Rat Intestinal α-Glucosidase Inhibitory Activities of Leguminous Seed Extracts

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The methanol extracts of 25 leguminous seeds *in vitro* was evaluated for inhibitory activities against the small intestinal α-glucosidase of Sprague Dawley male rats. The responses varied both with leguminous seed types and concentrations used. At the concentration of 0.5 mg/ml, the methanol extracts of *Cassia obtusifolia*, *Glycine max* var. *yagkong*, *Glycine max* var. *hooktae*, *Glycine max* var. *geumdu*, *Glycine max* var. *mejukong*, *Glycine soja*, *Phaseolus multiflorus*, *Pisum sativum*, and *Vigna sinensis* inhibited over 50% of the enzyme activity. The extracts of *G. max* var. *yagkong* and *V. sinensis* showed relatively strong inhibitory activities against α-glucosidase at the concentration of 0.1 mg/ml. The activity of each solvent fraction from *G. max* var. *yagkong* and *V. sinensis* was determined, and potent activities were detected from chloroform and butanol fractions, respectively. IC_{50} values of *G. max* var. *yagkong* and *V. sinensis* were 0.06 and 0.19 mg/ml, respectively. As a naturally occurring therapeutic agents, leguminous seeds examined could be useful for developing new types of antidiabetic agents.

Key words: *legume, α-glucosidase, seed extract,* Glycine max *var.* yagkong, Vigna sinensis, *antidiabetic agent.*

Diabetes mellitus affects approximately 300 million people worldwide and is the leading cause of blindness, kidney failure, heart attack, stroke, and amputation among adults.[1)] Achieving blood glucose levels as close to normal as possible has been considered as one of the major goals of therapy for those with diabetes mellitus, as high blood glucose level is implicated in the development of macro- and microvascular complications associated with diabetes.[2)] However, in clinical practice, normalizing blood glucose levels is a formidable challenge. Even more difficult is the control of postprandial hyperglycemia. Both dietary and pharmacological tools are now available for the management of postprandial hyperglycemia. The pharmacological agents with the greatest effect on postprandial hyperglycemia include insulin lispro, amylin analogues, and α-glucosidase inhibitors.[3)]

α-Glucosidase (EC 3.2.1.20) catalyzes the final step in the digestive process of carbohydrates. Its inhibitors can retard the uptake dietary carbohydrates and suppress postprandial hyperglycemia, and could be useful for treating diabetic and/or obese patients.[4)] α-Glucosidase inhibitors such as acarbose, miglitol, and voglibose are known to reduce postprandial hyperglycemia primarily by interfering with the carbohydrate digesting enzymes and delaying glucose absorption.[3)] In addition, numerous α-glucosidase inhibitors have been screened from plants, some of which are of clinical importance.[4-8)] Although several drugs targeted for carbohydrate-hydrolyzing enzyme are in clinical use, a large inhibitor pool is required as diabetic patients can develop resistance to current regimens.

Plants constitute a rich source of bioactive chemicals.[9,10)] Since many of them are largely free from adverse effects and have excellent pharmacological actions, they could lead to the development of new classes of possibly safer antidiabetic agents. Additionally, some flavonoids and polyphenol as well as sugar derivatives are found to be effective on the inhibition of α-glucosidase.[8,11)] Therefore, much efforts have been focused on the plants for potentially useful products as commercial antidiabetic agents or lead compounds. However, relatively little work has been done on α-glucosidase inhibitory activities of leguminous seed extracts compared to other food[12,13)] and plant origins[14,15)] in spite of their excellent nutritional, pharmacological, and industrial significances.[16-19)] In this study, we assessed α-glucosidase inhibitory activities of the extracts prepared from 25 leguminous seeds to develop potentially new safer types of α-glucosidase inhibitory agents.

Materials and Methods

Chemicals. Bovine serum albumin and *p*-nitrophenyl-α-D-glucopyranoside were purchased from Sigma Chemical (St.

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Table 1. List of leguminous plants tested.

Scientific name	Chracteristics				
	Seed colour	Flower colour	Size (cm)	Shape	Yielda (%)
Amphicarpaea edgeworthii	Purple	Light-purple	0.5	Ellipse	10.7
Arachis hypogaea	Dark-brown	Yellow	1.3	Ellipse	5.3
Canavalia lineata	Brown	Purple	0.9	Rod	12.0
Cassia obtusifolia	Dark-brown	Yellow	0.4	Rod	13.3
Dunbaria villosa	Light-brown	Yellow	0.9	Ellipse	5.6
Glycine max var. solitae	Black	White	1.1	Ellipse	10.0
Glycine max var. yagkong	Black	White	0.5	Spherical	5.5
Glycine max var. hooktae	Black	Purple	0.8	Spherical	6.6
Glycine max var. bangkong	Dark-brown	Purple	1.1	Ellipse	5.4
Glycine max var. geumdu	Dark-purple	Purple	0.6	Spherical	4.8
Glycine max var. chungtae	Light-green	White	0.8	Spherical	11.1
Glycine max var. wooltalikong	Purple	Purple	1.1	Ellipse	1.9
Glycine max var. mejukong	Yellow	White	0.8	Spherical	7.1
Glycine soja	Brown	Light-purple	2.0	Rod	10.7
Lathyrus japonica	Black	Red	1.5	Ellipse	12.0
Phaseolus multiflorus	Dark-purple	Red	1.2	Rod	5.3
Phaseolus nipponensis	Dark-green	Yellow	2.1	Ellipse	5.7
Phaseolus radiatus var. geodu	Black	White	0.5	Spherical	7.8
Phaseolus radiatus var. aurea	Green	Yellow	0.5	Rod	5.2
Pisum sativum	Light-green	White-blue	0.7	Spherical	3.6
Rhynchosia volubilis	Brown	Yellow	1.1	Ellipse	5.3
Vicia hirsuta	Black	Light-purple	1.2	Ellipse	11.8
Vicia tetrasperma	Light-purple	Light-purple	1.1	Ellipse	12.3
Vigna angulasis	Red	Yellow	0.6	Spherical	4.8
Vigna sinensis	Light-yellow	Yellow	0.7	Ellipse	6.2

[a](Dried weight of methanol extract/dried weight of sample) × 100.

Louis, MO, USA). Sprague Dawley male rats were purchased from Dae Han Laboratory Animal Research Center Co. (Umsung, Chungbuk, Korea), and all other chemicals were of reagent grade.

Plant materials and sample preparation. The leguminous seeds were randomly and anecdotally collected (Table 1). They were dried in an oven at 60°C for 3 days and finely powdered using a blender. Each sample (50 g) was extracted twice with 500 m*l* methanol at room temperature and filtered (Toyo filter paper No. 2, Toyo Roshi, Japan). The combined filtrate was concentrated *in vacuo* at 35°C using a rotary vacuum evaporator (Model: N-3NW, EYELA, Japan). The yields of the seed extractions are shown in Table 1.

Isolation of α-glucosidase from the intestine of Sprague Dawley rats. α-Glucosidase was prepared from the small intestines of 4-week-old rats weighing 180-200 g each. The rats were starved for 16-18 h prior to the study but were allowed access to water *ad libitum*. Small intestinal brush border was removed from the rats and carefully homogenized for 5 min in 5 volumes (w/v) of 5 mM EDTA (pH 7.0) containing 0.5 M NaCl and 0.5 M KCl using a Potter-Elvehjem homogenizer (Wheaton Co., IL, USA). The homogenate was centrifuged at 20,000 × g for 30 min. The precipitate was dissolved with 5 mM EDTA (pH 7.0) and centrifuged at 20,000 × g for 30 min. It was subsequently redissolved with 5 volumes of 0.9% NaCl and centrifuged at 1,000 × g for 30 min. The supernatant was retained for the enzyme preparation. All procedures were carried out at 4°C.

Enzyme inhibitory assay. α-Glucosidase activity was assayed according to the method described by Kim[20] with slight modifications. α-Glucosidase (0.6 U) was dissolved in 100 mM phosphate buffer (pH 7.0) containing 2 g/L bovine serum albumin and 0.2 g/L NaN_3 and was used as an enzyme solution. *p*-Nitrophenyl-α- D-glucopyranoside (5 mM) in the same buffer (pH 7.0) was used as a substrate solution. Enzyme solution (50 μ*l*) and test extracts (10 μ*l*) dissolved in DMSO at a concentration of 5 mg/m*l* were mixed in a microtiter plate well and measured for titer (Abs 405 nm) at zero time using a microplate reader (model 550, BioRad, Hercules, CA, USA). After incubation for 5 min, the substrate solution (50 μ*l*) was added and incubated for additional 5 min at room temperature. The increase in absorbance at zero time was measured. Inhibitory activity was expressed as 10 minus relative absorbance difference (%) of test compounds to absorbance change of the control, where the test solution was replaced by a carrier solvent. All determinations were performed in triplicates. The protein content of the enzyme preparation was determined through Lowry method[21] using bovine serum albumin as a standard.

Results and Discussion

The methanol extracts of 25 leguminous seeds and acarbose potent α- glucosidase inhibitor, for comparison, were determined for inhibitory activities against small intestinal α-glucosidase isolated from Sprague Dawley male rats (Table 2). The responses against α-glucosidase varied both with leguminous seed types and concentrations used. The methanol extracts of leguminous seeds exhibited α-glucosidase inhibition rate ranging from 11 to 91% at the concentration of 1 mg/ml, while 17 to 75% at 0.5 mg/ml (Table 2).

For tests at the concentration of 1 mg/ml, the methanol extracts of *Amphicarpaea edgeworthii*, *Cassia obtusifolia*, *Glycine max* var. *yagkong*, *Glycine max* var. *hooktae*, *Glycine max* var. *bangkong*, *Glycine max* var. *geumdu*, *Glycine max* var. *mejukong*, *Glycine soja*, *Phaseolus multiflorus*, *Phaseolus nipponensis*, *Pisum sativum*, and *Vigna sinensis* inhibited over 70% enzyme activities, whereas the extracts of *Glycine max* var. *solitae*, *Glycine max* var. *wooltalikong*, *Vicia tetrasperma*, and *Vigna angulasis* inhibited 50-70% (Table 2). Among the 25 samples, the methanol extracts of *G. max* var. *geumdu*, *P. multiflorus*, and *V. sinensis* inhibited 90-91% α-glucosidase activity. For tests at the concentration of 0.5 mg/ml, over 50% inhibitory activities were exhibited in the methanol extracts of *C. obtusifolia*, *G. max* var. *yagkong*, *G. max* var. *hooktae*, *G. max* var. *geumdu*, *G. max* var. *mejukong*, *G. soja*, *P. multiflorus*, *P. sativum*, and *V. sinensis*. Nine extracts showing potent inhibition rate against α-glucosidase were evaluated for inhibitory activities at the concentration of 0.1 mg/ml (Fig. 1). The extracts of G. max var. yagkong and V. sinensis exhibited high inhibition rate (>50%). However, the remaining leguminous seeds exhibited low or no inhibition rate (<40%). These results suggest that various compounds such as alkaloids, phenolics, and terpenoids in leguminous seeds[19] may contribute to the inhibition of α-glucosidase.

The activities of the solvent fractions from the methanol extracts of *G. max* var. *yagkong* and *V. sinensis* were evaluated (Table 3). Chloroform fraction from the extract of *G. max* var. *yagkong* showed a potent inhibition of α-glucosidase, whereas the other fractions exhibited little or no inhibition. In the fractionation of the methanol extract from *V. sinensis*, strong inhibitory activity was observed in the butanol fraction, whereas not detected in other fractions. IC_{50} values of *G. max* var. *yagkong* and *V. sinensis* were compared to the α-glucosidase inhibitor, acarbose (Table 3). IC_{50} value of the chloroform fraction from *G. max* var. *yagkong* extract (IC_{50}, 0.06 mg/ml) was similar to that of acarbose (IC_{50}, 0.05 mg/ml), whereas IC_{50} value of acarbose was fivefold stronger than that of *V. sinensis* (IC_{50}, 0.19 mg/ml). These results suggest that *G. max* var. *yagkong* and *V. sinensis* may contain potent α-glucosidase inhibitors such as alkaloids, phenolics, and terpenoids.[19]

It has been well-acknowledged that plant-derived extracts and phytochemicals are potential alternatives to synthetic inhibitors against α-glucosidase.[4,6,7,22] Barclay and Perdue[14] suggested that the most promising botanicals, as sources of novel plant-based α-glucosidase inhibitors for present and future uses (1976), are species of the families *Apocynaceae, Celestraceae, Cephalotaxaceae, Euphorbiaceae, Leguminosae, Liliaece, Menispermaceae, Podocarpaceae, Rutaceae,*

Table 2. α-Glucosidase inhibitory activities of methanol extracts of leguminous seeds.

Sample tested	Final Conc. (mg/ml)	Inhibition (%)
A. edgeworthii	1	75
	0.5	46
A. hypogaea	1	42
	0.5	31
C. lineata	1	24
C. obtusifolia	1	79
	0.5	52
D. villosa	1	11
G. max var. solitae	1	58
	0.5	37
G. max var. yagkong	1	87
	0.5	73
G. max var. hooktae	1	85
	0.5	53
G. max var. bangkong	1	79
	0.5	43
G. max var. geumdu	1	90
	0.5	75
G. max var. chungtae	1	43
	0.5	21
G. max var. wooltalikong	1	58
	0.5	17
G. max var. mejukong	1	86
	0.5	71
G. soja	1	78
	0.5	61
L. japonica	1	36
P. multiflorus	1	91
	0.5	62
P. nipponensis	1	78
	0.5	45
P. radiatus var. geodu	1	25
P. radiatus var. aurea	1	31
P. sativum	1	77
	0.5	65
R. volubilis	1	26
V. hirsuta	1	30
V. tetrasperma	1	53
	0.5	25
V. angulasis	1	68
	0.5	49
V. sinensis	1	91
	0.5	73
Acarbose	1	98
	0.8	76
	0.5	68

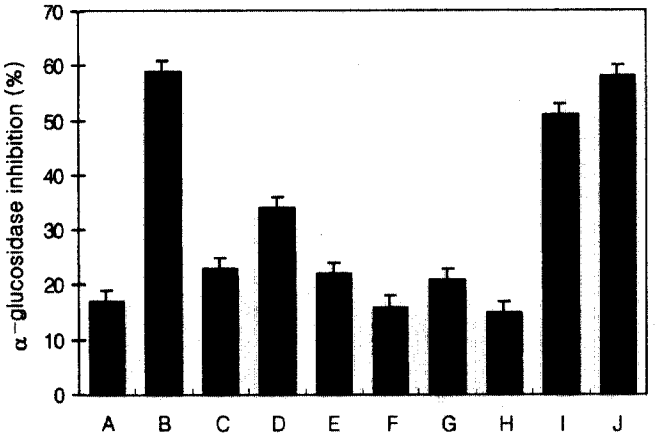

Fig. 1. α-Glucosidase inhibitory activities of methanol extracts of leguminous seeds at a concentration of 0.1 mg/ml. A, *C. obtusifolia*; B, *G. max* var. *yagkong*; C, *G. max* var. *hooktae*; D, *G. max* var. *geumdu*; E, *G. max* var. *mejukong*; F, *G. soja*; G, *P. multiflorus*; H, *P. sativum*; I, *V. sinensis*; J, Acarbose.

Simanubaceae, Taxaceae, and *Thymelaeaceae*. In this studty, *G. max* var. *yagkong* and *V. sinensis* seeds showed potent inhibitory activities against α-glucosidase, an indication of at least one of their pharmacological actions. Although the active principles of these seeds remain unknown at present, soybean seed-derived isoflavones, proglycinins, glycopeptide, and aglucones exhibit antitumorigenesis and pharmacological functions.[23-25]

α-Glucosidase inhibitors are currently the most commonly used oral agents for improving postprandial hyperglycemia due to the lack of hypoglycemic threat, and, more importantly, the prospect of blood glucose control without hyperinsulinemia and body weight gain.[3] Inhibition of α-glucosidase and amylase should result in delayed carbohydrate digestion and glucose absorption with attenuation of postprandial hyperglycemic excursions. It has been reported that α-glucosidase inhibitors generally do not alter the total amount of carbohydrate absorbed and, therefore, do not cause any net nutritional caloric loss, although they slow down the carbohydrate digestion. As mentioned earlier, α-glucosidase inhibitors including acarbose, miglitol, and voglibose are currently available for the treatment of patients with type II diabetes mellitus. In addition to these drugs, flavonoids, N-*p*-coumaroyl tyramine, and kotalanol isolated from plants have been reported to strongly inhibit α-glucosidase.[6,7]

For several years, many studies on screening α-glucosidase inhibitors were done with yeast α-glucosidase. However, a controversy exists regarding the use of yeast α-glucosidase in screening potential agents of clinical importance, since the yeast α-glucosidase inhibitors may not work on the mammalian enzymes as much as they do on the yeast enzyme.[26] In this study, α-glucosidase prepared from the small intestinal brush border of Sprague Dawley male rats was used for screening potential agents of clinical importance. It might be worthwhile to evaluate practical inhibition against mammalian α-glucosidase.

Table 3. α-Glucosidase inhibitory activities of solvent fractions of methanol extracts from *Glycine max* var. *yagkong* and *Vigna sinensis*.

Legume Fraction	Final Conc. (mg/ml)	Inhibition (%)	IC_{50} (mg/ml)
G. max var. yagkong			
Hexane	1	3	
Chloroform	1	93	
	0.5	76	
	0.1	60	
	0.01	31	0.06 ± 0.006
Ethyl acetate	1	5	
Butanol	1	0	
Water	1	0	
V. sinensis			
Hexane	1	0	
Chloroform	1	0	
Ethyl acetate	1	3	
Butanol	1	92	
	0.5	79	
	0.1	41	
	0.01	19	0.19 ± 0.02
Water	1	0	
Acarbose	1	98	
	0.8	76	
	0.5	68	
	0.1	58	
	0.01	38	0.05 ± 0.003

In conclusion, although *in vivo* efficacy and the clinical usefulness of the leguminous seeds showing strong inhibitory activity remain to be evaluated, the strong inhibitory activities of leguminous seeds examined confirm their superiority and usefulness as antidiabetic agents. The isolation and characterization of the components against α-glucosidase are in progress.

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