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Rat Lens Aldose Reductase Inhibitory Activities of Leguminous Seed Extracts

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Abstract

The methanol extracts of 25 leguminous seeds *in vitro* were evaluated for inhibitory activities against lens aldose reductase of Sprague Dawley male rats. The responses varied with both leguminous seed and concentration used. At the concentration of 0.1 mg/mL, the methanol extracts from *Amphicarpaea edgeworthii*, *Canavalia lineata*, *Glycine max* var. *solitae*, *Glycine max* var. *yagkong*, *Glycine max* var. *hooktae*, *Glycine max* var. *bangkong*, *Glycine max* var. *geumdu*, *Glycine max* var. *chungtae*, *Glycine max* var. *mejukong*, *Glycine soja*, *Phaseolus radiatus* var. *geodu*, *Vicia tetrasperma*, *Vigna angulasis*, and *Vigna sinensis* inhibited enzyme activity by greater than 60%. In following study, at the concentration of 0.01 mg/mL, the extracts of *C. lineata* and *V. tetrasperma* had relatively strong inhibitory activity against aldose reductase. Because of their potent inhibitory activities, the activity of each solvent fraction from *C. lineata* and *V. tetrasperma* was determined, and the potent activity was showed from chloroform and hexane fractions, respectively. IC_{50} values of *C. lineata* and *V. tetrasperma* were 0.004 and 0.006 mg/mL, respectively. As a naturally occurring therapeutic agent, leguminous seeds described could be useful for developing new agents of antidiabetic complications.

Key words: legume, lens aldose reductase, *Canavalia lineata*, *Vicia tetrasperma*

INTRODUCTION

Aldose reductase (EC 1. 1. 1. 21) catalyzes the reduction of glucose to the corresponding sugar alcohol, sorbitol. Sorbitol is subsequently metabolized to fructose by sorbitol dehydrogenase. The conversion of glucose to fructose by this means constitutes the polyol pathway of glucose metabolism. Under normal physiological conditions, this pathway plays a minor role in glucose metabolism in most tissue. In hyperglycemia associated with diabetes, however, cells undergoing insulin-independent uptake of glucose produce significant quantities of sorbitol. These cells accumulate sorbitol because of its poor penetration through cellular membranes and its slow metabolism by sorbitol dehydrogenase. The resulting hyperosmotic stress to cells is postulated to be the primary cause for the development of such diabetic complications as retinopathy, cataracts, neuropathy and nephropathy (1). These observations suggest that the inhibition of aldose reductase would represent a novel, potentially direct pharmacological approach toward the treatment of certain diabetic complications (2).

Plants constitute a rich source of bioactive chemicals (3,4). Many plants and crude drugs have recently been tested for their effects on aldose reductase (5-9). 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 flavonone glucosides as well as sugar related components are found to be effective on the inhibitory activities of aldose reductase (10-12). Therefore, much effort has been focused on the plants for potentially useful products as commercial antidiabetic agents or as lead compounds. However, relatively little work has been done on the aldose reductase inhibitory activities of leguminous seed extracts compared to food (13,14) and plant origins (15,16) in spite of their excellent nutritional, pharmacological and industrial significances (17-20).

To find naturally occurring substances which could prevent and treat diabetic complications, the extracts prepared from 25 leguminous seeds were examined for their inhibitory effects on lens aldose reductase activity.

MATERIALS AND METHODS

Chemicals

Bovine serum albumin, DL-glyceraldehyde, imidazole, lithium sulfate, NADPH, and phenylmethylsolfonyl fluoride were purchased from Sigma Chemical (St. Louis, MO, USA). Sprague Dawley male rats were purchased from Dae Han Labo-

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ratory Animal Research Center Co. (Umsung, Cungbuk, 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 two times with 500 mL 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 aldose reductase from Sprague Dawley rat lenses

Crude aldose reductase was prepared from rat lenses. Lenses were removed from the eyes of 8-week-old Sprague-Dawley male rats (Dae Han Laboratory Animal Research Center Co., Umsung, Chungbuk, Korea) weighing 100~150 g and homogenized in 12 volumes of a 135 mM Na, K-phosphate buffer (pH 7.0) containing 0.5 mM phenylmethylsolfonyl fluoride and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 100,000×g for 30 min, and the resulting supernatant was retained as an enzyme preparation. All procedures were carried out at 4°C. The protein content of the enzyme preparation was determined by Biuret method (21), using bovine serum albumin as a standard. The activity of this preparation was determined by measuring the amount of NADP converted from NADPH per unit time at 37°C and pH 7.0. One unit (U) of activity is defined as the amount of the enzyme catalyzing the oxidation of 1 μmol of NADPH per minute under the above experimental conditions.

Enzyme inhibitory assay

Aldose reductase activity was assayed according to the method described by Dufrane et al. (22) with slight modifications. The incubation mixture contained a 135 mM Na, K-phosphate buffer (pH 7.0), 100 mM lithium sulfate, 0.03 mM NADPH, 0.04 mM DL-glyceraldehyde and 50 μL of an enzyme preparation, with or without a plant extract, in a total volume of 1.0 mL. Each plant extract was dissolved in dimethyl sulfoxide, which was found to have no effect on the enzyme activity at less than a 1% concentration. Appropriate blanks contained all of the above-mentioned compounds, except DL-glyceraldehyde. The reaction was initiated by adding NADPH at 37°C and stopped by adding 0.3 mL of 0.5 N hydrochloric acid. Then, 1 mL of 6 N NaOH containing 10 mM imidazole was added, and the mixture incubated at 60°C for 10 min to convert NADP to a fluorescent product. The fluorescence was measured at room temperature with a spectrofluorophotometer (Aminco Bowman series 2, Spectronic Instruments, Rochester, NY, USA) with an excitation wavelength of 360 nm and an emission wavelength of 460 nm. Standards of NADP (0.1~5 μM) were treated in the same manner. All determinations were performed in triplicate. The concentration of each test sample giving 50% inhibition of the enzyme activity (IC_{50}) was estimated from the least-squares regression line of the logar-

Table 1. List of leguminous plants tested

Scientific name	Characteristics				
	Seed colour	Flower colour	Size (cm)	Shape	Yield[1] (%)
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. *woolitalkong*	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

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

ithmic concentration plotted against the remaining activity. The protein content of the enzyme preparation was 0.023 g/L, and the aldose reductase activity in the preparation was 7.41 U/L or 373.4 U/g of protein at 37°C.

RESULTS AND DISCUSSION

The methanol extracts of 25 leguminous seeds and potent aldose reductase inhibitors for the comparison, quercitrin and tolrestat, were determined for the inhibitory activity against lens aldose reductase isolated from Sprague Dawley male rats (Table 2). The inhibition at a final concentration of 0.100 mg/μL was determined for each of the extracts prepared. This concentration for most of the plant extracts was the highest used in the assay mixture for two reasons: one was the limited solubility of the extracts in dimethyl sulfoxide, and the other was the limited amount of dimethyl sulfoxide that could be added to the assay mixture without affecting the enzyme activity. The responses varied with both leguminous seed and concentration used. The inhibitory effect was determined as the concentration of a plant extract was decreased step by step: for those extracts whose inhibition was above 60% at 0.1 mg/mL, the inhibition was again measured at a concentration of 0.05 mg/mL (Table 2); for those whose inhibition was above 60% at 0.05 mg/mL, the inhibition was measured at 0.01 mg/mL (Fig. 1); finally, for those whose inhibition was above 80% at 0.010 mg/mL, the inhibition was tested at 0.005 and 0.001 mg/mL (Table 3). Only those samples which exerted significant inhibition at 0.001 mg/mL were evaluated for their IC_{50} values.

Table 2. Aldose reductase inhibitory activities of methanol extracts of leguminous seeds

Sample tested	Final conc. (mg/mL)	Inhibition (%)
A. edgeworthii	0.1	74
	0.05	61
A. hypogaea	0.1	32
C. lineata	0.1	98
	0.05	94
C. obtusifolia	0.1	44
D. villosa	0.1	13
G. max var. *solitae*	0.1	62
	0.05	34
G. max var. *yagkong*	0.1	86
	0.05	68
G. max var. *hooktae*	0.1	74
	0 05	30
G. max var. *bangkong*	0.1	60
	0.05	25
G. max var. *geumdu*	0.1	85
	0.05	72
G. max var. *chungtae*	0.1	65
	0.05	37
G. max var. *wooltalikong*	0.1	54
G. max var. *mejukong*	0.1	62
	0.05	21
G. soja	0.1	61
	0 05	20
L. japonica	0.1	34
P. multiflorus	0.1	21
P. nipponensis	0.1	38
P. radiatus var. *geodu*	0.1	84
	0.05	72
P. radiatus var. *aurea*	0.1	25
P. sativum	0.1	49
R. volubilis	0.1	16
V. hirsuta	0.1	39
V. tetrasperma	0.1	97
	0.05	94
V. angulasis	0.1	61
	0.05	32
V. sinensis	0.1	90
	0.05	74
Quercitrin	0.1	98
	0.05	92
Tolrestat	0.1	97
	0.05	95

The methanol extracts of leguminous seeds exhibited inhibitory activity against aldose ranging from 13 to 98% at a concentration of 0.1 mg/mL. At a concentration of 0.05 mg/mL the methanol extracts showed inhibitory activity ranging from 20 to 94% (Table 2). For tests with a concentration of 0.1 mg/mL, the methanol extracts from *Amphicarpaea edgeworthii*, *Canavalia lineata*, *Glycine max* var. *solitae*, *Glycine max* var. *yagkong*, *Glycine max* var. *hooktae*,

Table 3. Aldose reductase inhibitory activities of solvent fractions of methanol extracts from *C. lineata* and *V. tetrasperma*

Legume / Fraction	Final conc. (mg/mL)	Inhibition (%)	IC_{50}[1] (mg/mL)
C. lineata	0.1	15	
Hexane	0.1	98	
Chloroform	0.05	94	
	0.01	83	
	0.005	55	
	0.001	28	0.004
	0.1	8	
Ethyl acetate	0.1	0	
Butanol	0.1	0	
Water			
V. tetrasperma	0.1	97	
Hexane	0.05	94	
	0.01	85	
	0.005	45	0.006
Chloroform	0.1	0	
Ethyl acetate	0.1	0	
Butanol	0.1	0	
Water	0.1	0	
Quercitrin	0.1	98	
	0.05	92	
	0.01	90	
	0.005	83	
	0.0001	34	0.0004
Tolrestat	0.1	97	
	0.05	95	
	0.01	91	
	0.005	85	
	0.0001	80	0.00001

[1]IC_{50}: 50% Inhibition

Glycine max var. *bangkong*, *Glycine max* var. *geumdu*, *Glycine max* var. *chungtae*, *Glycine max* var. *mejukong*, *Glycine soja*, *Phaseolus radiatus* var. *geodu*, *Vicia tetrasperma*, *Vigna angulasis*, and *Vigna sinensis* revealed strong inhibitory activity over 60% inhibition, whereas moderate activity (50% <inhibition<60%) was obtained in the extract of *Glycine max* var. *wooltalikong* (Table 2). Among 25 samples the methanol extracts of *C. lineata* (98%), *V. tetrasperma* (97%), and *V. sinensis* (90%) exhibited highly effective inhibition against aldose reductase. For tests with a concentration of 0.05 mg/mL, over 60% inhibitory activity showed in the methanol extracts of *A. edgeworthii*, *C. lineata*, *G. max* var. *yagkong*, *G. max* var. *geumdu*, *P. radiatus* var. *geodu*, *V. tetrasperma*, and *V. sinensis*. Because of their potent inhibitory activity against aldose reductase, the activity of 7 extracts selected from 25 leguminous seeds was evaluated at the concentration of 0.01 mg/mL (Fig. 1). In this study, the extracts of *C. lineata* and *V. tetrasperma* exhibited effective inhibition (>80% inhibition). However, the other leguminous seeds exhibited weak or no inhibitory activity against aldose reductase (<50% inhibition). In this study, it can be expected that there would be differences in the secondary metabolites produced from various leguminous seeds.

In a further study, the activity of each solvent fraction from the extracts of *C. lineata* and *V. tetrasperma* was evaluated (Table 3). The chloroform fraction from the extract of *C. lineata* showed potent inhibitory activity, whereas the other fractions exhibited little or no inhibitory activity against lens aldose reductase. In the fractionation of the methanol extract from *V. tetrasperma*, strong inhibitory activity was observed in the hexane fraction, whereas inhibitory activity was not detected in other fractions. IC_{50} values of *C. lineata* and *V. tetrasperma* are 0.004 and 0.006 mg/mL, respectively (Table 3). On the basis of their potent inhibitory activity, IC_{50} values of *C. lineata* and *V. tetrasperma* were compared to aldose reductase inhibitors, quercitrin and tolrestat. In this study, even though the IC_{50} value of *C. lineata* and *V. tetrasperma* against lens aldose reductase was approximately 10 times lower than the value of quercitrin, these crude extracts include not only active substances but also non-active components. Therefore, the inhibitory activity of two crude extracts against lens aldose reductase could be improved by purification.

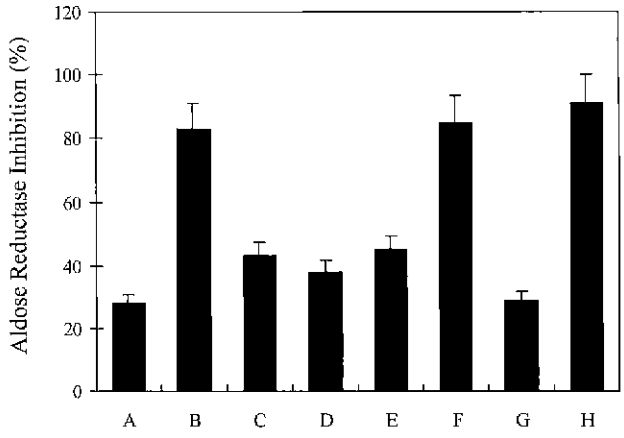

Fig. 1. Aldose reductase inhibitory activities of methanol extracts of leguminous seeds at a concentration of 0.01 mg/mL.
A, *A. edgeworthii*; B, *C. lineata*; C, *G. max* var. *yagkong*; D, *G. max* var. *geumdu*; E, *P. radiatus* var. *geodu*; F, *V. tetrasperma*; G, *V. sinensis*; H, Quercitrin

It has been well acknowledged that plant-derived extracts and phytochemicals are potential alternatives to synthetic inhibitors against aldose reductase (6-10). Currently, the compounds isolated from plants as aldose reductase inhibitors are classified in flavonoids and flavonoid-related compounds. These include 5,7,4'-trihydroxy-3,6-dimethoxyflavone isolated from *Acanthospermum australe* (8), myricetin 3-O-(4″ - acetyl)-fucoside from *Anthocepharus chinensis* (23), and astilbin, neoastilbin (dihydroflavonol rhamnosides) and quercitrin (quercitrin 3-rhamnoside) from *Engelhardtia chrysolepis* (24). In this study, although the active principles of *C. lineata* and *V. tetrasperma* seeds as aldose reductase inhibitor remain unknown at present, leguminous seed-derived isoflavones, glycopeptide, and aglucones exhibit antitumorigenesis and pharmacological functions (25,26). It might be expected that the active components of these seeds would be flavonoids or flavonoid-related compounds, suggesting an indication of at least one of their pharmacological actions.

Aldose reductase inhibitors including quercitrin and tolrestat are currently the most commonly used oral agents for good penetration of sorbitol through cellular membranes and fast metabolism of sorbitol by sorbitol dehydrogenase. And they are considered more importantly as the therapeutic prospect of patient treatment associated with diabetic complications as retinopathy, cataracts, neuropathy and nephropathy (1). In conclusion, the strong inhibitory activity of leguminous seeds tested confirms their superiority and usefulness on antidiabetic agents, although *in vivo* efficacy and the clinical usefulness of them remain to be evaluated. Additionally, the isolation and characterization of the components against lens aldose reductase are in progress.

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