CHAPTER I

INTRODUCTION

Background and rationale

Diabetes is one of the most common non-communicable diseases globally. It is estimated that some 194 million people worldwide suffering from diabetes mellitus. Between 1995 and 2025 the number of people with diabetes worldwide is projected to increase by 122%.[1-5] These people are at increased risk of developing a number of complications which lead to a reduction in quality of life and an increase in morbidity and mortality. The complication from diabetes, such as coronary artery and peripheral vascular disease, stroke, diabetic neuropathy, amputations, renal failure, and blindness result in increase disability, reduce life expectancy and enormous health costs in many societies.[5]

The diabetic complications are considered to be multifactorial origin. One of the major pathways involved the development and progressive of both macrovascular and microvascular diseases that involved the biochemical process of advanced glycation, which is accelerated in diabetes as a result of chronic hyperglycemia and increased oxidation. The accumulation of advanced glycated end products (AGE) or its receptors that mediate their biological actions could potentially involve end-organ injury.[6]

AGE is a complex group of compounds formed via a nonenzymatic reaction between reducing sugars and amine residues on proteins, lipids, or nucleic acids. Some of the best chemically characterized AGE in humans include pentosidine and N (carboxymethyl) lysine (CML). AGE often accumulates intracellularly as a results of their generation from glucose-derived dicarbonyl precursors. It is likely that these intracellular AGE play important roles as stimuli for activating intracellular signaling pathways as well as modifying the function of intracellular protein. In addition, AGE modified proteins may be more resistant to enzymatic degradation, and it is likely that this further promotes local tissue AGE accumulation.[7]

Extracellular matrix (ECM) proteins are susceptible to AGE modification because of their slow turnover rate. The formation of intermolecular and intramolecular cross-links with collagen as a result of the glycation process leads to structural alterations, leading to increased stiffness and resistance to proteolytic digestion. For example, AGE cross-linking on type I collagen and elastin leads to increased stiffness of blood vessels.

The effects of AGE are partially mediated through their interactions with surface receptors. Among these receptors, RAGE or receptor for AGE is the best characterized. This 35 kDa polypeptide receptor is a member of the Ig superfamily of receptors. It is present in a range of cell types like endothelium, mesangial cell, vascular smooth cell and monocytes. Through the interaction with RAGE, AGE triggers the activation of secondary messenger pathways such as protein kinase C. A key target of RAGE signaling is nucleus factor-κB (NF-κB), which is translocated to the nucleus where it increases transcription of a number of proteins. The promoter region of RAGE contains functional binding elements for NF-κB, and one consequence of NF-κB translocation is the up-regulation of RAGE itself. [7]

Recent *in vivo* studies confirm the pathogenic effects of ligand-RAGE interactions. A role of RAGE in the pathogenesis of diabetic nephropathy has been most demonstrated in transgenic mice overexpressing RAGE in vascular cells. These animals develop glomerulosclerosis more rapidly compared to non transgenic mice, an effect blocked by an AGE inhibitor. In parallel, most structural and functional abnormalities associated with diabetic nephropathy were both prevented by pharmacologic blockage of RAGE by soluble RAGE and in the model of RAGE null mice. [8]

The hypothesis that variants in the RAGE gene may influence the development of diabetic complications has been shown. Variants of the RAGE gene could alter RAGE expression, then affecting disease development. The gene for RAGE (6p21.3) has 11 exons and encodes a protein of 404 amino acids. Most of the several polymorphisms that have been identified in the RAGE gene are rare coding changes or located in noncoding regions. Two functional polymorphisms recently described in the promoter of RAGE gene, namely -429T/C, and -374T/A were highlighted as being

involved in the pathogenesis of diabetic complication, because they were shown to have a marked effect on transcriptional activity.[8]

However, the few studies which analyzed the relationship between these two polymorphisms and the diabetic complications have shown conflicting results. For example, -429 T/C and -374 T/A gene polymorphisms of RAGE are not risk factors for coronary disease and retinopathy in Slovene population (Caucasians) with type 2 diabetes, but the -374 A alleles of RAGE gene is associated with a decreased risk of ischemic heart disease in African-Brazilians with type 2 diabetes and also a protective factor against cardiovascular lesions in English T2D patients. Therefore, one aim of this study is to evaluate the association between -492T/C and the -374 T/A polymorphisms in the RAGE gene, and the presence of diabetic nephropathy, cardiovascular disease, and neuropathy in Thai with type 2 diabetes.[9-10]

Despite the psychosocial implications, as well as the financial burden associated with the management of diabetes, existing treatment options are costly, and have limited palliative effects. In addition, many current therapies to control glycemia have harmful side effects, such as hypoglycemia and liver damage. One treatment that is emerging as a potential strategy for the management of diabetes is medicinal herb.

Surveys indicate that the North American public has an avid interest in the potential benefit of complementary and alternative therapies, as evidenced by the world spread use of herbal and natural substances. There is an increasing trend towards reimbursement of herbal medicines by insurance companies, which further encourage their utilization. Herbs are listed under the "supplement" category by the Food and Drug Administration. The Dietary Supplement and Health Education Act signed into law in October 1994, require no proof of efficacy, no demonstration of safety, and set no standards for quality control for the products labeled as "supplements" thereby increasing the risk of adverse effects of these herbs. Plant derivatives with hypoglycemic properties have been used in the alternative or traditional medicine around the world. However, there is relatively little knowledge about efficiency and safety of herb supplements for diabetes.[11-16]

Ayurveda ("science of life") is a system of medicine that combines natural therapies with a personalized approach to the treatment of disease. For some

3,000 years, the traditional medicinal practice of Ayurveda has been used in India and has since found acceptance in other parts of Asia as well as the West. According to folklore, it has therapeutic benefits for numerous conditions, including diabetes mellitus, through a combination of approaches including diet, exercise, herbs, massage, and medication. The herbal components have been proposed to possess antidiabetic properties and used either singly or in specified mixtures[16].

Gymnema sylvestre (GS), the Asclepiad plant which grows in tropical forests of south and south-eastern Asia, is the well-known herb that has been used for diabetic treatment for more than 2000 years in Ayuravedic medicine. This plant was called Meshashringi or "ram's horn" in Sanskrit. Its use in snake bite as a remedy was well known to the natives of the Konkan in India and the natives of Southern India. Gymnema leaves have been used for centuries in the traditional Indian system of Ayurvedic medicine. The term "destroyer of sugar" is traditionally used for Gymnema because chewing the leaves will abolish the taste of sweetness. That is, sweet foods no longer tasted sweet, but rather became almost completely tasteless. The medicinally active parts of the plant are the leaves and the roots. Recent clinical trials conducted in India have shown that an extract of *Gymnema sylvestre* is useful for controlling blood sugar.[17]

The hypoglycemic (blood sugar-lowering) action of gymnema leaves was first documented in the late 1920s. This action is attributed to members of a family of substances called gymnemic acids. Gymnema leaves raise insulin levels, according to research in healthy volunteers. Based on animal studies, this may be due to regeneration of the cells in the pancreas that secrete insulin, or by increasing the flow of insulin from these cells. Other animal research shows that gymnema can also reduce glucose absorption from the intestine, improve uptake of glucose into cells, and prevent adrenal hormones from stimulating the liver to produce glucose, thereby reducing blood sugar levels.[18-20]

Many studies showed that GS extract can inhibit glucose absorption in intestines of both animal models and human by suppressing potassium-induced contraction of ileal longitudinal muscle. Oral administration of gymnema extract reduced postprandial serum glucose and improved glucose tolerance in mildly diabetic rats. The

water-extracted fraction of GS leaves can return fasting blood glucose levels to normal after a long period of oral administration by revitalization of pancreatic beta cells. However, GS suppresses sweet taste on taste buds and has bitterness, therefore it may irritate the flavor of food or drink.[21-25]

Gymnema inodorum (GI), also a member of Asclepiad strain, is found ubiquitously in south-eastern Asia including Thailand. GI does not have gustatory modifying action, so its leaves and stems have been used as vegetables in Thai food especially in the northern and eastern parts of the country. GI has been shown to have an ability to inhibit glucose absorption in guinea pig intestines. However, the study about hypoglycemic effect of GI has never been done in human. For this reason, we will study the hypoglycemic effect of GI in human, using commercial GI tea. Since the underlying mechanism of hypoglycemic effect of GI is not well understood, we will investigate and characterize the underlying mechanism of hypoglycemic effect of *Gymnema inodorum*.

Research Questions

A. What is the effect of *Gymnema inodorum* tea consumption on blood glucose concentration?

B. What are the underlying mechanisms of hypoglycemic effect of *G. inodorum*?

C. Are there any associations between -492T/C and -374T/A polymorphisms in the RAGE gene, and the presence of diabetic complication in Thai with type 2 diabetes?

Objectives

A. To study and characterize the hypoglycemic effect of G. *inodorum*.

B. To understand the mechanism of hypoglycemic effect of *G. inodorum*.

C. To evaluate the associations between -492T/C and -374T/A polymorphisms in RAGE gene and diabetic complication in Thai T2D patient.

Hypothesis

A. *Gymnema inodorum* can lower blood sugar concentration.

B. *Gymnema inodorum* can stimulate insulin secretion of pancreatic beta cell.

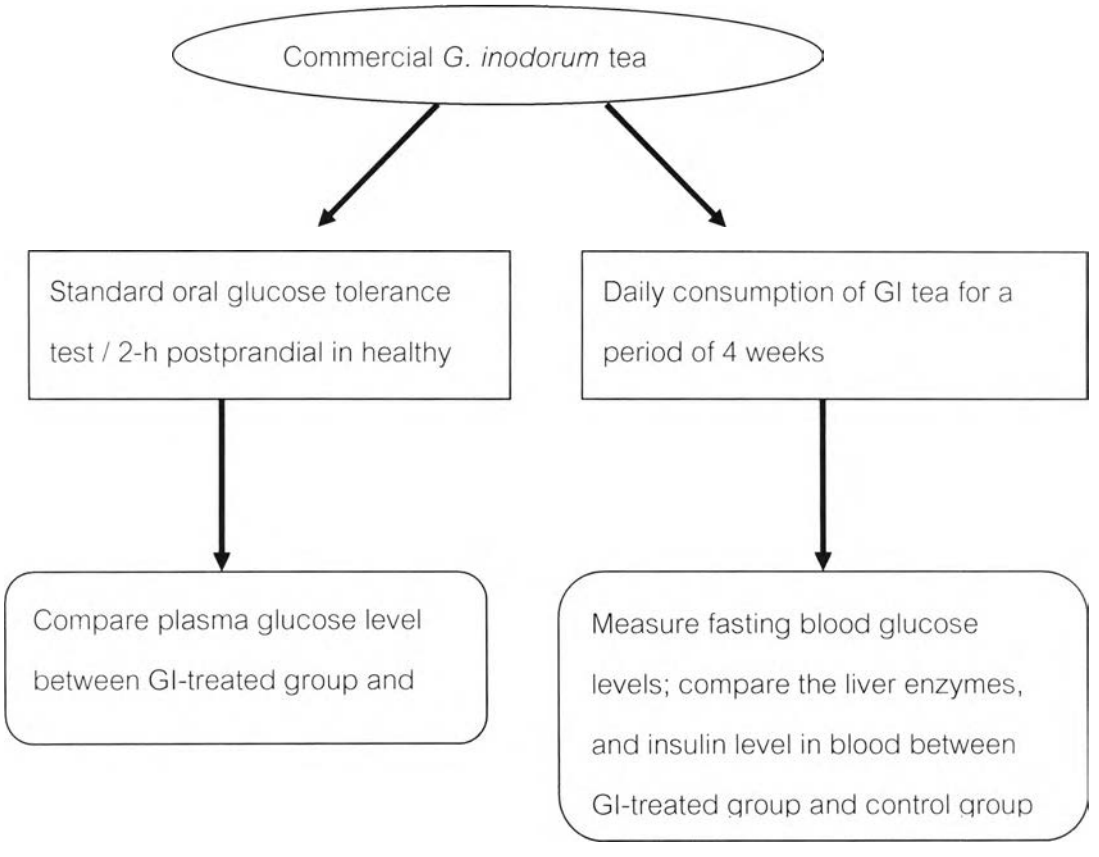
C. *Gymnema inodorum* extract contain alpha glucosidase inhibitor.

D. There is an association between -492T/C polymorphism in RAGE gene and diabetic complications in T2D patients.

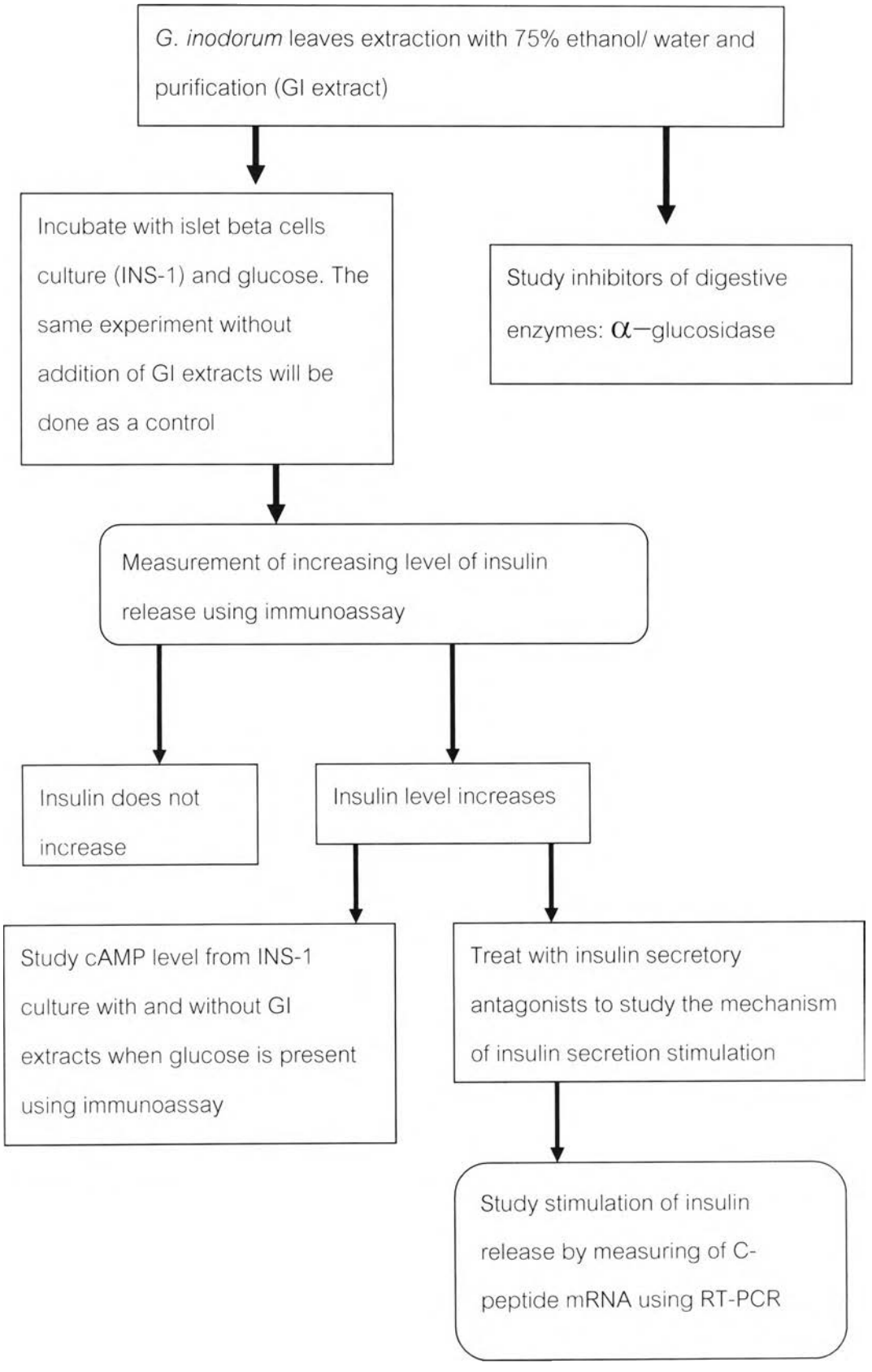
E. There is an association between -374T/A polymorphism in RAGE gene and diabetic complications in T2D patients.

Conceptual Framework

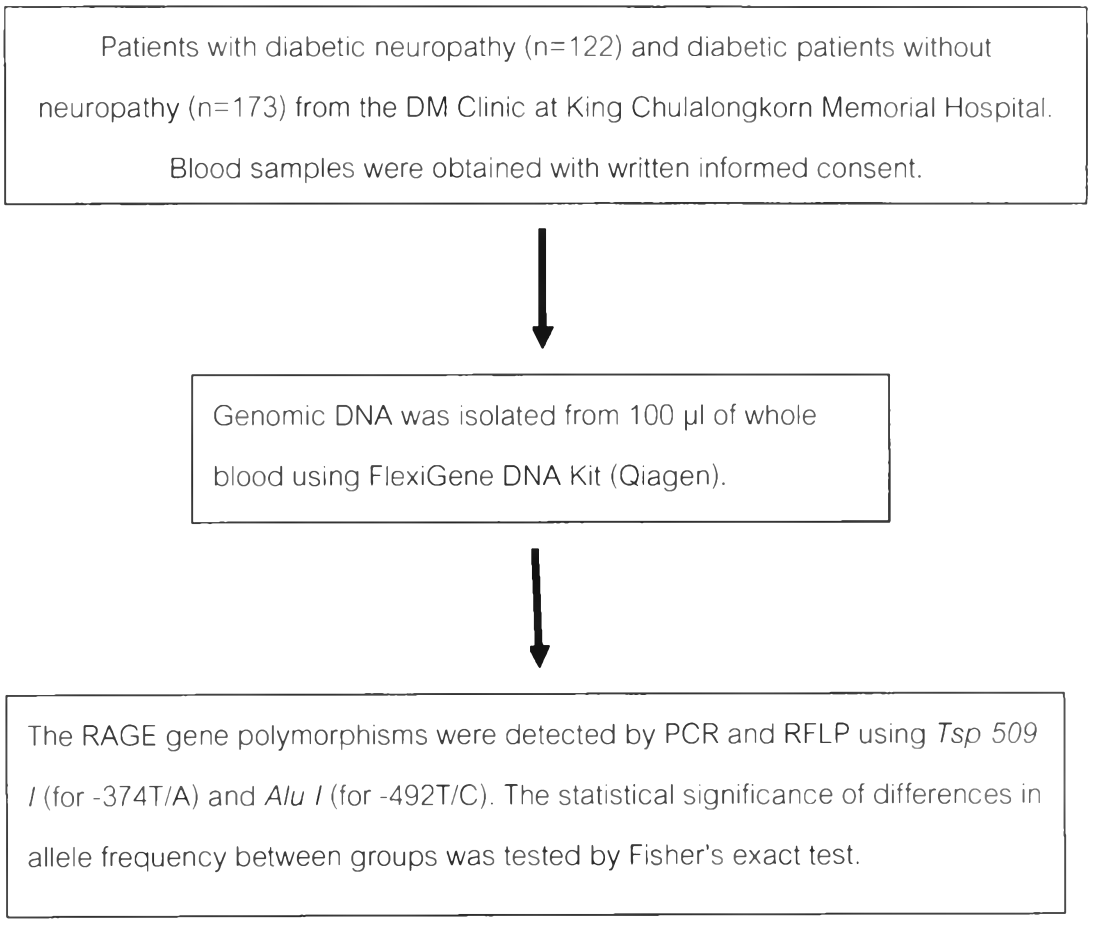
1. Study hypoglycemic effect of *G. inodorum* extracts in human



2. Study mechanisms of hypoglycemic effect of *G. inodorum*



3. Study association of RAGE polymorphisms and diabetic complication



The Benefit of this study

A. This study would be an important fundamental in basic sciences for further study.
B. This study would indicate the hypoglycemic effect and underlying mechanism of *Gymnema inodorum* extract.
C. This study would indicate the association of RAGE polymorphism and the risk of developing complication in diabetic patients.