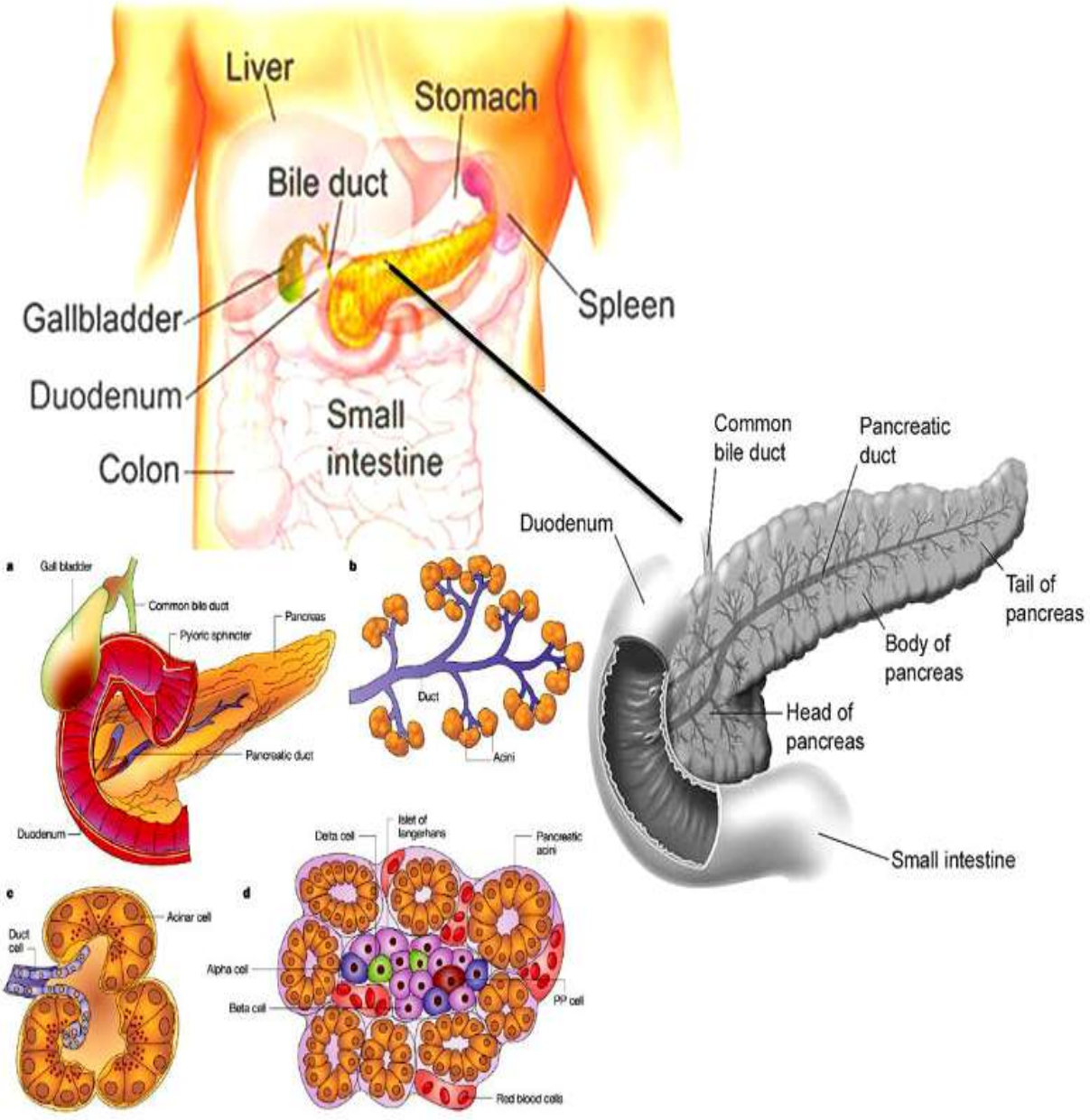


Figure 1.1. The pancreas system (National cancer institute, Pancreatic Cancer, 05-07-2014. Retrieved from http://www.cancer.gov/types/pancreatic) with modification.

1.4.1 Glucagon

Glucagon is secreted by the alpha cells of the pancreas. It increases the concentration of cyclic AMP, derived from ATP in the liver cells, thereby causing the enzyme (phosphorylase) to be activated when the blood glucose level is low. For instance, glucagon will be higher between meals and during exercise. Glucagon as a polypeptide hormone is released in response to hypoglycemia and plays a role as a physiological regulator of the effects caused by insulin (Constantin et al., 1995).

When the concentration of glucose falls in the blood and glucagon gets secreted, the liver responded by converting glycogen into glucose (glycogenolysis) to raise the blood glucose concentration. Glucagon stimulates the release of fatty acids and glycerol from the adipose tissue. This enzyme also separates glucose units from the branched glycogen molecules, and the free glucose units then enter the bloodstream to maintain the blood level of glucose at 70-110 mg/dl. For this reason, glucagon is considered to be hyperglycemic factor (DiCostanzo et al., 2007)

1.4.2 Insulin

Insulin is synthesized as pre-pro insulin. It contains A and B chains connected by 51 amino acids, and linked by disulfide. Insulin as a peptide hormone released and synthesized from pancreatic β-cells at low basal rates. However, it gets stimulated to reach at much higher levels in response to a variety of stimuli, especially glucose. Insulin analogues have been commercially available for years as they have been synthesized to mimic human insulin (Ljungqvist, 2012).

1.4.2.1 Mechanism

Beta cells in pancreas naturally produce insulin. The hormone sending signals to the cell receptors to absorb glucose from the blood and raises blood glucose levels in the cells (hypoglycemic effect). Insulin does not have an effect beyond that of lowering blood glucose concentration with respect to altering ris (Stratton et al., 2000). However, insulin is effective in increasing protein synthesis. Furthermore, it increases the conversion of glucose into fatty acids and promotes amino acid transport into the cells.

1.5 Antidiabetic Drugs

Modern day research on drug development associated in β-cell function of pancreas has produced several number of new drug compounds in the treatment of Type II or insulin dependent diabetes mellitus (Zhao et al., 2010). However, In Type I diabetes some treatment prevents the development of microvascular and produces neurologic complications (Ohkubo et al., 1995).

1.5.1 Biguanides

Biguanide refers to a class of drug which are using as an oral antihyperglycemic drugs for diabetes mellitus. The most popular example and the only widely available antidiabetic biguanide drug is metformin. It can also improve glycaemic control in overweight to obese individuals with type 2 diabetes (Gilbert et al., 2006). Biguanides mechanism of action is to inhibit the liver from producing glucose and to help the body secrete insulin (Potenza et al., 2009). Except metformin other biguanides derivatives have been withdrawn from the market due to their toxic effects.

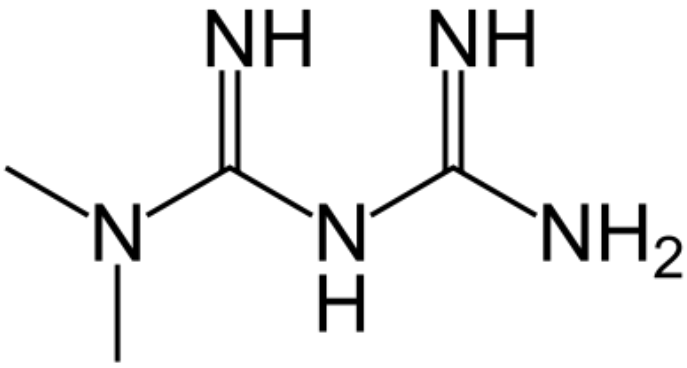


Figure 1.2. Chemical structure of metformin.

1.5.2 Sulphonylureas

Sulphonylurea derivatives is one of the earliest class of anti-diabetic drugs, that are used to management of diabetes mellitus type II. This group of drugs are metabolized by the liver. Sulphonylureas are known to be the 'principal oral hypoglycemic agents' in type II diabetes treatment. This class works by stimulating the β-cells in the pancreas to produce more endogenous insulin, and by increasing the circulating insulin to levels sufficient to overcome insulin resistance. This class of drug is generally taken one to twice a day before meals during treatment. Hypoglycemia is the possible side effect in all type of sulphonylurea. However, glipizide and glimepiride showing less hypoglycemic effects than the other drugs of this class (Pieber et al., 2003). Considering the fact that cardiac myocytes also express the same sulphonylurea receptor as the β-cells, these drug compounds may

potentially lead to cardiovascular toxicity. In this drug category most of the drugs names end with ‘ide’. Examples of sulphonylureas are glibenclamide which are also known as glyburide, glipizide, gliquidone, glyclopyramide, glimepiride , gliclazide, tolbutamide and tolazamid.

1.5.3 Alpha-glucosidase inhibitors

Alpha-glucosidase is an enzyme responsible for the breakdown of complex carbohydrates into di- and mono-saccharides in the distal duodenum and the proximal jejunum. Alpha-glucosidase inhibitors help the body to lower blood glucose level by blocking the break down of starches into glucose in the intestine.

Miglitol, acarbose and voglibose are examples of alpha-glucosidase inhibitors. However, acarbose is the only commercialized α-glucosidase inhibitor. It is an oligosaccharide produced by cultured strains of actinomycetes. It is a competitive inhibitor with low affinity for glucoamylase, and high affinity for sucrase and pancreatic α-amylase (Braun et al., 1995). The major effect of acarbose is reducing the risk for myocardial infarction in type 2 diabetic patients by controlling and improving the body weight and triglyceride levels (Hanefeld et al., 2004).

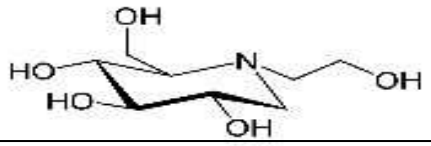
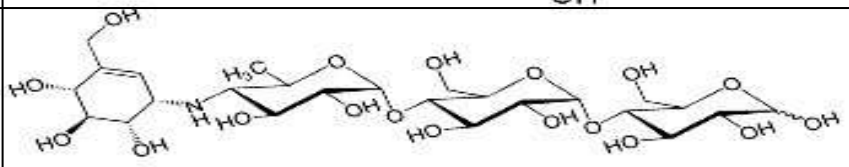
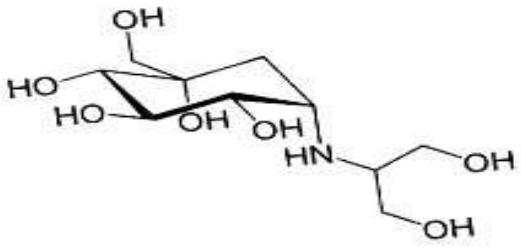
DRUG COMMON NAME	STRUCTURE
1 Miglitol	
2 Acarbose	
3 Voglibose	

Figure 1.3. Chemical structures of important alpha-glucosidase inhibitor drugs.

1.5.4 Thiazolidinedione

Glitazones or thiazolidinediones are anti-diabetic drugs which improve insulin sensitivity in the muscles and also reduce glucose production in the liver. Thus, they are used in the treatment of type II diabetes. They also help to decrease the levels of triglycerides in the blood. Avandia and pioglitazone are the most popularly prescribed drugs of this class. However, due to some adverse reaction incidents, which included bladder cancer and heart attacks, they were banned by the European Medicines Agency in September 2010. Nevertheless, for pioglitazone, the adverse effect has not been deemed sufficient to justify restriction on its treatment use in the UK (Dunn et al., 2011).

1.5.5 Incretin mimetics / GLP-1 analogues

Incretin mimetics or glucagon like peptide-1 receptor agonists (GLP-1) analogues are injectable drugs used for the treatment of Type II diabetes. As the level of glucose in the body increases more hormone of GLP-1 also will be produced. While food is consumed, the body releases GLP-1, and the hormone moves to the pancreas. They will increase the amount of insulin released from the beta cells of the islets of Langerhans and decrease the release of glucagon. They are known to be inhibitors that stop the protein dipeptidyl peptidase-4 from destroying the incretin hormones (Ellingsgaard et al., 2011).

1.5.6 Meglitinide

Meglitinides are from the class of drugs which stimulate β-cells to release more insulin and this class drugs are used to treat diabetes type II, The mechanism of action is similar to sulphonylureas. Since meglitinides and sulphonylureas both stimulate to release insulin, hypoglycemia or low blood glucose is the possible side effect on patients using them. Some example of this class are nateglinide and repaglinide (Lorenzati et al., 2010).

1.5.7 Dipeptidyl peptidase-4 inhibitor / Gliptins

Dipeptidyl peptidase-4 inhibitor (DPP-4 inhibitors) or Gliptins represent an innovative approach and are a class of oral hypoglycemic which are used for treatment of type II diabetes mellitus. DPP-4 inhibitors are attached to the DPP-4 enzyme and prevent them from assisting in the breaking down of Glucagon-like peptide-1 (GLP-1), (Monami et al., 2011).

Drugs of this class stimulate the production of insulin and reduce the production of glucagon, especially during digestion where it decreases gastric emptying, thus it helps decreasing the blood glucose levels. The effect of this agent inhibitor is that DPP-4 will be released throughout the day and their levels increased at meal times (Ahrén et al., 2011). Example of some drugs belonging to this class are sitagliptin, vildagliptin, saxagliptin and linagliptin. Linagliptin was approved as a treatment for Type II diabetes by the U.S. Food and Drug Administration (FDA) in May 2011.

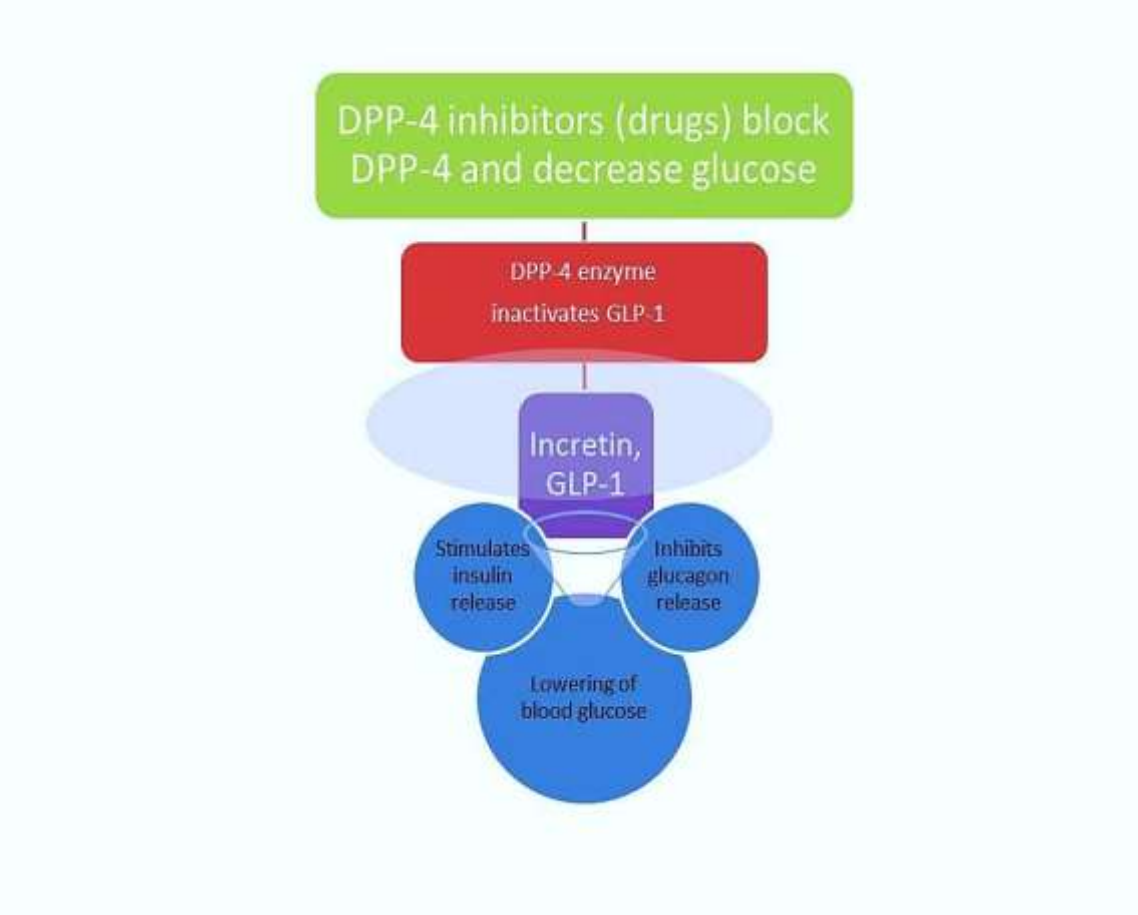


Figure 1.4. Mechanism of DPP-4 inhibitors and GLP-1.

1.5.8 Insulin

Insulin is one of the main hormones that regulate blood glucose levels. It can directly lower the glucose levels by increasing the uptake of glucose to the muscle and fatty tissue, and reducing the release of glucose from the liver. A number of different types of insulin preparations are available in the medications. Insulin is usually prescribed for patients suffering from Type I DM; however, it may also be prescribed for patients with Type II DM who have not responded well to strict diets, exercise and oral antidiabetic drugs. Some insulin act only for a few hours duration, but some exert their effects for as long as one whole day (Sussman et al., 2012).

1.5.8.1 Rapid action insulin

To control blood glucose levels promptly, rapid-acting insulin is administered for adults with type II diabetes, and also for adults and children (4 years and above) with type I diabetes. These types are usually taken just before or during meal. They act very quickly to minimise the increase of blood glucose level after eating. This type of insulin should be given within 15 minutes before, or 20 minutes after starting a meal. The action of rapid-acting insulin lasts for about 4 hours. As they act very quickly, they can lead to an increased chances of hypoglycemia (Heinemann, 2010).

1.5.8.2 Short action insulin

Regular insulin is short-acting or specifically, it has an onset of action that normally reaches the bloodstream within 30 minutes to an hour after injection and a duration of action of 4 to 8 hours (Siebenhofer et al., 2006).

1.5.8.3 Intermediate action insulin

Neutral Protamine Hagedorn (NPH) insulin which is also known as Humulin N, Novolin N, Novolin NPH, NPH Iletin II, and iso-phane insulin, is an intermediate acting insulin product given to help control the blood sugar level of those with diabetes. This type of insulin is absorbed slowly and generally reaches the bloodstream from about 2 to 4 hours after it is injected. Thus, it has a formulation that contains a substance of action, and it lasts longer. Its duration of action is between 10 and 20 hours and after approximately 20 hours, the whole dose has been absorbed. In 1936, Nordisk formulated pig insulin by adding neutral protamine to regular insulin, and NPH was created (Peterson, 2006).

1.5.8.4 Long action insulin

Long acting insulin preparations are prescribed for a number of different types of diabetes and they have an onset of action of around 1 hour, and a duration of action of 14 to 24 hours. However, the action of those insulin products may vary substantially from one person to another. Given to certain patients, it may vary by one day. Lantus is a clear insulin preparation. It was the first real basal insulin that lasted for 24 hours, almost with no peak. Long acting insulins are available in animal and analogue forms, in the UK analogue insulins are more preferred by the National Health Service because the insulins have no

peak activity. As such, it allows for a consistent delivery of activity through the day. However, it may not be used concurrently with rapid insulin. Some patients may need to use this product twice daily for an action duration of 24 hours (Peterson, 2006).

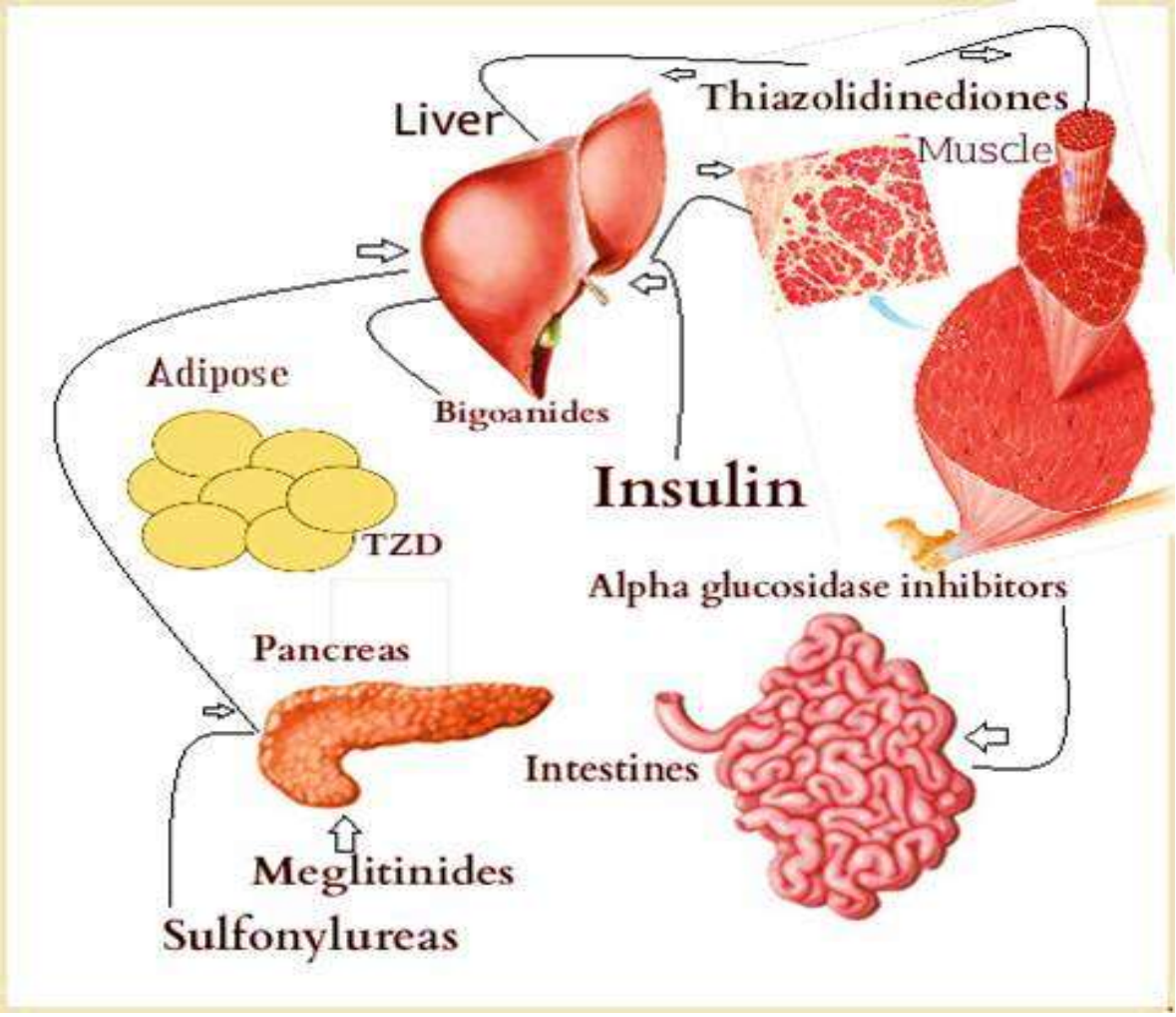


Figure 1.5 Antidiabetic drugs action on different organs

1.6 The plant, *Tinospora crispa*

1.6.1 Botanical aspects

Kingdom	:	Plantae
Subkingdom	:	Tracheobionta
Division	:	Magnoliophyta
Class	:	Magnoliopsida
Scientific name	:	*Tinospora crispa*
Synonym	:	*Tinospora cordifolia*
Family	:	Menispermaceae
Local name	:	Akar patawali
Part used	:	Stem

Figure 1.6 The stem of *Tinospora crispa* plant

Figure 1.7 The *Tinospora crispa* plant

1.6.2 Background

Tinospora crispa is an indigenous medicinal plant that belongs to the Menispermaceae family. Locally, it is known as akar patawali and akar seruntum. It is a medicinal plant that commonly grows wildly in the Asian countries, such as Malaysia, Indonesia, Thailand, India and the Philippines. The leaves, the roots and the stems, all have been claimed to have miraculous properties in curing a spectrum of diseases.

In rural southern India, *Tinospora crispa* has a reputation of being a hypoglycemic agent. For many years, *Tinospora crispa* has also been used in southern eastern Asia, especially in China and Malaysia as a treatment for various ailments, such as prurigo, eczema, impetigo and oxidative stress conditions. It also has been known to have anti-allergic and antiviral properties as well. A decoction of *Tinospora crispa* as a whole is used in the treatment of cholera throughout the Malay communities, and a decoction of its stem is used for various therapeutic purposes such as managing hypertension, stimulating the appetite, and protecting against mosquito bites. It is also used to treat ailments like jaundice, wounds, intestinal worms, skin infections, tooth and stomach aches, coughs, asthma, and pleurisy (Rahman et al., 1999). In Thailand, a decoction from the stems has been used as antipyretic syrup, for the treatment of internal inflammations, to reduce thirst, to cool down body temperatures, and to maintain good health (Dweck & Cavin, 2006).

Tinospora crispa was also used as an anti-parasitic agent for humans and for domestic animals (Noor & Ashcroft, 1989). This plant was able to cause a reduction in serum glucose level in diabetic rats and its hypoglycemic effect was probably due to an insulinotropic activity. It was also shown to increase peripheral utilization of glucose and to inhibit hepatic glucose release.

Tinospora crispa has been scientifically demonstrated to possess anti-filarial, anti-malarial, anti-pyretic (Kongkathip et al., 2002), and antihyperglycemic properties (Noor & Ashcroft, 1989). Also antinociceptive and anti-inflammatory activities of the ethanol extract of *Tinospora crispa* stem has been demonstrated (Sulaiman et al., 2008). In Indonesia (Borneo), it is used as a treatment for lumbago (Dweck and Cavin, 2006), in South-East Asia, in countries like Malaysia and China, an aqueous extract of *Tinospora crispa* is taken orally as a diabetes treatment.

Previous studies conducted on this herb showed that its aqueous extract was able to lower blood glucose levels in moderately diabetic rats. Its hypoglycemic effect was attributed to its insulinotropic activity (Noor & Ashcroft, 1989), However, according to a human research on glucose and insulin levels in healthy subjects and diabetic patients with type 2 diabetes mellitus in Mahidol university of Bangkok, ingestion of 250 g ethanolic extract of *Tinospora crispa* obtained using Soxlet, did not reduce the blood glucose and insulin levels of healthy and diabetic human volunteers and have no ability to sensitize β cells to secrete insulin (Klangjareonchai & Roongpisuthipong, 2011).

1.7 Antihyperglycemic drug methodologies

To investigate the activity of a drug and its use as a treatment for diabetes, more systematic approaches are require. Certain approaches of bioassay-guided drug discovery to optimize time and resources are available, which can be divided into the two main classes of *in vitro* and *in vivo* techniques.

1.7.1 *In vitro* techniques

To study on extracts and fractions of *Tinospora crisp* and to assess the potential of hypoglycemic agents on the release of insulin, many different *in vitro* techniques have been developed such as intact isolated islets techniques, perfuse pancreas methods and Scattered islet cell techniques. The features of insulin and glucagon released as a result of the use of agents had been comparatively studied (Weir et al., 1986).

The majority of researchers who are active in the drug discovery industry prefer to use *in vitro* studies as first line screening models (Berkowitz & Katzung, 2001). The recent research on the mechanisms of sulfonylureas the biochemical basis and diabetes the cellular and sub-cellular levels was mostly done with cultured β-cells (Lienhard et al., 1992). Furthermore, the results of animal experiments are sometimes impossible to be extrapolated to humans. Nevertheless, when conducted properly, animal experiments could provide vital information on the drugs being examined. In order to study on *in vitro* insulin internalization and glucose transport in peripheral tissues and insulin resistance, the most common techniques involve cultures of adipocytes derived from rat epididymal pads, skeletal or abdominal muscle strips or cells (Reecy et al., 2003). Also, the effect of natural products on glucose absorption has also been studied by using fragments or a homogenate

of the rat's small intestine Jejenum (Hikino et al., 1989). Eventually, in several of the bioassays, the measurement of insulin levels shows a determining effect. However, in some situation *in vitro* techniques are not able to simulate the real life conditions owing to the effect of the different instrument using in study or the chemicals on cell culture and isolated tissue. Therefore having *in vivo* models, using animals and other pharmacological methods is inevitable (Lipinski & Hopkins, 2004)

1.7.2 *In vivo* techniques

In vivo techniques are applied for the study of hypoglycemic activity which make use of animals for activity assessments with the aim to study the effects of therapeutic agents by monitoring the blood glucose levels of animals with normoglycaemia and/or induced-hyperglycaemia by loading with glucose, also an isolated nitrosourea glycoside from Streptomyces achromogenes that causes degeneration of pancreatic β-cells (Srivastava et al., 2007).

Diabetic humans may sometimes be grouped for *in vivo* studies. However, in animals, diabetes gets experimentally induced by using drugs such as alloxan and streptozotocin which selectively destroying β-cells. A single subcutaneous injection in a normal rat can produce an experimental model of Type I or II diabetes mellitus. However, results of adipocyte insulin binding and glucose transport (Fantus et al., 1987), concluded that the streptozotocin injected diabetic rats did not provide a representation of human non-insulin dependent diabetes mellitus (NIDDM) characterized by deficient insulin biosynthesis and release in response to glucose and diminished pancreatic insulin content (Fantus et al., 1987). The history and the mechanisms of action of alloxan, a pyrimidine derivative, have been reviewed by Lenzen and Panten (1988). Considering the fact that

animal tests always reveal little information on the mechanism of action of the compound, and involve with financial restrictions on maintaining animals, and social restrictions on the extensive use of animals in experimentation, shortage of perfect models for Type I and II has always been observed.

1.8 Hypoglycemia studies

1.8.1 Mechanisms of action of hypoglycemic agents

Hypoglycemic properties denote the qualities of certain agents in rapidly reducing the concentration of glucose in the blood and the term hypoglycemia "low blood glucose levels" experiment classifying on acute tests. An inadequate supply of glucose to the nervous system may however cause serious brain problems, from mild dysphoria to more serious issues such as seizures unconsciousness. It can occur due to a wide range of internal factors and permanent brain damage or death. It also may cause a variety of other side effects. The activity of herbal extracts in controlling blood sugar levels may be attributed to certain properties, thereby allowing them to act at the pancreatic level and stimulate β-cells to release insulin.

1.8.2 Glucose loading tests

To evaluate the anti-hypoglycemic activity of a drug following oral administration of glucose, traditionally, two tests have been used on animals: a hypoglycemic test spanning a duration of 7 hours on normal groups and a glucose tolerance test of a duration of 2 hours on glucose loaded on normal groups. IPGTT and OGTT tests are the two most classical tests used in diagnosing diabetes Glucose Tolerance Test. According to the American Diabetes Association, to verify the diagnosis of Diabetes Mellitus and the