CHAPTER 1

INTRODUCTION

The prevalence of obesity has increased dramatically worldwide. This increase has a major impact on public health, because obesity is a known risk factor for metabolic and chronic ailments including heart disease, cancer, arthritis, obstructive sleep apnea, hypertension, hyperlipidemia, and type 2 diabetes mellitus (Giusti, 2007). These chronic diseases have currently become the leading causes of morbidity and mortality in various countries. In the United State, roughly 300,000 death per year are directly related to obesity, and more than 80% of these are the patients with a body mass index (BMI) over 30 (http;//www.medicinenet.com/obesity_weight_loss/article.htm). In Thailand prevalence of overweight and obesity has also become a public health problem among the population. Thai hospital admission rate increase from 56.5 and 33.3 in 1985 per 100,000 population for heart disease and diabetes to 285.4 and 250.3 in 2000, respectively. Data comparing overweight and obesity in 1991 and 1997 was 13.7% and 19.5% in men and 25.1% and 28.0% in women. And, the prevalence of overweight had increased twice as high in women aged between 30 and 59 years old (Cited by Kitvorapat *et al*., 2004). Current lifestyles with less excercise plus the consumption of high caloric foods is the main factor of the increase in the number of overweight people. In 2007 the Ministry of Public Health of Thailand has launched a campaign "Thai people is not fat" to promote the improvement of the population weight. The World Health Organization (WHO) has made a recommendation for obesity prevention in community. This includes strategies for the change of the population behavior which achieve longterm improvement incidence of chronic diseases (WHO, 1998).

Weight loss of approximately 5-10% of body weight has been reported to reduce risk factors for various diseases related to obesity (Lauber and Sheard, 2001). The standard conservative treatments of obesity including control of diet, physical activity, cognitive-behavioural therapy and drugs treatment are effective in the short term, but ineffective in the long-term in 95% of patients (Giusti, 2007). Diet

has been the most common treatment used to induce weight loss and it is still frequently used. Patients seeking weight loss have many diet options: low fat, low carbohydrate. Pharmacological treatment provides valid support for management of obesity, combined with diet or behavioural therapy. Many researches on various plant extracts or their purified compounds on the reduction of blood glucose levels because of their anti-digestive enzymes function have been investigated, for example, juices of onion and galic (El-Demerdash *et al*., 2005), wheat amylase inhibitor (Koike *et al*., 1995), oregano extract of Oregano (*Origanum vulgare*) (McCue *et al*., 2004). Proteinaceous α-amylase inhibitor from white kidney bean purified as described by Layer *et al.* (1985) have been commercially introduced as a nutracutical food for controlling weight and blood glucose levels (Chokshi *et al*., 2007; http://www.nutrilite.com/th-TH/Nature/Products/carb-blocker.aspx).

Royal red kidney beans, the important commercial crop which commonly consumed among Thai people have been identified as a source of α-amylase inhibitor for both proteinaceous and nonproteinaceous types, developed for their purification processes and studied on some properties of the proteinaceous inhibitor by Sitthipong (2005). This studied thus aims to further investigate for biochemical properties of nonproteinaceous inhibitor and the effect of both inhibitor types in controlling blood glucose level *in vivo* using the developed purification processes as a tool in obtaining the inhibitors.

Review of literatures

1. Alpha-amylase (EC 3.2.1.1)

Alpha-amylase (EC 3.2.1.1) is an endo-acting enzyme that catalyzes the hydrolysis of the (1→4)-α-D-glycosidic linkages of starch, amylose, amylopectin, glycogen, and various maltodextrins. Alpha-amylases are produced by a diverse variety of organisms including bacteria, fungi, plants and animals. Two kinds of α-amylases are produced by many mammals, salivary α-amylase from the parotid gland and pancreatic α-amylase from the pancreas. The digestion of food starch begins with salivary α-amylase in the mouth, stops by the low pH of gastric juice in the stomach, the food bolus from the stomach is neutralized in the small intestine and

the starch is completly digested by an α-amylase secreted from the pancreas. Bacteria and fungi use starch for their growth by secreting α-amylases into their environment and the hydrolyzed product *i.e.* maltodextrins transported into the cells for further converted to D-glucose and metabolized. Plants produce α-amylases to degrade the starch, which is synthesized from photosynthesis for their energy needs. Digestion of starch by α-amylases is a very important process in the utilization of the sun's energy by nonphotosynthesizing organisms (Yoon and Robyt, 2003). Enzymetic hydrolysis of carbohydrates is very important in nutrition and technological processes. For example, amylases, pectinases and xylanases are often added in many food processes such as bakery products, beer and production of glucose, fructose or dextrins. (S∅rensen *et al*., 2004).

2. Alpha-amylase inhibitor

Amylase inhibitor (AI) is a substance which decreased amylase activity, so that it can reduce or delay carbohydrate digestion and glucose absorption. Alpha-amylase inhibitors are classified into two major groups, proteinaceous α-amylase inhibitor and nonproteinaceous α-amylase inhibitor.

2.1 Proteinaceous α-amylase inhibitor

The proteinaceous α-amylase inhibitor are found in cereals and legumes, such as rye (Iulek *et al*., 2000), barley (B∅nsager *et al*., 2003), wheat germ (Sharma and Gupta, 2001) and common beans (*Phaseolus valgaris*) (Gibbe and Alli, 1998; Lee *et al*., 2002). The molecular weight (MW) of AI in many plants are different in size such as 13.76 kDa in Barley (Bønsager *et al*., 2003) and 12, 24, 60 kDa in various species of wheat (Payan, 2004). Different plant α-amylase inhibitor exhibit different specificities against α-amylases from diverse sources, but not inhibits amylase in plants as shown in Table 1. Based on their tertiary structure proteinaceous α-amylase inhibitor were classified into six structural classes as shown in Table 2.

Table 1 Specificity of amylase inhibitors from different plant sources against α-amylases.

Source of amylase inhibitor	Specificity of α-amylase inhibitor against α-amylase
α-AI1 from *P. vulgaris*	Mammalian : porcine pancreatic α-amylase (PPA) Insect : *Callosobruchus maculates* *Callosobruchus chinensis Diabrotica virgifera virgifera* *Hypothenemus hampei* *Tenebrio molitor*
α-AI2 from *P. vulgaris*	Mammalian: no activity Insect : *Zabrotes subfasciatus*
wheat extract from *T. aestivum*	Mammalian : PPA and human salivary α-amylase (HSA) Insect : *Diabrotica virgifera virgifera* *Lygus Hesperus* *Lygus lineorali*
WRP25 from *T. aestivum*	Mammalian: no activity Insect : *Sitophilus oryzae* *Tribolium castaneum* *Tenebrio molitor* *Callosobruchus maculates* *Zabrotes subfasciatus*
WRP26 from *T. aestivum*	Mammalian: no activity Insect : *Tenebrio molitor* *Sitophilus oryzae* *Tribolium castaneum* *Callosobruchus maculatus*
WRP27 from *T. aestivum*	Mammalian: no activity Insect : *Tenebrio molitor* (low) *Sitophilus oryzae*

AA1 from *A. hypochondriacus*	Mammalian: no activity Insect : *Tenebrio molitor* *Hypothenemus hampei* *Prostephanus truncatus*
PAI from *C. cajan*	Mammalian : HSA and PPA Insect : *Helicoverpa armigera* (low)
Zeamatin from *Z. mays*	Mammalian: no activity Insect : *Tribolium castaneum* *Sitophilus zeamais* *Rhyzoperta dominica*
SIα1, SIα2 and SIα3 from *S. bicolor*	Mammalian : HSA (low) Insect : *Locusta migratoria* *Periplaneta americana*

From: Franco *et al*., 2002

Table 2 Different structural classes of α-amylase inhibitors

Structural class	Source	Amino acid residue numbers	Disulfide bonds	Names
Lectin-like	Common beans	240-250	5	α-AI1 & α-AI2
Knottin-like	Amaranth	32	3	AA1
Cereal-type	Wheat, barley, millet	124-160	5	WRP25, WRP26, WRP27 & RBI
Kunitz-like	Barley,wheat, rice	176-181	1-2	BASI, WASI & RASI
Thaumatin-like	Maize	173-235	5-8	Zeamatin
γ-Purothionin-like	Sorghum	47-48	5	SIα1, SIα2, SIα3

From: Franco *et al*., 2002

2.2 Nonproteinaceous α-amylase inhibitor

The nonproteinaceous α-amylase inhibitor contains diverse types of organic compounds such as acarbose, acarbose analogues, acarviosine-glucose, hibiscus acid, tannins, flavonoid, glucopyranosylidene-spiro-thiohydantoin. The hibiscus acid, the acarviosine-glucose, the acarbose are highly active against porcine and human pancreatic α-amylase (PPA and HPA). The inhibitory activity of these compounds against α-amylase is due to their cyclic structures, which resemble substrates that enter into the catalytic sites of α-amylase (Franco *et al*., 2003).

Hibicus acid from a roselle (*Hibiscus sabdariffa* Linn.) tea extract was found to have high inhibitory activity against porcine pancreatic α-amylase. Hibiscus acid and its derivative hibiscus 6-methyl ester (Figure 1) were respectively isolated as active principles from the 50% methanol and acetone extracts of rosella tea (Hansawasdi *et al.*, 2000).

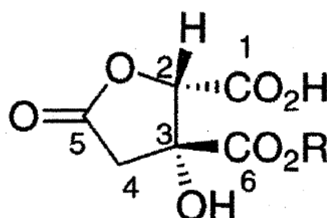

Figure 1 Molecular structure of hibiscus acid: hibiscus acid (R = H); hibiscus acid 6-methyl ester (R = CH_3) (From: Hansawasdi *et al.*, 2000)

Acarbose is a natural product produced by *Actinoplanes* sp. fermentation. It is a pseudotetrasaccharide with an unsaturated cyclitol [2, 3, 4-trihydroxy-5-(hydroxymethyl)-5, 6-cyclohexane in a D-*gluco*-configuration] attached to the nitrogen of 4-amino-4, 6-dideoxy-D-glucopyranose, which is linked α- (1→ 4) to maltose (Figure 2). Acarbose is a strong competitive inhibitor of α-glucosidase, α-amylase, cyclomaltodextrin glucanyltransferase (CG-Tase), glucoamylase and glucansucrases. The mechanism of inhibition for these enzymes has been postulated

to be due to the cyclohexane ring and the nitrogen linkage that mimics the transitions state for the enzymatic cleavage of glycosidic linkges (Yoon and Robyt, 2002).

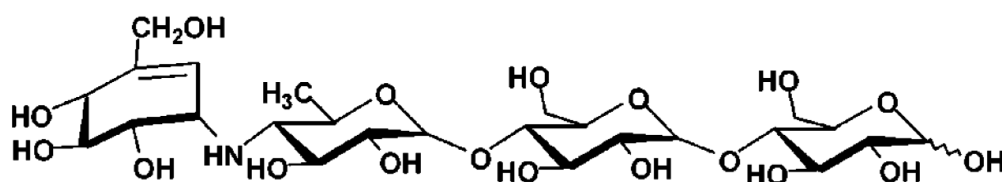

Figure 2 Molecular structure of acarbose (From Kim *et al*., 2002)

Matsuura *et al*. (2004) found inhibitory activity of the active principles in aqueous methanol extract from dried hyssop (*Hyssopus officinalis*) leaves against α-glucosidase, prepared from rat small intestine acetone powders. These are (7*S*, 8*S*)-syringoylglycerol 9-*O*-β-D-glucopyranoside (**1**) and (7*S*, 8*S*)-syringoylglycerol-9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside (**2**) by physical and spectroscopic data (Figure 3).

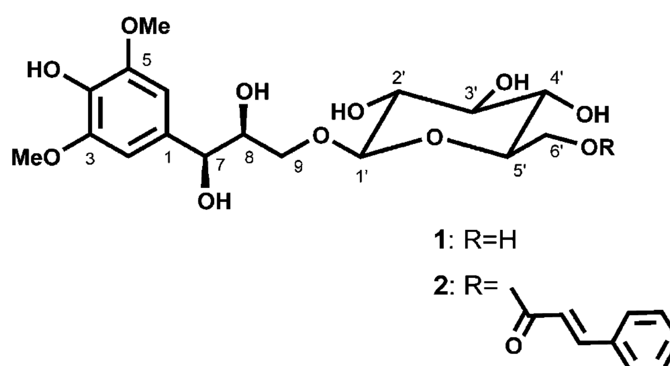

Figure 3 α-Glucosidase inhibitors isolated from hyssop (*Hyssopus officinalis)* (from; Matsuura *et al*., 2004)

Different plant nonproteinaceous α-amylase inhibitor exhibit different specificities against α-amylases from diverse sources, but not inhibits amylase in plants as shown in Table 3.

Table 3 Specificity of nonproteinaceous amylase inhibitors from different plant sources against α-amylases.

Soures of amylase inhibitor	Specificity of α-amylase inhibitor against α-amylase	Reference
Cedrus libani woods oil	human salivary amylase	Loizzo *et al*. (2007)
tannin from the gall nut of Aleppo oak	human salivary amylase	Zajácz *et al*. (2007)
acetone extract of *Spatoglossum schroederi* (brown seaweed)	human salivary amylase	Teixeira *et al*. (2007)
acetone extract of *Caulerpa racemosa* (green macroalga)	human salivary amylase	Teixeira *et al*. (2007)
methanol extract of *Tournefortia hartwegiana* (a Maxican medicinal plant)	α-glucosidase from rat small intestine	Ortiz-Andrade *et al*. (2007)

3. Extraction and purification of proteinaceous α-amylase inhibitor

Proteinaceous α-amylase inhibitors are generally extracted from plant material by either heat or non-heat method.

Heat extraction method: Grant *et al.* (1995) extracted the proteinaceous inhibitor from various seeds available in Europe with sodium phosphate buffer pH 6.9 containing NaCl (9 g/litre^{-1}) (1 : 5 w/v) and centrifuged. The supernatant was heated at 70 ºC for 10 min. Iulek *et al.* (2000) extracted the proteinaceous inhibitor from rye seeds with 70% ethanolic solution (v/v) and the supernatant was heated at 70 ºC for 1 h. Sasikiran *et al*. (2002) extracted the proteinaceous inhibitor from sweet potato by homogenizing it in 0.01 M sodium phosphate buffer pH 8.0 (ratio 1 : 5 w/v) in the

presence of 1.0% polyvinyl pyrrolidone (PVP). The native proteases and other unwanted proteins were inactivated and precipitated at 70 ºC for 10 min and further were removed by centrifugation at 1,000 x g for 10 min.

Non-heat extraction method: The inhibitor from wheat (*Triticum aestivum*) was extracted with 0.15 M NaCl (1: 5 w/v) (Franco *et al*., 2000). The inhibitor from common bean seeds (*P. vulgaris*) were extracted with 0.15 M NaCl and 0.1% HCl (1: 5 w/v) (Dayler *et al*., 2005).

Proteinaceous α-amylase inhibitors were purified by various method such as precipitated with ammonium sulfate and further purified by column chromatography. Iulek *et al*. (2000) purified α-amylase inhibitor from rye by ammonium sulfate at 20-25% saturation, followed by centrifugation at 9,400 x g for 1 h, then redissolved in 20 mM phosphate buffer pH 6.9 and extensively dialyzed against 20 mM phosphate buffer pH 6.9. The resulting solution was first applied to a DEAE-Sepharose ion exchange. Fractions were eluted stepwise using 20, 30 and 200 mM phosphate buffer pH 6.9, followed by gradient of 0-1.0 M NaCl in the same buffer. A flow rate of 144 ml/h was used throughout. Fractions with α-amylase inhibitory activity were then dialyzed against 50 mM acetate buffer pH 5.0 and submitted to CM-Sepharose ion exchange column. Fractions were eluted using steps of 50, 100 and 500 mM acetate buffer followed by gradient of 0 - 1.0 M NaCl in the same buffer, at flow rate of 138 ml/h.

Yamada *et al.* (2001) purified αAI-Pa1 and αAI-Pa2 from tepary bean (*Phaseolus acutifolius* A. Gray) by ammonium sulfate precipitation and column chromatography of DEAE-sephacel and CM-Sepharose.

Dayler *et al*. (2005) also precipitated the proteinaceous inhibitor from crude extract of common bean seeds by ammonium sulfate, dialyzed and applied the fraction obtained between 0% and 85% saturation onto ionic exchange DEAE-cellulose column equilibrated with 20 mM KPO_4 buffer, pH 6.7 at a flow rate of 30 ml/h. The retained proteins were removed with a 0-0.2 M NaCl linear gradient and further applied onto reverse phase analytical HPLC (Vydac 218 TP 1022 C-18 column) at flow rate of 1.0 ml/min.

Kluh *et al.* (2005) precipitated proteinaceous α-amylase inhibitor from acid extraction of seeds of common bean (*Phaseolus vulgaris* cv. Magna) by 35-65% saturation of ammonium sulfate and further purified using DEAE-cellulose and Biogel P-100 columns.

Farias *et al*. (2007) purified α-amylase inhibitor from *Carica papaya* seeds by 100% saturation ammonium sulfate precipitation, dialyzed and applied onto a CM-cellulose column equilibrated with 50 mM $CaCl_2$ and 10 mM NaCl. Retained proteins were eluted with a linear gradient of 0-1.0 M NaCl in the same buffer. The eluted peak of retained protein was further identified by a reverse phase HPLC (Vydac 218 TP 1022 C-18 column) using a flow rate of 1.0 ml/min and measured at 216 nm.

4. Extraction and purification of nonproteinaceous α-amylase inhibitor

Nonproteinaceous α-amylase inhibitor contains diverse types of organic compounds such as acarbose, polyphenol, tannin and flavonoid. A large proportion of nonproteinaceous α-amylase inhibitors were extracted from sources by various types of solvents and extraction procedures depending on types of the inhibitors and the sources. Similary the purification methods were quite varies depending on types and the sources of the inhibitors.

Hansawasdi *et al*. (2000) extracted hibiscus acid from a roselle tea (*H. sabdariffa* Linn.) with 50% aqueous methanol (10 ml/g fresh weight) for 24 hours at room temperature. The methanol was evaporated from one part of the extract, the resulting residue was redissolved in dimethyl sulfoxide (10 ml/g fresh weight) and subjected to a porcine pancreatic amylase (PPA) inhibitory activity assay.

Kim *et al*. (2002) isolated acarbose from *Actinoplanes* sp. broth by adding 80 ml of methanol to its 10 folds concentrated supernatant (20 ml). After the removal of the sediment by centrifugation at 4,000 x g for 5 min, 800 ml of ethanol was added to 100 ml of the supernatant. The high molecular weight acarbose was collected by centrifugation and dissolved in 200 ml of 50 mM sodium acetate buffer

pH 6.0and further purified. Thin layer chromatography coated with K6F silica gel and a solvent system of ethyl acetated/isopropyl alcohol/water (1:3:1, v/v/v) were used. After the developing, the plate was visualized by spraying with a solution of 0.3% (w/v) *N*-(1-naphthyl)-ethylenediamine and 5% (v/v) H_2SO_4 in methanol and heated at 110 ºC for 10 min. Each of the detected spots were compared with maltodextrin standards *i.e*. G1 to G7.

Yoon and Robyt (2003) prepared acarbose analogues by adding *B. macerans* CGTase (EC 2.4.1.19) to acarbose and cyclomaltohexaose. The enzyme reaction was carried out at 35 ºC for 6 days with the periodic, stepwise addition cyclomaltohexaose solution every 1-2 days. After stopping the enzyme reaction by heating in boiling water, insoluble matter was removed by centrifugation at 4,000 rpm 10 min and then the supernatant was concentrated to 1.2 ml by rotary vacuum evaporation. The major reaction products, maltohexose-Aca, maltododecaose-Aca and maltooctadecaose-Aca, were purified by Bio-gel P2 column (1.5 × 100 cm) chromatography (flow rate 0.06 ml/min, fraction size 1.0 ml).

Matsuura *et al*. (2004) extracted α-glucosidase inhibitor from air-dried leaves of hyssop (*Hyssopus officinalis*) with aqueous methanol by soaking the leaves in MeOH : H_2O (7 : 3, v/v) and filtrated through filter paper (No. 1, Toyo). The filtrate was concentrated under vacuum and the extract was partitioned between ethyl acetate and distilled water

To purify α-glucosidase inhibitor a solution of ethanol was added into the aqueous soluble layer of the concentrated extract. The supernatant and precipitate were separated with filter paper (No. 1, Toyo). The solution of the supernatant was subjected to Diaion HP-20 resins. The eluate with α-glucosidase inhibitory activity was purified by Sephadex LH-20, MPLC and HPLC, to obtain (7*S*, 8*S*)-syringoylglycerol 9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside. The ethyl acetate-soluble layer of the crude extract was concentrated and subjected to Diaion HP-20. The eluate with the inhibitory activities was purified by silica gel column, Sephadex LH-20, MPLC, HPLC, to obtain (7*S*, 8*S*)-syringoylglycerol-9-*O*-(6′-*O*-cinnamoyl)-β-D-glucopyranoside.

5. The role of α-amylase inhibitor in controlling postprandial plasma glucose levels

Key enzymes involved in the breakdown of complex carbohydrates, salivary α-amylase, pancreatic α-amylase, sucrase and intestinal α-glucosidase are targeted for modulation of type 2 diabetes-associated post-prandial hyperglycemia. Various researches showed the potency in using amylase inhibitor, both proteinaceous and nonproteinaceous types in lowering plasma glucose level through *in vitro* and *in vivo* studies.

For *in vitro* studies, there are many reports on the inhibitory effect of plant extract on carbohydrate hydrolysis. Iulek *et al.* (2000) reported that the purified proteinaceous α-amylases inhibitor from rye showed inhibitory activity against α-amylases from porcine pancreas and human saliva. Franco *et al.* (2002) reviewed that α-AI1 from *P. vulgaris*, the extract wheat (*Triticum aestivum*), and α-amylases inhibitor from Pigeonpea (*Cajanus cajan*) inhibit porcine pancreatic α-amylases and human salivary α-amylases, while *S. bicolor* α-amylases inhibitor (SIα1, SIα2 and SIα3) show low inhibition to human salivary α-amylases. Mancinelli *et al.* (2003) purified CM2 and CM3 α-amylase inhibitor from wheat feed chromatin and demonstrated their inhibitory activity against human salivary α-amylase *in vitro*. Araújo *et al.* (2004) found that the dialysates obtained from 90% ammonium sulfate precipitation of yellow mombim (*Spondias lutea* L.) and cupuassu (*Theobroms grandeflorum*) pulps inhibited pancreatic α-amylase with a concentration at 0.0345 and 0.0335 mg inhibitor/g pulp, respectively. The dialysates of melon (*Cucumis melo* L.) and assai (*Euterpe oleracea*) pulps inhibited salivary α-amylase with a concentration at 0.042 and 0.142 mg inhibitor/ g pulp, respectively. Haq *et al.* (2005) found that the purified of *Phaseolus aureus* Roxb (Mung bean) was a bi-funtional protein proteinase/amylase inhibitor which had ability in decreasing α-amylase activity by approximately 60% at inhibitor concentration as low as 0.5 μg.

Matsuura *et al.* (2004) found that structure of compounds; (7*S*, 8*S*)-syringoylglycerol 9-*O*-β-D-glucopyranoside and (7*S*, 8*S*)-syringoylglycerol-9-*O*-(6′-

O-cinnamoyl)-β-D-glucopyranoside from extract of hyssop inhibit α-glucosidase (which was prepared from rat small intestinal acetone powder), by 53 and 54% at its concentration of 3×10^{-3} M. McCue *et al*. (2004) reported that 50% ethanol extract of Oregano (*Origanum vulgare*), a rich source of natural phenolic, showed the inhibition against porcine pancreatic amylase by 9-57%. Kim *et al.* (2005) found pine bark extract potency against salivary and pancreatic α-amylase and yeast *Saccharomyces cerevisiae* α-glucosidase with more than 90% inhibition. Loizzo *et al.* (2007) reported that the phytochemical composition of *Cedrus libani* essential oils obtained from wood exhibited an IC_{50} value of 0.14 mg/ml against α-amylase, whereas the leaves and cones oils were devoid of any significant activity. Ortiz-Andrade *et al*. (2007) extracted α-glucosidase from rat small intestine and used for inhibition assay with the methanolic extract of *Tournefortia hartwegiana.* The results revealed a concentration for the inhibition at IC_{50} of 3.13 mg/ml.

For *in vivo* studies, many studies on the effect of proteinaceous and nonproteinaceous amylase inhibitor, and plant extracts on blood glucose levels in animals have been reported. Layer *et al*. (1986) reported that the effect of purified proteinaceous α-amylase inhibitor obtained from *P. vulgaris* have been demonstrated to substantially reduce postprandial increases in the plasma concentration of glucose and insulin in both normal and diabetic patients. Koike *et al*. (1995) determined longterm effect ingestion of a wheat amylase inhibitor on carbohydrate digestion, absorption and pancreatic growth in 8 dogs. The results showed that ingestion of 1.5 g/day of the inhibitor for 9 weeks reduced the postprandial glucose levels through the delay of carbohydrate digestion and absorption without any altering pancreatic growth.

For the nonproteinaceous inhibitor Kim *et al*. (2005) showed that the inhibitory activity of pine bark extract (PBE) against human salivary, porcine pancreatic α-amylase and yeast α-glucosidase *in vitro* was also effect the decrease of blood glucose level after meal *in vivo*. Eight-week-old Lep^{ob}(*ob/ob*) mice were orally administered with 250 mg/kg of PBE once and then let the animals accessed food freely for 20 min before blood collection at 15. 30 and 60 min in the absence of food. The results of postprandial blood glucose level were significantly suppressed in the

group administered with PBE than the control group and the positive control group fed with 50 mg/kg. Yamagishi *et al.* (2005) reported that acarbose could reduce body mass index and waist circumference in nonalcoholic steatohepatitis (NASH) patients. Furthermore, a meta-analysis of long-term studies also showed that intervention with acarbose improved triglyceride levels, body weight and systolic blood pressure and subsequently prevented myocardial infraction in type 2 diabetic patients. Since acarbose improves postprandial hyperglycemia by delaying the release of glucose from complex carbohydrates in the absence in insulin secretion.

Murai *et al.* (2002) found that of 4^2-*O*-β-D-galactosyl maltobio-nolactone (LG2O) with anti-amylase activity was able to control postprandial blood glucose concentration in mice. LG2O was postulated suppress any steep increase in postprandial blood glucose concentration in mice.

Murthy *et al*. (2004) test the seed powder of *Datura metel* was tested for its hypoglycemic activity in normal and alloxan-induced diabetic rats. The results revealed that 25, 50 and 75 mg/kg of the seed powder given to both normal and diabetic rats reduced significantly blood glucose with in 8h. This effect was found to be dose dependent with all treatment at the doses administered.

Heacock *et al.* (2005) study the effect of *Salacia oblonga* extract on postprandial glycemic, insulinemic in healthy adults. The 1,000 mg *S. oblonga* extract dose reduced the plasma glucose and serum insulin incremental areas under the curve (0 to 120 min postprandial) by 23% and 29% recpectively.

Lee *et al*. (2005) fed streptozotocin induced diabetic male Sprague-Dawley rats with the powdered of Du-zhong leaves (PDZ) and its water extract (WDZ). Blood glucose levels were significantly lower in the DM-PDZ and DM-WDZ groups than in the diabetic control group (20.05 ± 0.88 and 18.96 ± 1.23 mmol/l versus 24.42 ± 1.07 mmol/l)($p<0.05$). These results demonstrated the possibility in using this plant powder and its water extract for the improvement of blood glucose level in diabetes. Du-Zhong leaves have been reported to contain polyphenolics, flavonoids and triterpines and these phytochemical compounds have been also reported as anti-diabetic agents without knowning the exact mechanism (Ali *et al.*, 2002, Kawasaki *et al.*, 2000).

McAnuff-Harding *et al*. (2006) fed diabetic male Wistar rats with 1% sapogenin extract of bitter yam (*Dioscorea polygonoides*) tubers for 3 weeks. After the removal of each duodenal parts as proximal, mid and distal the effect of the fed substance on intestinal enzymes activity were investigated. The results revealed that supplement of the bitter yam sapogenin extract significantly reduced activity of maltase and sucrase in all intestinal regions of the diabetic rats in comparison to the diabetic control rats and the normal rats. The authors suggested that the reduction of both disaccharidases activity was the indicative of lowered level of absorbable glucose being formed from carbohydrate digestion leading to the reduction of blood glucose level. Ortiz-Andrade *et al*. (2007) extracted α-glucosidase inhibitor from *Tournefortia hartwegiana* with methanol (METh). The METh 310 mg/kg was fed to Wistar rats before administration of substrate at a dose 2 g/kg of substrate (glucose, sucrose and maltose) solution to each rat. The postprandial blood glucose was lower at 60 min and this activity was similar during 90 and 120 min post-ingestion of all three carbohydrates. The effect was more significant in the presence of sucrose and maltose than glucose at $p< 0.01$. The blood glucose lowering effect of METh was lower than that of acarbose, a therapeutic drug used as a positive control (3 mg/kg). The authors concluded that the control of postprandial glucose level showed by METh might involve an anti-hyperglycemic effect, mediated by the regulation of glucose uptake from the intestinal lumen, through the inhibition of carbohydrate digestion or absorption. Results of phytochemical investigation of METh extract led the authors to concluded that triterpenoid, oleanolic acid which was described by Ali *et al.* (2002) as a α-glucosidase inhibitor may be the main anti-hyperglycemic agent of the METh extract.

Therefore, it is interested in knowing if the proteinaceous and nonproteinaceous inhibitor of Royal red kidney beans had any potency in lowering blood glucose level through *in vitro* and *in vivo* studies.

Objectives

1. To prepare proteinaceous amylase inhibitor and nonproteineous amylase inhibitor from Royal red kidney bean (*Phaseolus vulgaris*).
2. To study properties of nonproteinaceous amylase inhibitor on salivary α-amylase activity.
3. To study potential application of the inhibitor on blood glucose reduction *via* luminal enzymes *in vitro*.
4. To study potential application of the inhibitor on blood glucose reduction *via* luminal enzymes *in vivo*.