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Inhibitory Effects of Crude Drugs on α-Glucosidase

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The inhibitory activity of several crude drugs on α-glucosidases, which are the key enzymes for carbohydrate digestion and the prevention of diabetic complications, was investigated. Several crude drugs including Terminaliae Fructus, Mori Cortex Radicis, Caesalpiniae Lignum and *Gyrophora esculenta* potently inhibited maltase and sucrase isolated from rat intestine, while Arecae Semen and Corni Fructus remarkably inhibited α-amylase. Caesalpiniae Lignum and *Gyrophora esculenta* exhibited significant reductions of blood glucose elevation in mice loaded with maltose and sucrose.

Key words: Hyperglycemia, α-Glucosidase inhibitor, Crude drugs, *Gyrophora esculenta*

INTRODUCTION

Diabetes mellitus is classified into insulin-dependent diabetes mellitus(IDDM) and non-insulin-dependent diabetes mellitus(NIDDM). Diabetes mellitus can create serious problems due to its subsequent complications rather than by its own symptoms (Winegrad, 1987). Therefore, several therapeutic methods to achieve near-normal glucose control in IDDM and NIDDM have been steadily developed, because the mortality of the poor glucose control group is about two and one-half times of that of well-controlled group, and the life expectancy of a well controlled diabetics is considered to be approximately that of the normal individual (Goodkin, 1975). However, one of the most difficult components of blood glucose control in diabetics is the restriction of postprandial increases to normal levels. Postprandial increases in blood glucose are dependent on a number of factors, which are comprised of intrinsic factors (gastric emptying, pancreatic enzyme secretion, intestinal mucosal enzyme content, intestinal absorptive capacity and intestinal motility) and extrinsic factors (carbohydrate source, processing prior to ingestion) and other components of the meal (Horowitz *et al.*, 1996; Madariaga *et al.*, 1988). α-Glucosidases are located in the brush-border surface membrane of intestinal cells, and are the key enzymes of carbohydrate digestion (Caspary, 1978). De Boer *et al.* (1993) and Rosenstock *et al.* (1988) reported that oral administration of specific α-glucosidase inhibitors could effectively improve hyperglycemia as well as diabetic complication. Many researchers have isolated hypoglycemic agents or α-glucosidase inhibitors from natural products, for example, the methanolic extract of *Myrcia multiflora* inhibited the increase of serum glucose levels in sucrose-loaded rats and alloxan-induced diabetic mice (Yoshikawa *et al.*, 1998), and Mori Folium ethanol soluble fraction and Cortex Mori radicis in *db/db* mice improved the hypoglycemic and reduced the triglyceride activity (Ryu *et al.*, 1998; Kim *et al.*, 1999). However, these studies on the α-glucosidase-inhibitory activity of herbal medicines were not complete. Therefore, we here investigated the inhibitory activity of two hundred and fifty crude drugs on α-glucosidases of rat intestine and blood glucose elevation in mice.

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MATERIALS AND METHODS

Materials

Glucose oxidase, starch azure and α-nitrophenyl-α-$_D$-glucopyranoside were purchased from Sigma Co. (U.S.A.), o-phenylenediamine and peroxidase were from Wako Co. (Japan) and blood glucose test strip from Johnson & Johnson Co. (U.S.A.). The other chemicals were of analytical grade. Crude drugs were purchased by Shinsungyakupsa (Korea) and their descriptions are listed in Table I.

Animals

Male ICR mice (25 ± 2 g) and male Sprague-Dawley rats (200 ± 20 g) were housed in plastic cages with wire

Table I. List of herbal medicines and mushrooms examined for effects upon *in vitro* β-glucosidase activity

Acanthopanacis Cortex	Caryophylli Cortex	Fritillariae Bulbus
Aconiti Ciliare Tuber	Caryophylli Flos	Galla Rhois
Acori Graminei Rhizoma	Cassiae Semen	Galli Stomachichum Corium
Aconiti Koreani Tuber	Castaneae Semen	Gardeniae Fructus
Achyranthis Radix	Celosiae Semen	Gastrodiae Rhizoma
Adenophorae Radix	Cervi Cornu Pantotrichum	Gentiana macrophyllae Radix
Agastachis Herba	Chaenomelis Fructus	Gentiana scabrae Radix
Ailanthi Radicis Cortex	Chelidonii Herba	Geranii Herba
Akebiae Caulis	Chrysanthemi Flos	Ginkgo Semen
Albizziae Cortex	Cibotii Rhizoma	Ginseng Radix
Alismatis Rhizoma	Cicadae Periostracum	Gleditsiae Spina
Alpiniae Fructus	Cimicifugae Rhizoma	Glycyrrhizae Radix
Alpiniae Katsumadaii Semen	Cinnamomi Cortex Spissus	Halloysitum Rubrum
Alpiniae officinari Rhizoma	Cinnamomi Ramulus	Herpertritdis Radix
Amomi Cardamomi Fructus	Cinnamomi Cortex	Hoelen
Amomi Semen	Cirsii Herba	Hoelen Cum Radix
Amomi Tsao-ko Fructus	Cistanchis Herba	Holotrichia
Ampelopidis Radix	Clematidis Radix	Ignati Semen
Amydae Carapax	Cnidii Rhizoma	Imperatae Rhizoma
Anemarrhenae Rhizoma	Codonopsis Radix	Inulae Flos
Anethi Fructus	Coicis Semen	Juglandis Semen
Angelicae Dahuricae Radix	Coptidis Radix	Junci Medulla
Angelicae Gigantis Radix	Coptidis Rhizoma	Kalopanacis Cortex
Angelicae Koreanae Radix	Corni Fructus	Kansui Radix
Angelicae Tenuissimae Radix	Corydalis Tuber	Kochiae Fructus
Antelopis Cornu	Crataegi Fructus	Laminariae Japonicae Thallus
Anthrisci Radix	Curculiginis Rhizoma	Ledebouriellae Radix
Araliae Cordatae Radix	Curcumae Longae Rhizoma	Leonuri Herba
Arctii Semen	Curcumae Rhizoma	Leonuri Semen
Arecae Pericarpium	Cuscutae Semen	Ligustri Fructus
Arecae Semen	Cynanchi Radix	Lilii Bulbus
Arisaematis Rhizoma	Cynomorii Caulis	Linderae Radix
Aristolochiae Fructus	Cyperi Rhizoma	Liriopis Tuber
Aristolochiae Radix	Dendrobii Herba	Lonicerae Herba
Armeniacae Semen	Dianthi Herba	Loranthi Ramulus
Artemisiae Asiaticae Herba	Dictamni Radicis Cortex	Lycii Fructus
Artemisiae Capillaris Herba	Dioscorea Rhizoma	Lycii Radicis Cortex
Asiasari Radix	Dolichoris Semen	Lycopi Herba
Asparagi Tuber	Drabae Semen	Lygodii Spora
Asteris Radix	Drynariae Rhizoma	Magnoliae Cortex
Astragali Radix	Ecliptae Herba	Magnoliae Flos
Atractylodis Rhizoma	Elsholtziae Herba	Malvae Semen
Atractylodis Rhizoma Alba	Ephedrae Herba	Manitis Squama
Aurantii Fructus	Ephedrae Radix	Mantidis Ootheca
Aurantii Immatri Pericarpium	Epimedii Herba	Massa Medicata Fermentata
Aurantii Nobilis Pericarpium	Equiseti Herba	Meliae Cortex
Bambusae Caulis in Taeniam	Eriobotryae Folium	Meloae Fructus
Belamcandae Rhizoma	Erycibae Caulis	Menthae Herba
Betulae Cortex	Eucommiae Cortex	Mori Cortex Radicis
Bletillae Rhizoma	Euryales Semen	Mori Folium
Bombycis Corpus	Evodiae Fructus	Mori Ramulus
Borneolum	Fagarae Fructus	Moutan Cortex Radicis
Caesalpiniae Lignum	Farfarae Flos	Mume Fructus
Cannabis Semen	Forsythiae Fructus	Myristicae Semen
Carthami Flos	Fossilia Ossis Mastodi	Nelumbinis Folium

Table I. Continued

Nelumbinis Semen	Prunijaponicae Semen	Stemonae Radix
Nepetae Spica	Pteropi Faeces	Taraxaci Herba
Notoginseng Radix	Puerariae Flos	Terminaliae Fructus
Olibanum	Puerariae Radix	Testdinis Plastrum
Orostachys Herba	Quisqualis Fructus	Thujae Folium
Paeoniae Radix	Raphani Semen	Tiglii Semen
Paeoniae Radix Rubra	Rehmanniae Radix	Tokoro Rhizoma
Pasoraliae Semen	Rehmanniae Radix(Dried)	Torilidis Fructus
Patriniae Radix	Rehmanniae Radix Preparata	Tribuli Fructus
Perillae Herba	Rosae Laevigatae Fructus	Trichosanthis Radix
Perillae Semen	Rubi Fructus	Trichosanthis Semen
Persicae Semen	Salviae Radix	Trigonellae Semen
Pharbitidis Semen	Sanguisorbae Fructus	Typhae Pollen
Phaseoli Angularis Semen	Santalini Lignum Rubrum	Uncariae Ramulus et Uncus
Phellodendri Cortex	Saussureae Radix	Viticis Fructus
Phlomidis Radix	Scirpi Rhizoma	Xanthii Fructus
Phragmits Rhizoma	Scolopendrae Corpus	Zedoariae Rhizoma
Phyllostachys Folium	Scrophulariae Radix	Zingiberis Rhizoma(Dried)
Picrorrhizae Rhizoma	Scutellariae Radix	Zingiberis Rhizoma
Pinelliae TuberPiperis Longi Fructus	Sepiae Os	Zizyphi Fructus
Plantaginis Semen	Sesami Semen	Zizyphi Spinosi Semen
Remotiflorae Radix	Siegesbeckiae Herba	Auricularia auricular
Rhei Undulati Rhizoma	Sinapis Semen Alba	*Coriolus versicolor*
Polygalae RadixPolygonati Rhizoma	Sinomeni Caulis et Rhizoma	*Flammulina velutipes*
Polygoni Avicularis Herba	Smilacis Rhizoma	*Ganoderma lucidum*
Polygoni Cuspidati Radix	Solani Nigri Herba	*Gyrophora esculenta*
Polygoni Multiflori Radix	Sophorae Flos	*Lentinus edodes*
Polyporus	Sophorae Fructus	*Pleurotus ostreatus*
Ponciri Fructus	Sophorae Radix	*Tricholoma caligatum*
Portulacae Herba	Sophorae Subprostratae Radix	
Prunellae Spica	Spirodelae Herba	

tops. All animals were fed a standard pellet diet (Samyang Co., Korea) and tap water *ad libitum*, and housed at a temperature of 23 ± 1°C, humidity of 50% with a light-dark cycle from 06:30 to 18:30 h.

Extraction of crude drugs

Two hundred and forty clinically used crude drugs and eight specimens of edible mushrooms were extracted with water at 100°C for 4 h. Each water extract was filtered and adjusted to a final concentration of 0.2 mg/ml. Seven α-glucosidase inhibitory herbal medicines were fractionated with 50% cold methanol, and the supernatants adjusted to a final concentration of 2 g/kg, for use in the *in vivo* experiments.

Preparation of crude enzyme solution

The brush-border mucosal layer of the small intestine from a overnight fasted rat was obtained by careful scraping with a thin spatula, and diluted with cold saline. After breakdown on a sonicator for 15 sec, the suspension was centrifuged at 10,000 rpm, at 4°C for 30 min and the supernatant used as the crude enzyme.

Enzyme assay

Maltase activity was measured according to the micromethod of Dahlqvist (Dahlqvist, 1970). The maltase reaction mixture contained 0.1 ml of crude enzyme solution, 0.1 ml of 2 mM maltose, 0.1 of sample and 0.2 ml of 0.1 M phosphate buffer (pH 7.0). After incubation for 40 min at 37°C, the reaction mixture was inactivated on a hot water bath for 2 min, and then centrifuged at 3,000 rpm for 5 min. 0.1 ml of supernatant was added to the glucose reagent, consisting of *o*-phenylenediamine 0.05 mg/ml, peroxidase 2 unit/ and glucose oxidase 0.384 unit/ml, and incubated for 30 min. 0.5 ml of 1N HCl were added to the reaction mixture and the liberated glucose measured colorimetrically at 492 nm (Lee *et al.*, 1983; Tandon *et al.*, 1975).

Sucrase activity was measured according to the micromethod of Dahlqvist (Dahlqvist, 1970). The reaction mixture for the sucrase determination contained 0.1 ml of crude

enzyme solution, 0.1 ml of 10 mM sucrose, 0.1 ml of sample and 0.2 ml of 0.1 M phosphate buffer (pH 7.0). After incubation for 180 min at 37°C, the enzyme inactivated in a hot water bath for 2 min, then centrifuged at 3,000 rpm for 5 min, and 0.1 ml of supernatant was then added to 0.1 ml of the glucose reagent. And it was incubated for 30 min. 0.5 ml of 1N HCl were added to the reaction mixture and the liberated glucose measured colorimetrically at 492 nm (Lee *et al.*, 1983; Tandon *et al.*, 1975)

α-Amylase activity was measured according to the method of Rinderknecht (Rinderknecht *et al.*, 1967). 0.1 ml of reaction mixture containing the crude enzyme in solution, 0.2 ml of sample and 0.75 ml of starch azure (1 unit/20 mM phosphate buffer (pH 7.0)) were incubated at 37°C for 1 h. After addition of 0.5 ml of 0.1 N HCl, the reaction mixture was centrifuged at 3,000 rpm for 10 min, and 1.0 ml of the supernatant was measured against a reagent blank colorimetrically at 620 nm (Rinderknecht *et al.*, 1967).

Nonspecific α-glucosidase activity was measured according to the previous published method (Dahlqvist, 1970). The reaction mixture containing 0.05 ml of crude enzyme solution, 0.1 ml of sample, 0.2 ml of 20 mM phosphate buffer (pH 7.0) and 0.25 ml of *p*-nitrophenyl-α-$_D$-glucopyranoside (2 mM) was incubated for 30 min at 37°C. The reaction was stopped by adding 0.5 ml of 1 M glycine-NaOH (pH 9.0) and centrifuged at 3,000 rpm for 10 min. The supernatant was analyzed at 405 nm spectrophotometrically.

Inhibition of blood glucose elevation in carbohydrate loaded mice

Each group consisted of five mice. After overnight fasting for 16 h, fasting blood glucose concentrations were determined in all mice with a blood glucose meter. Carbohydrate (2 g/kg) and the materials under test (2 g/kg) were simultaneously injected by oral inoculation. Thirty min (60 min for starch) later, blood glucose levels were rechecked.

Statistics

The significance of differences between the groups was calculated using Student's paired *t*-test.

RESULTS

In vitro inhibitory activity of crude drugs on α-glucosidases

To isolate α-glucosidase inhibitors from natural products, the inhibitory activity of two hundred and forty herbal medicines and eight mushrooms on rat intestinal α-glucosidases were determined. Terminaliae Fructus, Bombycis Corpus, Mori Cortex Radicis, Mori Folium, Mori Ramulus, Caesalpiniae Lignum, Galla Rhois and *Gyrophora esculenta* inhibited maltase by more than 90%. Bombycis Corpus and Gastrodiae Rhizoma inhibited the sucrase by more than 80%, and Dictamni Radicis Cortex, Mori Ramulus, Polyporus, Mori Cortex Radicis, Mori Folium, *Lentinus edodes* and *Gyrophora esculenta* inhibited sucrase by more than 90%. Arecae Semen and Corni Fructus inhibited α-amylase by 90%. In addition, some crude drugs including Bombycis Corpus, Mori Cortex Radicis, Mori Folium, Mori Ramulus and *Gyrophora esculenta* strongly inhibited nonspecific α-glucosidase using *p*-nitrophenyl-α-$_D$-glucopyranoside as substrate (Table II).

Table II. *Inhibitory effects of various crude drugs and mushrooms on rat intestinal α-glucosidases*

Materials	Inhibition(%)			
	Maltase	Sucrase	α-Amylase	Nonspecific α-glucosidase
Arecae Semen	43	13	89	18
Bombycis Corpus	92	83	23	76
Caesalpiniae Lignum	95	52	12	7
Corni Fructus	-[a)]	12	94	7
Dictamni Radicis Cortex	18	95	13	6
Gastrodiae Rhizoma	-	86	10	17
Galla Rhois	100	74	56	37
Mori Cortex Radicis	100	100	11	99
Mori Folium	93	94	4	92
Mori Ramulus	99	100	4	84
Polyporus	67	98	4	46
Terminaliae Fructus	91	77	28	-
Lentinus edodes	2	95	12	-
Gyrophora esculenta	97	96	-	80

Each crude drug and mushroom extract was adjusted to a final concentration of 0.2 mg/ml.
[a)]. not inhibited

Inhibition effects of some crude α-glucosidase-inhibitory drugs on blood glucose elevation in mice

Seven crude drugs, which strongly inhibited α-glucosidases were selected, were selected and fractionated with 50% cold methanol. The inhibitory effects of their supernatants on blood glucose elevation in carbohydrates loaded mice were investigated (Table III). *Gyrophora esculenta* and Caesalpiniae Lignum considerably reduced the blood glucose elevation in mice loaded with maltose. In the case of the sucrose loading test, most of the crude drugs tested howed strong inhibition, and Caesalpiniae Lignum, Galla Rhois, and *Gyrophora esculenta* each repressed blood glucose elevation by 72%. Arecae Semen, Corni Fructus and Galla Rhois strongly reduced blood glucose level in starch loaded mice.

DISCUSSION

In our experiment, several crude drugs were shown to have an inhibitory effect on some kinds of α-glucosidases *in vitro* as well as on blood glucose elevation in mice loaded wtih saccharides *in vivo*. They offer the possibility of being developed as successful -glucosidase inhibitors with fewer side effects, because they have been clinically used as natural plant drugs for a long time without serious problems.

α-Glucosidases are distributed in the small intestine. These enzymes are known to be adaptive, and specifically stimulated by certain dietary sugars in diabetics. Carbohydrates, which are the most fundamental of the energy supplying nutrients, have been shown to increase the specific activities of sucrase and maltase and also to increase the levels of lactase in diabetics. In addition to the enzyme activity, hexose absorption is also increased in human diabetics. Increased glucose transport in diabetics may be responsible for stimulating disaccharidase activity. These factors may be the cause of a sudden increase of postprandial blood glucose level in diabetic patients. Therefore, diabetic patients have been encouraged to avoid simple carbohydrates like sucrose in favor of the more starchy alternatives. Because the simple carbohydrates are more readily absorbed from the gastrointestinal tract and cause more pronounced hyperglycemia than the complex carbohydrates (Defronzo *et al.*, 1983; Tandon *et al.*, 1975; Lembcke *et al.*, 1990). It is particularly true that, in NIDDM patients, the control of post-meal hyperglycemia can be important in reducing the occurrence of complications. It may well be that intensive diabetes treatment with long-term normoglycemia should be considered preventive. Once significant diabetic complications occur, no degree of normoglycemia will cause a reversal, but some extent of slowing disease progression is possible (Raskin and Rosenstock *et al.*, 1986; Ceriello *et al.*, 1996). In addi-tion, diet control would seem to be one of the notorious noncompliances in diabetics. Therefore, we believe that *Gyrophora esculenta* and Caesalpiniae Lignumon, which were selected as α-glucosidase inhibitors in the present study could be used for controlling postprandial blood glucose elevation in patients.

Table III. Inhibitory activities of some crude drugs and mushrooms on blood glucose elevation in mice loaded with maltose, sucrose and starch

Groups	Blood glucose(/)		
	Maltose	Sucrose	Starch
Control	89 ± 12	93 ± 17	98 ± 19
Arecae Semen	71± 10 (20.2)	35 ± 7** (62.4)	24 ± 3*** (75.5)
Caesalpiniae Lignum	37 ± 12** (58.4)	26 ± 3*** (72.0)	58 ± 8** (40.8)
Corni Fructus	96 ± 22 (-[a])	61 ± 13* (34.4)	47 ± 11** (52.0)
Galla Rhois	73 ± 8 (18.0)	26 ± 3*** (72.0)	49 ± 17** (50.0)
Gyrophora esculenta	48 ± 10* (46.1)	26 ± 6*** (72.0)	66 ± 16* (32.7)
Polyporus	137 ± 21 (-)	79 ± 5 (15.1)	65 ± 3* (33.7)
Terminaliae Fructus	145 ± 29 (-)	39 ± 10** (58.1)	61 ± 11* (37.8)

Each group had five animals. Each crude drug and saccharide were simultaneously injected by *p.o.* at a dose of 2 g/kg of body weight. Thirty minutes (60 minutes for starch) later, blood glucose levels were rechecked. Each result is expressed as mean ± SD, and the inhibition of blood glucose elevation is showed in parenthesis. *,Statistically significant compared with the control data (*,$p<0.05$; **,$p<0.01$ and ***, $p< 0.001$).
a), not inhibited

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