J. Food Sci. Nutr.
Vol. 7, No. 1, p.62~66 (2002)

Inhibition of Carbohydrate-Digesting Enzymes and Amelioration of Glucose Tolerance by Korean Medicinal Herbs

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Abstract

As inhibitors of carbohydrate-digesting enzymes can prevent hyperglycemia that is known to cause many macrovascular complications, they may prove a useful adjunct to hypocaloric diets in patients with type 2 diabetes and obesity. Inhibitory activities of two hundred and fifteen kinds of medicinal herb extracts against α-glucosidase (EC 3.2.1.20) and α-amylase (EC 3.2.1.1) have been investigated *in vitro*. *Adenophora triphylla*, *Aneilema keisak*, and *Morus bombysis* significantly suppressed rat intestinal α-glucosidase activity *in vitro*. Porcine pancreatic amylase was efficiently inhibited by methanol extracts of *Epimedium koreanum*, *Campsis grandiflora* and *Salvia plebeia*. Methanol extract of *Epimedium koreanum* among the medicinal herbs tested showed the strongest inhibitory activity against porcine pancreatic α-amylase with 0.1 mg/mL of IC_{50}. The herb extract also improved glucose tolerance in ICR mice when loaded with 0.9 g soluble starch per kg body weight. Taken together, *Epimedium koreanum* merits further evaluation as a therapeutic measure.

Key words: α-glucosidase, α-amylase, inhibitory activity, *Epimedium koreanum*

INTRODUCTION

Despite periods of feeding and fasting, plasma glucose remains in a narrow range between 4 and 7 mM in normal individuals. This tight control is governed by the balance between glucose absorption from the intestine, production by the liver and uptake and metabolism by peripheral tissues (1). In diabetic or insulin-resistant patients plasma glucose level remains high, resulting in the development of macrovascular complications such as heart disease, kidney failure, blindness and even loss of limbs (2). Inhibition of carbohydrate-digesting enzymes such as α-glucosidase and amylase has been recognized as one of major tools to ameliorate hyperglycemia in diabetic and obese patients. α-Glucosidase is an enzyme that catalyzes the final step in the digestive process of carbohydrates, and hence α-glucosidase inhibitors could retard the use of dietary carbohydrates to suppress postprandial hyperglycemia (PPHG) (3). α-Glucosidase inhibitors such as acarbose, miglitol and voglibose are known to reduce PPHG primarily by interfering with the carbohydrate-digesting enzymes and delaying glucose absorption (2). Amylases, endoglucanases that catalyze the hydrolysis of internal α-1,4-glucosidic linkages in starch and other related polysaccharides, have also been target for suppression of PPHG (4). In fact, inhibitors of α-amylases are expected to be better suppressor of PPHG, since they would not result in an abnormal accumulation of maltose that causes side effects such as abdominal pain, flatulence, diarrhea, and soft feces in the colon (5). Although several drugs targeted for carbohydrate-hydrolyzing enzymes are in clinical use, it is necessary to have a large inhibitor pool as diabetic patients can develop resistance to current regimens. Medicinal herbs, which are widely distributed in the plant kingdom have been used since ancient times by physicians and laymen to treat a great variety of human diseases such as diabetes, coronary heart disease, and cancers (5). In this study we investigated α-glucosidase and α-amylase inhibitory activities of some domestic medicinal herbs to evaluate their potential as dietary supplements for diabetic or obese people.

MATERIALS AND METHODS

Porcine pancreatic α-amylase, *p*-nitrophenyl-α-D-glucopyranoside, and α-nitrophenyl-α-D-maltopentoglycoside were purchased from Sigma (St Louis, MO, USA), and α-glucosidase was prepared from rat intestinal powder (Sigma) as described elsewhere (6). Acarbose (purity: 96.1 %) was kindly provided by Bayer Korea Ltd. (Seoul, Korea).

Preparation of test sample

Medicinal herbs were obtained from the Medicinal Herb Experiment Station located in Kyungpook province of

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Korea and extracted with ten volumes of methanol, followed by rotary evaporation to dryness. Herb extracts were dissolved in dimethylsulfoxide (DMSO) at the concentration of 5 mg per mL and used as test samples.

Assays of α-glucosidase and amylase inhibitory activities

The α-glucosidase inhibitory assay was performed by the chromogenic method described by Watanabe (3) using a readily available rat intestinal enzyme. Briefly, α-glucosidase (0.7 U, Sigma) 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 used as an enzyme solution. 5 mM *p*-Nitrophenyl-α-D-glucopyranoside in the same buffer (pH 7.0) was used as a substrate solution. The 50 μL of enzyme solution and 10 μL of test compounds dissolved in dimethylsulfoxide at the 5 mg/mL concentration were mixed in a well of a microtiter plate and measured for titer (Abs 405 nm) at zero time with a microplate reader (model 550, Biorad, Hercules, California, USA). After incubation for 5 min, the substrate solution (50 μL) was added and incubated for another 5 min at room temperature. The increase in absorbance from zero time was measured. Inhibitory activity was expressed as 100 minus relative absorbance difference (%) of test compounds to absorbance change of the control where test solution was replaced by the carrier solvent. α-Amylase inhibitory activity was assayed in the same way as described for the α-glucosidase inhibitory assay except that porcine pancreatic amylase (100 U, Sigma) and blocked *p*-nitrophenyl-α-D-maltopentoglycoside were used as enzyme and substrate, respectively.

Glucose tolerance test

A glucose tolerance test was conducted after overnight food deprivation. Soluble starch (0.9 g) was dissolved in 5 mL warm water with and without 25 mg test sample. Dissolved starch was intubated into ICR mice, and peripheral blood was sampled at 0, 30, 60, 90, 120 min. Plasma concentrations of glucose were determined in triplicate using a commercial kit (Sigma Procedure No. 315, St. Louis, MO) and read at 505 nm using spectrophotometer (BU 7400, Beckman Instruments, Fullerton, CA) (7).

RESULTS

Edible parts of two hundred and fifteen medicinal herbs raised in Korea were extracted with methanol (10 mL/g fresh weight). The extracts were rotary-evaporated to dryness, and redissolved in dimethylsulfoxide at the concentration of 5 mg per mL. The inhibitory activities of the sample extracts against rat intestinal α-glucosidase and porcine pancreatic α-amylase are shown in Table 1. Although most of sample extracts were not effective in inhibiting α-glucosidase, methanol extract of *Adenophora triphylla*, *Aneilema keisak*, and *Morus bombysis* significantly suppressed rat intestinal α-glucosidase activity *in vitro*. The extract of *Epimedium koreanum* showed the strongest inhibitory activity against α-amylase. *Campsis grandiflora* and *Salvia plebeia* also showed moderate inhibitory activity against the enzyme activity although their inhibitory activities were less potent than that of *Epimedium koreanum*. However, none of the samples inhibited both enzyme activities simultaneously.

Epimedium koreanum repressed α-amylase activity in a dose-dependent manner with 0.1 mg/mL of IC_{50}, a concentration causing 50% inhibition of enzyme activity (Fig. 1). However, its inhibitory activity against α-amylase was one tenth of that of Acarbose, a prescription drug and a potent α-amylase inhibitor, with 0.01 mg/mL of IC_{50} (data not shown).

Time-dependent increase in plasma glucose concentration in mice by the intubation of starch solution was significantly suppressed by the extract of *Epimedium koreanum*, especially at 30 min after starch feeding (Fig. 2).

DISCUSSION

Non-insulin dependent diabetes mellitus (NIDDM) accounts for 90~95% of all diabetes. This heterogeneous disorder afflicts an estimated 6% of the adult population in Western society; its worldwide frequency is expected to continue to grow by 6% per annum, potentially reaching a total of 200~300 million cases in 2010 (8). The main force driving this increasing incidence is a staggering increase in obesity, the single most important contributor to the pathogenesis of diabetes (8). Therefore developing the measures to prevent obesity is essential for lessening the increasing rate of diabetes. One of the main features observed commonly in obesity and NIDDM is postprandial hyperglycemia. Such high blood glucose concentration accelerates lipogenesis and fat accumulation through hypersecretion of insulin as well as the development of chronic complications such as retinopathy and nephropathy (9). At present, therapy for NIDDM or type 2 diabetes relies mainly on several approaches intended to reduce the hyperglycaemia itself: sulphonylureas (and related insulin secretagogues), which increase insulin release from pancreatic islets; metformin, which acts to reduce hepatic glucose production; peroxisome proliferator-activated receptor-γ agonists (thiazolidinediones), which enhance insulin action; α-glucosidase inhibitors, which interfere with gut glucose absorption; and insulin itself, which suppresses glucose production and augments glucose utilization (8).

The present study indicates that some medicinal herbs

Table 1. Inhibitory activities of medicinal herb extracts against rat intestinal α-glucosidase and porcine pancreatic amylase

Scientific name	α-Glucosidase inhibitory activity (%)	α-Amylase inhibitory activity (%)	Scientific name	α-Glucosidase inhibitory activity (%)	α-Amylase inhibitory activity (%)
Acanthopanax sessiliflorus (leaves)	-2	-32	*Cichorium intybus* L.	-22	-94
Acanthopanax sessiliflorus (frutes)	13	-6	*Cirsium japonicum*	-55	-47
Achillea sibirica	-10	-58	*Cnidium officinale*	-13	-52
Achyranthes japonica	-29	-42	*Codonopsis pilosula*	-50	43
Aconitium carmichaehi	-14	-42	*Coix lachryma-jobi*	-38	-36
Acorus calamus (leaves)	-47	-97	*Commelina communis (flower)*	-4	-28
Actinidia arguta (leaves)	-12	-60	*Commelina communis (leaves)*	-14	-35
Adenophora triphylla	70	-24	*Convallaria keiskei*	12	-21
Agastache rugosa	-31	14	*Convallaria keiskei*	21	-22
Agrimonia pilosa	-17	-17	*Cyperus rotundus*	-3	-47
Akebia quinata (leaves)	-38	19	*Cyperus rotundus (seed)*	-9	11
Allium senescens L.	3	-51	*Dendrobium moniliforme*	-20	-34
Allium thunbergi	-11	-39	*Dianthus chinensis*	-10	-61
Allium tuberosum	-4	-56	*Dianthus superbus*	-4	-64
Allium victorialis	4	-2	*Dictamnus dasycarpus*	-3	16
Althaea rosea	-15	-50	*Dioscorea batatas*	-11	-19
Aneilema keisak	83	-32	*Dioscorea nipponica*	-57	-30
Anemarrhena asphodeloides	-40	-75	*Disporum sessile*	-18	41
Angelica autiloba	-3	-39	*Duchesnea chrysantha*	-20	-3
Angelica dahurica (leaves)	10	-33	*Echinops setifer*	8	-16
Angelica gigas (leaves)	-33	-81	*Elsholtzia splendens*	-33	18
Angelica tenuissima	7	-45	*Epimedium koreanum*	-13	86
Anglelica gigas (radis)	-11	-13	*Equisetum hyemale*	2	-53
Anglelica gigas (frutes)	-5	-23	*Euphorbia pekinensis*	5	-45
Aquilegia buergeriana	-21	8	*Euphorbia pekinensis (leaves)*	-11	-17
Aralia continentalis (frutes)	-2	-43	*Ficus carica*	-19	-6
Aralia continentalis (leaves)	-7	-48	*Ficus carica (leaves)*	-29	-8
Aralia continentalis (seed)	15	-52	*Foeniculium vulgare*	-15	-17
Aralia elata	-7	-14	*Forsythiae viridissima*	-12	1
Arctium lappa	-33	-73	*Geranium sibiricum*	-29	-81
Arisaema amurense	-4	-37	*Geranium sibiricum (leaves)*	-8	-56
Artemisia capillaris	-19	-54	*Geum japonicum*	-8	-17
Artemisia capillaris (leaves)	-16	-27	*Glycyrrhiza uralensis*	-10	-30
Artemisia keiskeana	-36	8	*Glycyrrhiza uralensis (seed)*	-28	-22
Artemisia melini	-32	23	*Gondrea*	-36	-8
Artemisia princeps	-13	-55	*Gossypium indicum (leaves)*	-67	-75
Aruncus dioicus	-27	13	*Gymnaster koraiensis*	1	-39
Asparagus cochinchinensis	21	-37	*Gynostemma pentaphyllum*	-8	-32
Asparagus oligoclonos	7	-29	*Hemerocallis fulva*	-11	-41
Asparagus oligoclonos Max	-3	-91	*Hemerocallis fulva var.*	-17	-77
Aster scaber	-28	-50	*Hemerocallis fulva (root)*	0	-29
Aster tataricus	3	-62	*Hemerocallis lilioasphodelus*	-10	-49
Astragalus membranaceus	-5	-41	*Hemerocallis lilioasphodelus (root)*	17	-21
Atractylodes japonica	-25	-27	*Hibiscus manihot*	-45	-44
Belamcanda chinensis	-108	-63	*Hibiscus manihot (flowers)*	-2	-76
Campsis grandiflora (leaves)	-34	49	*Hibiscus manihot (leaves)*	-12	-30
Canavalia gladiata	-23	-27	*Hibiscus manihot (frutes)*	1	-58
Canavalia gladiata (frutes)	10	-30	*Hibiscus manihot (leaves)*	-10	-19
Caragana sinica	-4	-34	*Hibiscus mutabilis*	-39	-111
Cassia tora	5	-30	*Hohyang*	-5	-48
Cassia tora (seed)	-29	-88	*Hosta lancifolia*	-5	-31
Cassiae Semen	-10	-30	*Hosta lancifolia (leaves)*	-6	-64
Cedrela sinensis (leaves)	-23	5	*Houttuynia cordata*	-15	-70
Chelidonium majus	-23	-6	*Impatiens textori*	-30	-35
Chloranthus japonicus	-11	-51	*Inula britannica*	-29	0
Chrysanthemum boreale	-26	12	*Inula helenium*	-60	-49
Chrysanthemum cinerariae folium	-1	-59	*Inula helenium var.*	-24	-48
Chrysanthemum indicum	-26	-36	*Iris koreana Nakai*	-12	-6
Chrysanthemum zawadskii	-21	-54	*Iris nertschinskia*	-16	-20

Table 1. Continued

Scientific name	α-Glucosidase inhibitory activity (%)	α-Amylase inhibitory activity (%)	Scientific name	α-Glucosidase inhibitory activity (%)	α-Amylase inhibitory activity (%)
Iris pallassii (leaves)	-18	-29	*Polyganatum stenophyllum*	-45	-77
Ixeris dentate	-23	1	*Polyganatum stenophyllum (root)*	-16	-4
Ixeris dentata (whole)	-1	-8	*Potentilla discolor Bunge*	-8	-26
Kirinwon	-18	-70	*Pteridium aquilinum*	27	-21
Leonurus sibiricus	-29	-58	*Pulsatilla koreana*	-2	-9
Leonurus sibiricus (aerial)	-2	-9	*Rehmannia glutinosa*	-14	-22
Ligularia fischeri	-34	-18	*Reynoutria elliptica*	-58	22
Ligusticum chuanxiong	13	-36	*Reynoutria elliptica (leaves)*	-27	-2
Lilium leichtlinii	19	-88	*Reynoutria elliptica (root)*	-26	-22
Lilium tigrinum	-14	-36	*Rheum undvlatum*	-24	-37
Liriope platyphylla (leaves)	-39	-43	*Rhododendron mucronulatum*	-30	-4
Liriope platyphylla (whole)	6	-29	*Rolyganatum stenophyllum (root)*	-15	-45
Liriope platyphylla (seed)	14	4	*Rubus crataegiflius*	-7	-7
Lonicera japonica (seed)	-5	-7	*Rumex acetocella*	-17	-63
Lonicera japonica (aerial)	-2	26	*Rumex crispus*	-5	-59
Lotus corniculatus	9	10	*Ruta graveolens (leaves)*	-8	-18
Lycium chinense	-7	-125	*Salvia plebeia*	7	62
Lycium chinense (frutes)	-7	-78	*Sanguisorba officinalis (seed)*	-24	-22
Lycium chinense (leaves)	8	-128	*Saururus chinensis*	-3	14
Lysimachia barystachys	-42	-36	*Schizandra chinensis (leaves)*	-32	21
Lysimachia davurica	-18	-153	*Scilla chinensis*	-11	-19
Mannunchung (leaves)	-7	-17	*Scilla chinensis (seed)*	-24	-44
Menispermum dauricum	-1	-41	*Scutellaria baicalensis*	-45	-46
Mentha arvensis	-20	-9	*Sedum aizoon*	-17	-66
Menthae Herba	-13	-52	*Sedum kamtschaticum*	-7	-40
Metaplexis japonica	-16	-13	*Sedum sarmentosum*	-28	-48
Morus bombysis	69	-40	*Sedum spectrabile*	-38	-16
Oenothera odorata	-52	-49	*Selaginella tamariscina*	-11	-74
Osmunda japonica	-14	-66	*Selaginella tamariscina (whole)*	-12	1
Ostericum koreanum	-32	21	*Sognamool*	16	-2
Paeonia suffruticosa	-15	20	*Sophora flavescens*	-26	-18
Paeonia suffruticosa (flower)	-32	29	*Symphytum officinale*	6	-79
Patrinia scabiosaefolia	4	-37	*Symphytum officinale (root)*	-30	-74
Patrinia villosa	-18	-1	*Syneilesis palmata*	-40	-41
Perilla frutescens	-13	-37	*Taraxacum platycarpum*	-18	-37
Persicaria filiforme	-41	14	*Trachelospermum asiaticum*	-8	6
Petasites japonicus	-28	-44	*Veronica rotunda*	-24	-31
Peucedanum japonicum	-19	-44	*Viola mandshurica*	-7	-66
Peucedanum japonicum (leaves)	-7	-26	*Viola mandshurica (aerial)*	-6	-115
Physalis alkekengi	2	-20	*Vitis coignetiae Pulliat*	-36	8
Physalis alkekengi (leaves)	-46	8	*Washabia japonica*	-2	-59
Phytolacca americana	1	-9	*Washabia japonica (leaves)*	-12	-41
Pimpinella brachycarpa	-20	-89	*Xanthium strumarium*	-9	-86
Plantago asiatica	5	-44	*Yacoon (leaves)*	-10	-35
Plantago asiatica (aerial)	-10	-36	*Youngchunwha*	-2	-30
Plantago asiatica (seed)	-8	-70	*Zanthoxylum schinifolium*	-15	-55
Platycodon grandiflorum	-16	26	*Zanthoxylum schinifolium (leaves)*	-39	7
Pleuropterus multiflorus	-3	38	*Zingiber mioga*	-16	-85
Pleuropterus multiflorus (leaves)	-14	40	*Zizyphus jujuba*	4	0

have potential to inhibit carbohydrate-digesting enzymes such as α-glucosidase and amylase. Some species of *Adenophora* and *Morus* inhibited α-glucosidase significantly, consistent with the previous study (10). It has been reported that the active components of these herbs were 1-deoxynojirimycin and other polyhydroxylated alkaloids such as (2*R*, 3*R*, 4*R*)-2-hydroxymethyl-3,4-dihydroxypyrrolidine-*N*-propionamide and 4-*O*-α-D-galactopyranosylcalystegine B2 (10).

It is generally accepted that α-amylase inhibitor has an advantage over α-glucosidase inhibitor due to its relatively low side effects such as flatulence and diarrhea (2). In fact, our study showed that Acarbose was a very potent α-amylase inhibitor while it is better known as a α-glu-

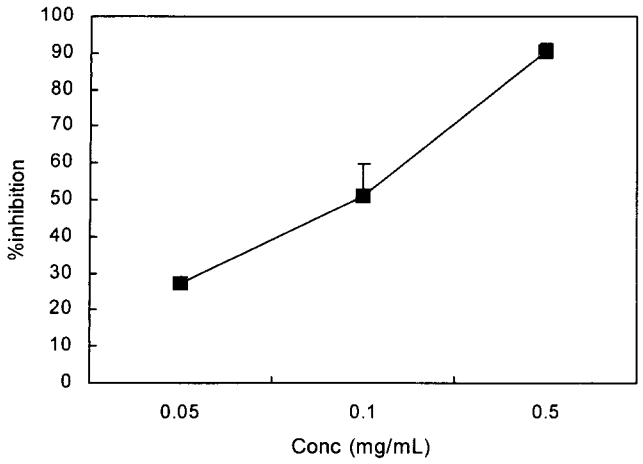

Fig. 1. Dose-dependent inhibition of porcine pancreatic α-amylase activity by *Epimedium koreanum* extract.

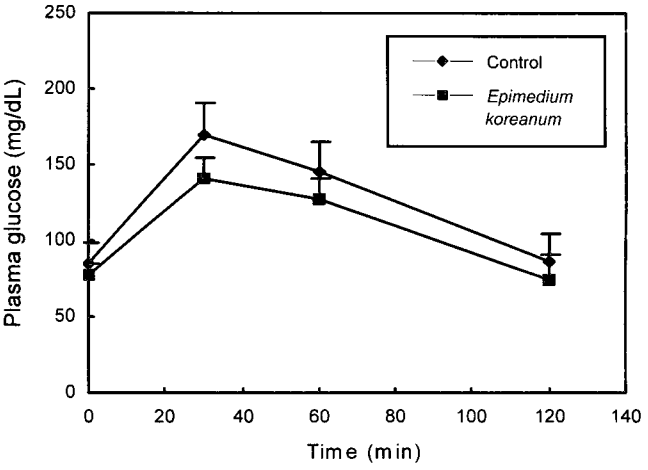

Fig. 2. Effect of *Epimedium koreanum* extract on plasma glucose level in starch-loaded ICR mice. Mice were intubated with 0.9 g soluble starch per kg body weight dissolved in water, followed by collecting blood from tail and measuring plasma glucose as described in Material and Methods. Mean ± SD (n=8). *p<0.05

cosidase inhibitor (data not shown). Therefore, this study focused on the medicinal herbs with a strong inhibitory activity against α-amylase. α-Amylase inhibitor is known to prevent postprandial hyperglycemia due to its ability to inhibit the conversion of starch into to maltose and its absorption in the small intestine (11). It is also expected to exert anti-obesity action as it lowers the utilization of starch, a major energy nutrient present in typical Korean diet. Some α-glycosidase inhibitors such as Acarbose have been proven to be effective in preventing obesity (8,12).

We found that the methanol extract of *Epimedium koreanum* effectively suppressed the α-amylase and improved hyperglycemia induced by oral starch load using both *in vitro* and *in vivo* studies. Therefore *Epimedium koreanum* extract deserves further clinical study for its development as a dietary supplement for obese and diabetic people.

ACKNOWLEDGEMENTS

This study has been supported by Agricultural Research Promotion Center, Ministry of Agriculture and Forestry (2001).

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(Received December 10, 2001; Accepted February 9, 2002)