CHAPTER V

DISCUSSION

Gymnema inodorum (GI), or *Chiengda* in Thai, is a local plant, regularly found in northern and north-eastern part of Thailand and is used in local cuisine for a long time. GI belongs to the *Asclepiadaceae* Family which is the same as *Gymnema sylvestre* (GS). GS is grown in India and has been used in Ayuraveda medicine for its therapeutic effects for some diseases including diabetes mellitus. Crude extract from GS leaves consists of gymnemic acids which is one of triterpene saponins [89] and has the pharmacological action in inhibiting glucose absorption and suppressing the increase of blood glucose level in oral glucose tolerance test. GI differs from GS because it does not suppress sweetness and is not bitter in taste.[90 91] The extraction from GI leaves can also suppress glucose absorption in guinea-pig intestine via Na+-glucose co-transport system.[33 34] The glucose suppression activity of GI has never been demonstrated in humans before.

In order to determine the hypoglycemic effect of GI tea on healthy subjects, we divided the subjects into 5 groups: group 1 or a control group, the standard OGTT was performed without GI tea consumption after 75 g glucose load; group 2 the treatment I group, the subjects were asked to drink one pack of GI tea (1.5 g in 150 ml hot water) immediately after oral glucose load; group 3 the treatment II group, the subjects were asked to drink 1 pack of GI tea 15 minutes after oral glucose load; group 4 the treatment III group, the subjects were asked to drink 1 pack of GI tea 30 minutes after oral glucose load; group 5 the treatment IV group, the subjects were asked to drink 2 packs of GI tea (3.0 g in 150 ml hot water) 15 minutes after 75 g oral glucose load.

The data in table 4 showed that drinking GI tea immediately and 15 minutes after oral glucose load (treatment I and treatment II) can significantly reduce plasma glucose ($p = 0.035$ and 0.004), while drinking GI tea 30 minute after glucose load (treatment III) cannot reduce plasma glucose when compare to control group ($p = 0.662$). When the double concentration of GI tea was used (treatment IV) in the study,

the data showed that it can reduce plasma glucose better than one pack of GI tea drinking ($p = 0.000$). The peak of glucose absorption of treatment I and II were significantly decreased approximately 10 and 11 % respectively, while treatment IV (double dosage) can reduce absorption peak about 25%. The peak of glucose absorption of treatment group III was unchanged, but at least it shows a tendency to reduce (2%) when compared with peak absorption of control group. Those data may indicate that GI tea has a hypoglycemic effect, because it can reduce plasma glucose of the study groups.

In Treatment III group, the subjects were asked to drink GI tea after 30 minutes of glucose load. We found that the GI tea cannot reduce peak of absorption. Natalucci et al. [106] studied a pattern of glucose absorption from OGTT and found that the average rate of glucose appearance into plasma will be highest at time 30-45 minutes after glucose load. This is one of the possible answers that why drinking GI tea at 30 minute after glucose load cannot reduce peak plasma glucose, because at 30 minutes, glucose is already absorbed and transported via blood circulation and it is too late to intervene that mechanism.

Upon food consumption, the process of starch or carbohydrate digestion begins in the mouth when amylase in saliva begins to break down carbohydrate into a disaccharide, maltose. After swallowing, the carbohydrates reach the stomach, which as a reservoir for food, squirting out small amounts into the intestines at intervals. In the small intestine, where most carbohydrate digestion occurs, the starch is processed by the enzyme amylase and converted into maltose and sucrose. The maltose and sucrose are then absorbed into the lining cells of the intestine and are further converted into monosaccharide, such as glucose.

Polysaccharides and disaccharides must be digested to monosaccharides prior to intestinal absorption and the key players in these processes are the brush border hydrolases, which include maltase, lactase and sucrase. Dietary lactose and sucrose are ready for digestion by their respective brush border enzymes. Starch is first digested into maltose by amylase in pancreatic secretions and saliva. Dietary lactose and sucrose, and maltose derived from digestion of starch, diffuse into

the small intestinal lumen and come into contact with the surface of absorptive epithelial cells covering the villi where they engage with brush border hydrolases:

- maltase cleaves maltose into two molecules of glucose
- lactase cleaves lactose into a glucose and a galactose
- sucrase cleaves sucrose into a glucose and a fructose

Alpha-glucosidase (EC 3.2.1.20) is an enzyme that hydrolyzes alpha glucosides to glucose. It is usually found at the brush border of the small intestinal epithelium, where it can hydrolyze oligosaccharides, trisaccharides, and disaccharides such as maltose to glucose and other saccharides for absorption. Alpha-glucosidase inhibitor can act as a competitive inhibitor of alpha-glucosidase, hence, it reduces the impact of carbohydrates on blood sugar.

In this study, we test the alpha-glucosidase inhibitory property from GI extract because alpha-glucosidase inhibition is one of possible mechanisms that can reduce blood sugar and many medicinal herb for diabetes have this property such as *Rosa damascene* (rose), *Rosmarinus offinalis* (rosemary), *Pistacia vera* (pistachio), *Entada rheedii* (African dream herb), and *Albizia lebbeck* (lebbeck-tree).[107-108] We found that *Gymnema inodorum* leaves extract, both with water and methanol extraction, cannot inhibit alpha-glucosidase activity.

Our finding is in accordance with the study of Sugihara et al.[109] They investigated the antihyperglycemic action of a crude saponin fraction and five triterpene glycosides (gymnemic acids I-IV) derived from the methanol extract of leaves of *Gymnema sylvestre* R. BR. (Asclepiadaceae) in streptozotocin (STZ)-diabetic mice. They also found that gymnemic acid IV (1 mg/mL) did not inhibit alpha-glucosidase activity in the brush border membrane vesicles of normal rat small intestines.

After digestion, glucose will be transported across the intestinal epithelium and absorbed into blood. Transportation of glucose through the apical of intestinal cells depends on the presence of secondary active Na+/glucose symporters, SGLT-1 and SGLT-2 which concentrate glucose inside the cells, using the energy provided by co-transport of Na+ ions down their electrochemical gradient.[110] The

essence of transport by the sodium-dependent hexose transporter involves a series of conformational changes induced by binding and release of sodium and glucose, and can be summarized as follows:

1. The transporter is initially oriented facing into the lumen. At this point it is capable of binding sodium, but not glucose.
2. Sodium binds, inducing a conformational change that opens the glucose-binding pocket
3. Glucose binds and the transporter reorients in the membrane such that the pockets holding sodium and glucose are moved inside the cell
4. Sodium dissociates into the cytoplasm, causing glucose binding to destabilize
5. Glucose dissociates into the cytoplasm and the unloaded transporter reorients back to its original, outward-facing position

The driving force for this symporter ia an electrochemical potential of Na+ across the cell membrane. [113] The mechanism responsible for maintaining the concentration of Na+ inside the cell membrane is Na^+/K^- pump. The Na^+/K^- pump is found in membrane of many types of cells and it plays a very important role in maintaining membrane potential. The Na^+/K^+ pump is a kind of active transport because normally, the cell contains relatively high concentration of potassium ions but low concentration of sodium ions. The Na^+/K^+ pump will move 3 Na^+ ions out of the cell for every 2 K^+ ions pump into the cell. In order to move the ions (Na^+ and K^+) against their gradients, energy is required and this energy is supplied by ATP (adenosine triphosphate).

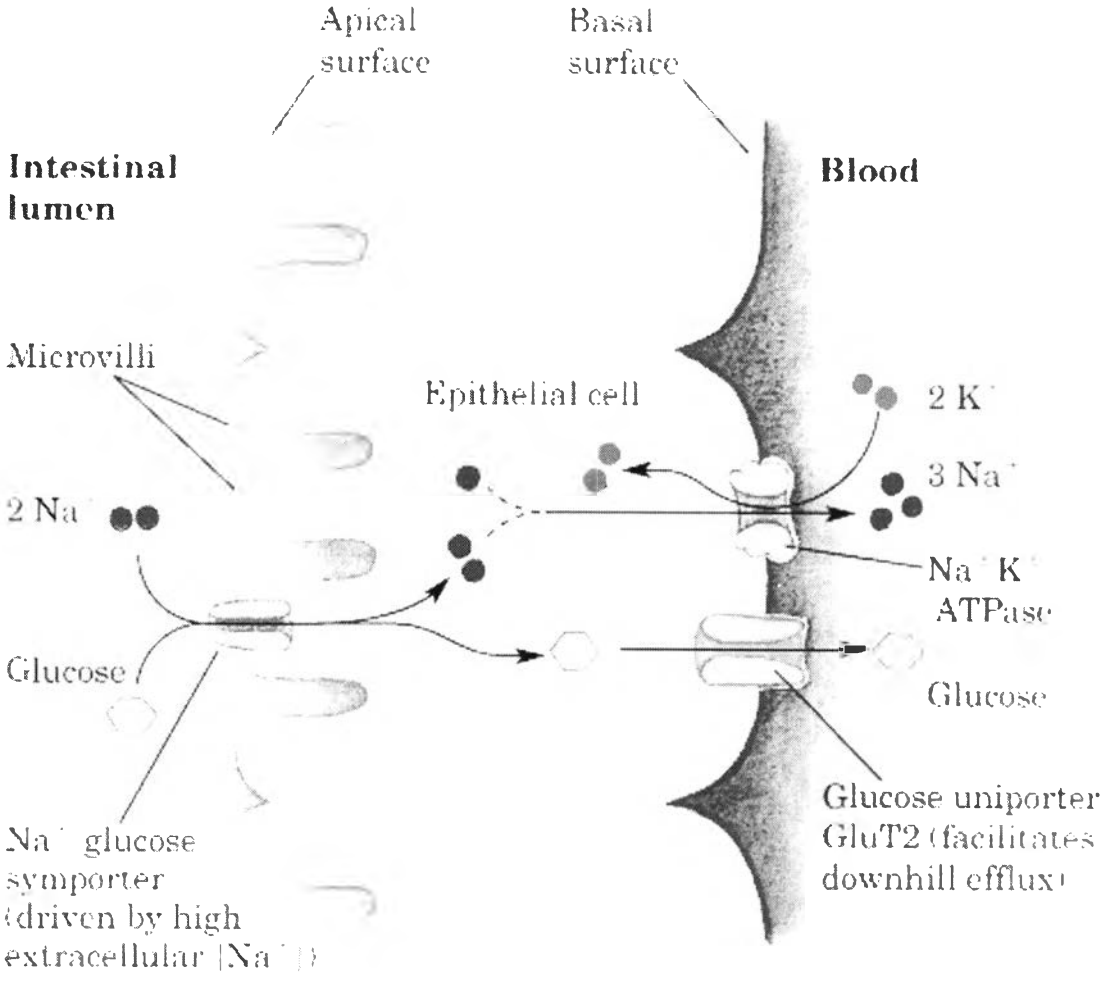

Figure 11 Sodium-depend glucose transport mechanism

From:

www.biochem.arizona.edu/classes/bioc462/462a/NOTES/LIPIDS/transport.html[111]

Shimizu et al.[65,66] showed that the crude saponin mixtures extracted from GI leaves can inhibit glucose absorption in the isolated intestinal tract and suppressed the increased blood glucose in rats. They found that triterpenoid saponin in GI extracts can suppress the high K^+-induced contraction of intestinal smooth muscle which affected Na^+/K^+ pump. When the pump is suppressed, the electrochemical potential of Na^+ inside the cell is changed and affects the Na^+-dependent co-transport system, and this is suggested to be the possible mechanism of the inhibitory effect of GI on glucose absorption from the intestinal tract.

The insulinotropic action of the extract of *G. inodorum* was further investigated using *INS-1* rat insulinoma cell line and immunoradioassay. We found that GI extracts fail to increase insulin secretion from the *INS-1* cell after incubating with GI extract for 1 hour.

Differ from *Gymnema sylvertre* (GS), the native plant of central and western India, which was known as a potent antidiabetic plant and used in treatment of diabetes since Ayaravedic period, GI cannot induce insulin secretion from beta cell of pancreas. GS, on the other hand, can reduce plasma glucose, using 4 possible mechanisms: 1) it increases secretion of insulin, [92,94] 2) it promotes regulation of islet cells, [92,94] 3) it increase utilization of glucose by insulin-dependent pathway, [93,94] and 4) it causes inhibition of glucose absorption from intestine.[93,94] The possible mechanism that GI is used to reduce plasma glucose level is the inhibition of glucose absorption from intestine.

Another possible mechanism for the lowering effect on blood glucose level of *Gymnema inodorum* may associate with incretin effect. Incretins are gut hormones which stimulate secretion of the endocrine pancreas and amplify glucose-induced insulin release[115]. Major incretins are GLP-1 (glucagons-like polypeptide-1) and GIP (glucose-dependent insulinotropic polypeptide). GLP-1 is released from the intestinal L-cell of distal small bowel in response to orally ingested nutrients and has effects on the endocrine pancreas, on the GI tract , and in the brain. Incretin hormones with potent glucose-dependent insulinotropic effects on the pancreatic β-cells, and inhibitory effects on the GI secretion and motiliy, all these effects combine to lower plasma glucose and reduce glycemic excursions.[116] In subjects with type 2 diabetes,

GLP-1 is active, but its plasma level decreases while GIP level is not decreased, but its activity is reduced or eventually lost.[116-119] The incretin effect refers to the increased insulin release from the beta cells of the pancreas in response to oral glucose and fat ingestion that is not found in intravenous glucose administration. [120] Nutrients entering the gut signals the pancreas to release more insulin and less glucagon. The incretin effect is likely related to the first-phase insulin response. Intravenous glucose challenges demonstrates a first-phase insulin release from the pancreas within 0-8 minutes, which is followed by a sustained delayed insulin response that is responsible for ultimate glucose homeostasis. The initial insulin response is blunted 3- to 4- folds in diabetic patients[117] and this suggested that restoration of this first-phase response may be important when treating type 2 diabetes. The first phase insulin response also augmented the second-phase. So when the first phase insulin release is lost, the second phase release is also blunted. The first phase insulin response may serve as a marker of beta cell function. Infusing GIP or GLP-1 into type2 diabetic patients recovers the first-phase insulin response[121] and this suggests an importance in beta cell function. *Gymnema inodorum* may decrease blood glucose peak level by enhancing glucose-dependent pancreatic secretion of insulin in response to nutrients intake and /or inhibit glucagon secretion. In our study the insulin level does not increase in glucose-induced INS-1 cells and this may be due to the in vitro condition and a short-term exposure. Further study on measurement of plasma insulin or C-peptide and glucagon level in human after meal and a longer period of GI consumption will help illustrating or confirming this possible mechanism.

Oxidative stress produced under diabetic conditions possibly causes various forms of tissue damage. Many researches show the benefit of antioxidant in diabetes. Kaneto et al. [111] found that pancreatic beta cell mass was significantly larger in the diabetic mice treated with the antioxidant than the untreated mice. As a possible cause, the antioxidant treatment can suppress apoptosis mechanism in beta cell without changing the rate of beta-cell proliferation. Muangman et al. [110] found that GI extracts, especially fresh juice prepared, are high in antioxidant activity, determined by DPPH radical scarvenging assay, deoxyribose degradation assay, hemolysis assay, and comet assay. The antioxidant property of GI may be one of the possible beneficial

effects of GI in diabetic patients, with preservation of beta-cell function and may increase the amount of insulin upon a long -term treatment.

In this study, we used commercial GI tea because it was approved by Thai FDA that there is no toxic substance and can be used as a regular beverage. To confirm the safety of GI tea, we asked a group of 20 healthy subjects to drink one cup of one pack of GI tea daily (1.5g GI extract in 150 ml hot water) for a period of 28 days and evaluate for fasting plasma glucose (FBG) and liver function enzyme profile (AST, ALT, ALP and GGT) at day 0,2,4,7,14, 21 and 28. We found that, GI tea has no toxicity to liver, because all subjects' liver profile enzymes still remain in normal level. Furthermore, GI tea did not reduce FBG level in healthy human after a period of 28 days (table 6) that means daily GI tea consumption will not induce hypoglycemic condition in healthy human.

The hyperglycemic milieu in diabetes results in the formation of advanced glycation endproducts (AGE) that predominantly act through specific receptors, particularly the receptor for AGE (RAGE). Two functional polymorphisms in the promoter of the RAGE gene (-429 T/C and -374 T/A) were investigated in this study.

A relationship between the -429 T/C and the -374 T/A gene polymorphisms of the receptor of advanced glycation endproducts (RAGE) gene and the development of diabetic complication in Thai with type 2 diabetes was investigated. In this study, no association between the -429C, and -374 A of the RAGE gene and DM complication was observed. We found that there is no association between -429C allele frequency and -374A allele frequency and complications of DM in Thai population. Our findings are in accordance with Xu et al.,[86] who studied the association between -429 T/C and diabetic retinopathy in Chinese patients with T2Dand Globocnik et al.[105] who studied the association between -429C and diabetic complication in Caucasian T2D population. Both studies found no association between -429 C allele frequency and the complication of diabetes. Furthermore, Kirbis et al., (2004) found that -429 T/C has no association with coronary artery disease in Slovene T2D patients. Dos Santos et al., (2005) also found no relationship between the -429C and -374 A of the RAGE gene and diabetic retinopathy, neuropathy, and ischemic heart disease in Caucasian T2D.[101]

When classify the complication of DM into groups of each complication, such as nephropathy, retinopathy, neuropathy, coronary artery disease, cerebrovascular disease, and skin disease, we still found no association between the -429C and -374A gene polymorphism and the complication of DM.

However, Hudson et al., (2001) reported a positive association between -429C allele and diabetic retinopathy. [84-85] The overall results seem to point to an absence of association between either -429 T/C or -374 T/A polymorphisms and diabetic complications in type 2 diabetes. Hudson also found no association between-429T/C polymorphism and macrovascular disease in DM patient. However, a study carried out on patients with type 1 diabetes has demonstrated a decreased risk of having proteinuria and cardiovascular disease among the AA homozygotes compared with the TT or TA genotype of -374 T/A polymorphism. (Pettersson-Fernholm et al.,2003). [88] Moreover, Ficalcone et al. (2004) [95] found an association between -374 AA genotype and a decreased risk of coronary artery disease in non-diabetic Italian.

Hudson et al. had observed that the -429C and -374A alleles had a marked effect on transcriptional activity [84-85], and a very clear difference in transcription factor binding was observed between the -374T and A alleles in vitro. [96-104] This finding suggests that the -374T/A polymorphism is in fact functional, and it may involve in the pathogenesis of diabetic complications. However, in our study, we have not found any association between the -429T/C and the -374T/A polymorphisms and the diabetic complication, so it is most likely that the genetic make up of different ethnics play important role for this variation.