Natural Product Sciences
16(4) : 285-290 (2010)

Inhibition of Aldose Reductase from Rat Lenses by Methanol Extracts from Korean Folk Plants

Hye Min Kim[1], So-Youn Mok[1], Jeong Min Lee[1], Eun Ju Cho[2], Kyung Choi[3], Jajung Ku[3], Kwang-Woo Park[3], and Sanghyun Lee[1,*]

[1]*Department of Applied Plant Science, Chung-Ang University, Anseong 456-756, Korea*
[2]*Department of Food Science and Nutrition, Pusan National University, Busan 609-735, Korea*
[3]*Department of Forest Resource Conservation, Korea National Arboretum, Pocheon 487-821, Korea*

Abstract – The inhibition of rat lens aldose reductase (AR) *in vitro* using methanol extracts from Korean folk plants was investigated. Among them, the extracts of *Saussurea grandifolia* and *Rumex crispus* showed highest inhibition of AR. IC_{50} values of the extracts from *S. grandifolia* and *R. crispus* were demonstrated 0.07 and 0.05 mg/ml, respectively. Korean folk plants such as *S. grandifolia* and *R. crispus* has a possibility of new natural resources for the inhibition of AR.
Keywords – Korean folk plant, rat lens aldose reductase, diabetic complications

Introduction

Aldose reductase (AR) is a rate limiting enzyme in the polyol pathway associated with the conversion of glucose to sorbitol. This reaction is vital for the function of various organs in the body and for the cataract formation in the lens (Van Heyningen, 1959). The enzyme is located in the eye (cornea, retina, and lens), kidney, myelin sheath, and also in other tissues less involved in the pathogenesis of diabetic complications such as neuropathy (Ward, 1973), nephropathy (Beyer-Mears *et al*., 1984; 1985), and retinopathy (Engerman and Kern, 1984). AR inhibitors can prevent or reverse early abnormalities in diabetic complications. The AR inhibitors such as zopolrestat, ponalrestat, sorbinil, tolrestat, epalretat, and ranireatat have been developed with promising results in the past years (Constantino *et al*., 1999; Sun *et al*., 2006; Drel *et al*., 2008; Hotta *et al*., 2006; Matsumoto *et al*., 2008). These AR inhibitors, however, have several problems such as side effects and limited efficacy (Ziegler, 2004; Chalk *et al*., 2007). Therefore, recently natural sources for AR inhibitors are spotlight for the treatment and prevention of diabetic complications due to safer and more effective phytochemicals (De la Fuente and Manzanaro, 2003; Kawanishi *et al*., 2003).

Folk plants are traditional plants widely known among the people in daily life for food, disease remedy, and personal preference for a long time. The use of traditional medicine has been expanded globally and gaining popularity during last decade. The World Health Organization (WHO) reported that the herbal medicine serve the health need about 80% of the World's population in 2001. Herbal medicines for disease remedy are an important part of the culture and traditions of Korean people. Today, most of the population in Korea is reliant on herbal medicines for their health care needs. Ethnobotanical investigation has revealed that *ca.* 700 species, out of a total of about 4,596 species identified in this territory, are used for different purposes related to local traditions (Kim, 2004). Therefore, there is a large scope for evaluations of naturally occurring AR inhibitors from Korean folk plants. In the present paper, as a preliminary step for the evaluations of potential of naturally occurring AR inhibitors, we tested the effects of Korean folk plants on rat lens AR inhibition.

Experimental

Plant materials – Korean folk plants were collected at Andong, Ansung, Kwangneung, Bonghyeon, Bonghwa, Gyeongju, Ilsung, Janghyun, Jeju, Kimcheon, Ulleung, Wangsukcheon, Yeoju, and Yongmun Station of KNA Useful Plant Resources Research Center (2010) by Korea National Arboretum (KNA), Korea.

*Author for correspondence
Tel: +82-31-670-4688; E-mail: slee@cau.ac.kr

General instruments and Reagents – Fluorescence was measured with a Hitachi U-3210 spectrophotometer. Solvents such as DL-glyceraldehyde, β-NADPH, sodium phosphate buffer, potassium phosphate buffer, and DMSO (Sigma-Aldrich Chemical Co.) were used for rat lens AR assay.

Extraction and Sample preparation – The samples were sliced and dried in an oven at 45 °C until use. Each three gram of dried, sliced Korean folk plant materials were extracted with methanol (300 ml) under reflux for 3 h. After filtration, the filtrates were concentrated *in vacuo* to produce methanol extracts. Each sample of MeOH extract 1.0 mg was dissolved in 1 ml DMSO solvent.

Measurement of AR activity – Rat lenses were removed from Sprague-Dawley rats (weighing 250 - 280 g) and preserved rat lenses by freezing it until use. These were homogenized and centrifuged at 10,000 rpm (4 °C, 20 min) and the supernatant was used as an enzyme source. AR activity was spectrophotometrically determined by measuring the decrease in absorption of NADPH at 340 nm for a 4 min period at room temperature with DL-glyceraldehydes as a substrate. The assay mixture contained 0.1 M potassium phosphate buffer (pH 7.0), 0.1 M sodium phosphate buffer (pH 6.2), 1.6 mM NADPH, and test extract sample (in DMSO) with 0.025 M DL-glyceraldehyde as substrate in quartz cell. IC_{50} values, the concentration of inhibitors giving 50% inhibition of enzyme activity, were calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity. Quercetin known as one of typical AR inhibitors was used as a positive control.

Table 1. Rat lens AR inhibitory activity of the extracts from Korean folk plants

Samples	Part used	Collection	Inhibition (%)
Acanthopanax sessiliflorus	leaf, branch	Kwangneung	54.2
Actinidia arguta	leaf	Bonghwa	14.1
Adenophora triphylla	root	Yeoju	29.5
Adriana acerifolia	leaf, branch	Yongmun*	83.2
Allium macrostemon	whole plant	Ulleung	70.0
A. victorialis	whole plant	Ulleung	1.5
Alopecurus aequalis	leaf, branch	Jeju	17.9
Anthriscus sylvestris	whole plant	Ulleung	17.1
Aralia cordata	leaf, stem	Ulleung	25.8
Aruncus dioicus var. *kamtschaticus*	leaf, stem	Ulleung	31.1
Aster glehni	leaf	Ulleung	64.3
A. tataricus	flower	Yongmun*	51.1
A. tataricus	leaf, branch	Yongmun*	49.4
Berberis amurensis var. *latifolia*	leaf, stem	Ulleung	33.1
Capsella bursa-pastoris	whole plant	Yongmun*	13.3
Cardamine leucantha	whole plant	Kwangneung	5.1
Cedrela sinensis	leaf	Ansung	75.9
Cercidiphyllum japonicum	leaf	Kwangneung	63.8
Chelidonium majus var. *asiaticum*	whole plant	Bonghyeon	35.7
Chrysanthemum boreale	leaf, branch	Yongmun*	63.4
C. indicum	flower	Yongmun*	54.4
C. indicum	leaf, branch	Yongmun*	31.7
C. pallasianum	leaf, branch	Yongmun*	75.0
C. zawadskii var.*latilobum*	leaf, branch	Yongmun*	31.6
Cirsium nipponicum	whole plant	Ulleung	18.4
Citrus unshui	leaf, stem	Jeju	23.9
Clerodendron trichotomum	leaf	Kwangneung	11.8
C. trichotomum	branch	Kwangneung	64.9
Cryptotaenia japonica	whole plant	KNA Station	27.6
Dracocephalum argunense	leaf, branch	Yongmun*	18.8
Dystaenia takesimana	leaf, stem	Ulleung	45.5
Elaeagnus macrophylla	leaf	Ulleung	9.7
E. umbellata	leaf	Kwangneung	31.8
E. umbellata	branch	Kwangneung	12.0
Equisetum arvense	leaf	Bonghyeon	28.7
Erigeron annuus	whole plant	Kwangneung	11.5
Euonymus alatus for. *striatus*	leaf, branch	Andong	46.2
Ficus lyrata	leaf	Kwangneung	12.5
Fragaria ananassa	whole plant	Yongmun*	34.4
Gypsophila oldhamiana	leaf, branch	Yongmun*	13.4

Table 1. continued

Samples	Part used	Collection	Inhibition (%)
Hedera rhombea	leaf, branch	Ulleung	31.1
Hibiscus hamabo	leaf	Yongmun*	16.8
Hovenia dulcis	branch	Yongmun*	47.0
Humulus scandens	leaf, stem	Kwangneung	40.7
Ixeris chinensis	whole plant	Pohang	50.9
I. chinensis var. *strigosa*	leaf, stem	Yongmun	26.8
I. chinensis var. *strigosa*	whole plant	Yongmun	16.4
Kalopanax pictus	leaf	Kwangneung	58.1
K. pictus	branch	Kwangneung	57.7
Lactuca indica var. *laciniata*	whole plant	Yongmun	29.2
Lamium amplexicaule	whole plant	Yongmun	27.8
Lespedeza cuneata	leaf, fruit	Kimcheon	52.2
L. cuneata	branch	Kimcheon	14.5
Ligustrum obtusifolium	leaf, stem	Pohang	34.8
Lindera obtusiloba	leaf	Bonghwa	58.6
Lonicera maackii	leaf	Gyeongju	79.2
Lycium chinense	leaf	Andong	7.0
Machilus thunbergii	branch	Yongmun*	2.7
Meehania urticifolia	leaf	Ulleung	22.7
Mentha sp.	leaf, branch	Yongmun*	12.9
Morus alba	leaf	Ilsung	54.0
Nepeta cataria	leaf, branch	Yongmun*	32.1
Oenothera odorata	leaf	Bonghwa	12.1
Petasites japonicus	leaf	Ulleung	59.9
Pimpinella brachycarpa	leaf, stem	KNA station	81.0
Pterocarya stenoptera	leaf	Kwangneung	71.0
Quercus variabilis	leaf	Bonghwa	45.8
Reynoutria sachalinensis	leaf, stem	Ulleung	15.1
Robinia pseudo-acacia	leaf, flower	Ansung	20.5
Rumex crispus	whole plant	Ulleung	47.5
R. crispus	stem	Kwangneung	39.3
R. crispus	rachis	Kwangneung	70.4
Sambucus sieboldiana var. *pendula*	leaf, stem	Ulleung	41.0
S. williamsii var. *coreana*	un-bloom flower	Janghyun	27.9
Saussurea grandifolia	whole plant	Ulleung	90.9
Sedum sarmentosum	whole plant	Kwangneung	66.0
Senecio vulgaris	whole plant	Pohang	42.0
Serratula coronata spp. *insularis*	leaf	Andong	71.4
Sophora japonica	testa	Yongmun*	37.5
Sorbus commixta	branch	Yongmun*	7.7
S. commixta	leaf	Ulleung	35.1
Staphylea bumalda	leaf, branch	Kwangneung	1.8
Syneilesis palmata	leaf	Kwangneung	15.2
Taraxacum officinale	whole plant	Pohang	33.9
Thymus quinquecostatus	leaf, branch	Yongmun*	54.0
Tilia mandshurica	leaf	Kwangneung	20.1
T. mandshurica	bract	Kwangneung	33.2
Trachelospermum asiaticum	leaf	Geoje	44.4
T. asiaticum	leaf	Yeosu	44.4
Tsuga sieboldii	leaf, branch	Ulleung	17.9
Ulmus davidiana var. *japonica*	leaf, stem	Jikdongri	29.4
Vitex negundo var. *incisa*	leaf, branch	Yongmun*	64.8
V. rotundifolia	leaf, branch	Yongmun*	52.1
Wasabia japonica	whole plant	Ulleung	1.5
Youngia japonica	leaf	Pohang	53.0
Zanthoxylum piperitum	leaf, stem	Ulleung	71.4
Z. schinifolium	branch	Wangsukcheon	51.9
Z. schinifolium	leaf	Kwangneung	10.6

Inhibition rate was calculated as percentage with respect to the control value.
* Yongmun Station of KNA Useful Plant Resources Research Center
KNA Station: Kwangneung Station of KNA

Results and Discussion

The methanol extracts from Korean folk plants were tested for their inhibitory effects on rat lens AR, and summarized in Table 1. Among Korean folk plants tested, the methanol extracts from *Acanthopanax sessiliflorus*, *Adriana acerifolia*, *Allium macrostemon*, *Aster glehni*, *A. tataricus*, *Cedrela sinensis*, *Cercidiphyllum japonicum*, *Chrysanthemum boreale*, *C. indicum*, *C. pallasianum*, *Clerodendron trichotomum*, *Kalopanax pictus*, *Lindera obtusiloba*, *Lonicera maackii*, *Morus alba*, *Petasites japonicus*, *Pimpinella brachycarpa*, *Pterocarya stenoptera*, *Rumex crispus*, *Saussurea grandifolia*, *Sedum sarmentosum*, *Serratula coronata* var. *insularis*, *Thymus quinquecostatus*, *Vitex negundo* var. *incisa*, *V. rotundifolia*, *Youngia japonica*, *Zanthoxylum piperitum*, and *Z. schinifolium* were demonstrated to show good inhibitory potencies on rat lens AR. Among them, the extracts of *A. acerifolia*, *A. macrostemon*, *C. sinensis*, *C. pallasianum*, *L. maackii*, *P. brachycarpa*, *P. stenoptera*, *R. crispus*, *S. grandifolia*, *S. coronata* var. *insularis*, and *Z. piperitum* were exhibited high degree of inhibition (> 70% at 1 mg/ml) on rat lens AR compared with other samples. The extract of *S. grandifolia* was particularly exhibited highest inhibitory value of 90.9% on rat lens AR.

To evaluate the rat lens AR inhibitory activity, their inhibitory percentage and IC_{50} values were calculated. Quercetin known as a very strong AR inhibitor (IC_{50} value, 0.19 mg/ml) was used as a positive control and the results were indicated in Table 2. The IC_{50} values of the extracts of *C. sinensis* leaf and *L. maackii* leaf were demonstrated 0.39 and 0.45 mg/ml, respectively. The leaf

Table 2. IC_{50} values of the extract from Korean folk plants on rat lens AR inhibition

Samples	Concentration (mg/ml)	Inhibition (%)	IC_{50} [a)] (mg/ml)
Acanthopanax sessiliflorus	1	54.2	0.85
	0.5	40.4	
	0.1	3.2	
Adriana acerifolia	1	83.2	0.62
	0.5	33.2	
	0.1	13.6	
Allium macrostemon	1	70.0	0.75
	0.5	23.2	
	0.1	21.0	
Aster glehni	1	64.3	0.70
	0.5	44.3	
	0.1	12.0	
A. tataricus	1	51.1	0.95
	0.5	30.6	
	0.1	6.0	
Cedrela sinensis	1	75.9	0.39
	0.5	65.2	
	0.1	30.5	
Cercidiphyllum japonicum	1	63.8	0.72
	0.5	42.1	
	0.1	11.9	
Chrysanthemum boreale	1	63.4	0.76
	0.5	37.4	
	0.1	9.6	
C. indicum	1	54.4	0.80
	0.5	48.4	
	0.1	0.9	
C. pallasianum	1	75.0	0.51
	0.5	59.5	
	0.1	19.4	
Clerodendron trichotomum	1	64.9	0.65
	0.5	49.1	
	0.1	17.1	
Kalopanax pictus (leaf)	1	58.1	0.89
	0.5	23.2	
	0.1	6.6	
K. pictus (branch)	1	57.7	0.78
	0.5	41.3	
	0.1	19.3	

Table 2. continued

Samples	Concentration (mg/ml)	Inhibition (%)	IC_{50} [a)] (mg/ml)
Lindera obtusiloba	1	58.6	0.82
	0.5	34.5	
	0.1	12.4	
Lonicera maackii	1	79.2	0.45
	0.5	56.0	
	0.1	28.2	
Morus alba	1	54.0	0.96
	0.5	23.3	
	0.1	12.4	
Petasites japonicus	1	59.9	0.78
	0.5	40.3	
	0.1	2.5	
Pimpinella brachycarpa	1	81.0	0.41
	0.5	62.6	
	0.1	28.3	
Pterocarya stenoptera	1	71.0	0.65
	0.5	33.1	
	0.1	29.9	
Rumex crispus	1	70.4	0.05
	0.5	65.7	
	0.1	48.3	
Saussurea grandifolia	1	90.9	0.07
	0.5	85.2	
	0.1	56.6	
	0.05	37.2	
Sedum sarmentosum	1	66.0	0.76
	0.5	33.6	
	0.1	1.6	
Serratula coronata spp. *insularis*	1	71.4	0.59
	0.5	53.7	
	0.1	14.2	
Thymus quinquecostatus	1	54.0	0.87
	0.5	36.0	
	0.1	0.9	
Vitex negundo var. *incisa*	1	64.8	0.56
	0.5	61.4	
	0.1	15.9	
V. rotundifolia	1	52.1	0.88
	0.5	40.6	
	0.1	15.4	
Youngia japonica	1	53.0	0.91
	0.5	32.7	
	0.1	2.8	
Zanthoxylum piperitum	1	71.4	0.59
	0.5	51.0	
	0.1	17.0	
Z. schinifolium	1	51.9	0.93
	0.5	39.4	
	0.1	31.8	
Quercetin*	0.5	73.3	0.19
	0.1	47.9	
	0.05	35.7	

[a)] IC_{50} value was calculated from the least-squares regression equations in the plot of the logarithm of at three graded concentrations *vs* % inhibition.
*Quercetin was used as a positive control.

and stem extracts from *P. brachycarpa* also had inhibitory activity with IC_{50} value 0.41 mg/ml. In particular, *R. crispus* and *S. grandifolia* extracts had predominant inhibitory activities with IC_{50} value 0.05 and 0.07 mg/ml, respectively, comparable to that of the positive control, quercetin.

R. crispus have been used as a folk medicine in the treatment of therapeutic agents of acute and chronic cutaneous diseases, cathartics, fever and jaundice (Lee *et al.*, 2007). Recently, the seed of *R. crispus* has known as a digestive, an anticancer agent and a remedy of acute hepatitis, among many traditional folk medicines (Lee *et al.*, 2007). The recent studies suggest that the butanol fraction of *R. crispus* Semen showed the highest activity in analgesic activity and the methanol extract of ripe fruits of *R. crispus* was determined for its antioxidant potential by assays for ferric-reducing antioxidant power, DPPH-free radical scavenging activity, and the influence on lipid peroxidation in liposomes (Lee *et al.*, 2007; Maksimović *et al.*, 2010). In addition, numerous phytochemicals including sterols, flavonoids, phemolic acids, and anthraquinones have been thus far isolated from this plant (Fan and Zhang, 2009; Başkan *et al.*, 2007; Wiese *et al.*, 1995). One of chwinamul, *S. grandifolia* is genus of the family Compositae and has traditionally been used as a vegetable in Korea to promote health (Ko and Jeon, 1983; Teixeira da Silva, 2004). Presently, analytical methods for simultaneous determination of caffeoylquinic acids in chwinamul were established (Nugroho *et al.*, 2009).

To the best of our knowledge, the rachis of *R. crispus* and whole plant of *S. grandifolia* were found to demonstrate high inhibitory activities on AR from *in vitro* data. Therefore, we suggest that Korean folk plants such as *R. crispus* and *S. grandifolia* has a possibility of new natural resources for the inhibition of AR. As a result, it can be used to study the preliminary data for new active substances. Further investigations on the bioactivity of constituents from *R. crispus* and *S. grandifolia* may prove the use of new medicinal plants for the prevention of diabetic complications.

Acknowledgement

This work was supported by grants from Scientific Research of Korea National Arboretum (KNA1-2-11, 10-2).

References

Başkan, S., Daut-Ozdemir, A., Günaydin, K., and Erim, F.B., Analysis of anthraquinones in *Rumex crispus* by micellar electrokinetic chromatography. *Talanta* **71**, 747-750 (2007).

Beyer-Mears, A. and Cruz, E., Reversal of diabetic cataract by sorbinil, an aldose reductase inhibitor. *Diabetes* **34**, 15-21 (1985).

Beyer-Mears, A., Ku, L., and Cohen, M., Glomerular polyol accumulation in diabetes and its prevention by oral sorbinil. *Diabetes* **33**, 604-607 (1984).

Chalk, C., Benstead, T.J., and Moore, F., Aldose reductase inhibitors for the treatment of diabetic polyneuropathy. *Cochrane Database Syst. Rev.* **4**, CD004572 (2007).

Constantino, L., Rastelli, G., Vianello, P., Cignarella, G., and Barlocco, D., Diabetes complications and their potential prevention: aldose reductase inhibition and other approaches. *Med. Res. Rev.* **19**, 3-23 (1999).

Drel, V.R., Pacher, P., Ali, T.K., Shin, J., Julius, U., El-Remessy, A.B., and Obrosova, I.G., Aldose reductase inhibitor fidarestat counteracts diabetes-associated cataract formation, retinal oxidative-nitrosative stress, glial activation, and apoptosis. *Int. J. Mol. Med.* **21**, 667-676 (2008).

De la Fuente, J.A. and Manzanaro, S., Aldose reductase inhibitors from natural sources. *Nat. Prod. Rep.* **20**, 243-251 (2003).

Engerman, R.L. and Kern, T.S., Experimental galactosemia produces diabetic-like retinopathy. *Diabetes* **33**, 97-100 (1984).

Fan, J.P. and Zhang, Z.L., Studies on the chemical constituents of *Rumex crispus*. *Zhong Yao Cai* **32**, 1836-1840 (2009).

Hotta, N., Akanuma, Y., Kawamori, R., Matsuoka, K., Oka, Y., Shichiri, M., Toyata, T., Nakashima, M., Yoshimura, I., Sakamoto, N., and Shigeta, Y., Long-term clinical effects of epalrestat, an aldose reductase inhibitor, on diabetic peripheral neuropathy. *Diabetes Care* **29**, 1538-1544 (2006).

Kawanishi, K., Ueda, H., and Moriyasu, M., Aldose reductase inhibitors from the nature. *Curr. Med. Chem.* **10**, 1353-1374 (2003).

Kim, M., Korean Endemic Plants, Solbook Press, Korea, 2004, p. 12-16.

Ko, K.S. and Jeon, U.S., Ferns, Fern-Allies and Seed-Bearing Plants of Korea, Iljinsa, Korea, 1983, p. 656-674.

Lee, S., Kim, D., Yim, D., and Lee S., Anti-inflammatory, analgesic and hepatoprotective effect of Semen of *Rumex crispus*. *Kor. J. Pharmacogn.* **38**, 334-338 (2007).

Maksimović, Z., Kovačević, N., Lakušić, B., and Cebović, T., Antioxidant activity of yellow dock (*Rumex crispus* L., Polygonaceae) fruit extract. *Phytother Res.* Published online in Wiley Inter Science (2010).

Matsumoto, T., Ono, Y., Kurono, M., Kuromiya, A., Nakamura, K., and Bril, V., Ranirestat (AS-3201), a potent aldose reductase inhibitor, reduces sorbitol levels and improves motor nerve conduction velocity in streptozotocin-diabetic rats. *J. Pharmacol. Sci.* **107**, 231-237 (2008).

Nugroho, A., Kim, K.H., Lee, K.R., Alam, M.B., Choi, J.S., Kim, W.B., and Park, H.J., Qualitative and quantitative determination of the caffeoylquinic acids on the Korean mountainous vegetables used for chwinamul and their peroxynitrite-scavenging effect. *Arch. Pharm. Res.* **35**, 1361-1367 (2009).

Sun, W., Oates, P.J., Coutcher, J.B., Gerhardinger, C., and Lorenzi, M., A selective aldose reductase inhibitor of a new structural class prevents or reverses early retinal abnormalities in experimental diabetic retinopathy. *Diabetes* **55**, 2757-2762 (2006).

Teixeira da Silva, J.M., Mining the essential oils of the Anthemideae. *African J. Biotechnol.* **3**, 706-720 (2004).

Van Heyningen, R., Formation of polyol by the lens of the rat with sugar cataract. *Nature* **184**, 194-196 (1959).

Ward, J.D., Advance in metabolic disorders (suppl. 2): The polyol pathway in the neuropathy of early diabetes, Academic press, New York, 1973, p. 425.

Wiese, B., Quiroga, O.E., Vigo, M.S., and Nolasco, S.M., Chemical composition of *Rumex crispus* L. seed. *JAOCS.* **72**, 1077-1078 (1995).

Ziegler, D., Polyneuropathy in the diabetic patient-updata on pathogenesis and management. *Nephrol. Dial. Transplant* **19**, 2170-2175 (2004).

Received October 13, 2010
Revised November 20, 2010
Accepted November 25, 2010