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Inhibition of Aldose Reductase on Rat Lens by Tartary Buckwheat

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Abstract – To evaluate the potential of naturally occurring aldose reductase (AR) inhibitors from food material, MeOH extract and stepwise polarity fractions from tartary buckwheat and two common Korean buckwheat cultivars, yangjul-maemil and daesan-maemil, were tested on AR inhibition in rat lens *in vitro*. The EtOAc fraction from tartary buckwheat exhibited good AR inhibitory activity (IC_{50} value, 8.19 μg/ml). A portion of the EtOAc fraction from tartary buckwheat led to the isolation of rutin by MeOH recrystalization. Rutin exhibited good AR inhibitory activity (IC_{50} value, 9.28 μM). These results suggest that tartary buckwheat could be a useful food material in the development of a novel AR inhibitory agent against diabetic complications.
Keywords – Aldose reductase, diabetic complications, rutin, tartary buckwheat

Introduction

Aldose reductase (AR) is a rate limiting enzyme in the polyol pathway associated with the conversion of glucose to sorbitol. The enzyme is located in the eye, kidney, myelin sheath, and other tissues that are less affected by diabetic complications (Narayanan, 1993). In a diabetic condition, sufficient glucose can enter the tissues, and the pathway operates to produce both sorbitol and fructose. These abnormal metabolic results have been reported to be responsible for diabetic complications such as cataracts, retinopathy, neuropathy, and nephropathy (Kato *et al*., 2009). Aldose reductase inhibitors (ARIs) can prevent or reverse early stage diabetic complications. Almost all ARIs developed in recent years have demonstrated undesired side effects during human clinical trials (Van Zandt *et al*., 2004). Therefore, evaluating natural sources of potential ARI may lead to the development of safer and more effective phytochemicals against diabetic complications (Yawadio *et al*., 2007). Several flavonoids such as quercetin and quercitrin have been reported to have inhibitory activity against AR (Mercader *et al*., 2008).

Buckwheat demonstrates biological activities that have cholesterol-lowering effects, anti-hypertension effects, and combat constipation and obesity (Cui *et al*., 2008; Li and Zhang, 2001). Tartary buckwheat (*Fagopyrum tataricum*) is a crop that is widely grown in Asia, including Nepal, India, Bhutan, and China (Wang and Campbell, 2004). Recently, tartary buckwheat has been gaining popularity as a functional food in Japan, where the grain is mainly processed for noodles, tea, and as a rice additive (Yuji *et al*., 2007). Phytochemical investigations of buckwheat have shown that tartary buckwheat contains flavonoids such as rutin, orientin, vitexin, quercetin, isovitexin, and isoorientin (Li and Zhang, 2001; Oomah and Mazza, 1996; Dubber *et al*., 2005; Joubert *et al*., 2004; Peng *et al*., 2005). Among these, rutin is a major compound in tartary buckwheat (Holasova *et al*., 2002). In addition, rutin contains various biological activities that are beneficial to human health (Koda *et al*., 2008).

The goal of this study is to take the preliminary step of evaluating the therapeutic potential of naturally occurring ARIs. In doing so, we tested the effects of tartary buckwheat and two common Korean buckwheat cultivars (yangjul-maemil and daesan-maemil) on AR inhibition in rat lenses.

Materials and Methods

Plant materials – Tartary buckwheat (*Fagopyrum tataricum*) and common Korean buckwheat cultivars (yangjul-maemil and daesan-maemil) were cultivated and collected at the Highland Agriculture Research Center, National Institute of Crop Science, RDA, Korea. A voucher specimen of tartary buckwheat (No. LEE 2010-02) was identified by Y. H. Yoon, Highland Agriculture

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Research Center, National Institute of Crop Science, Korea, and deposited at the Herbarium of Department of Integrative Plant Science, Chung-Ang University, Korea.

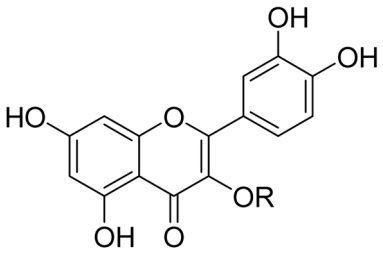
General instruments and reagents – Fluorescence was measured with a Hitachi U-3210 spectrophotometer. Solvents such as β-NADPH, sodium phosphate buffer, DL-glyceraldehyde potassium phosphate buffer, and DMSO (Sigma-Aldrich Chemical Co.) were used in the rat lens AR assay.

Fractionation and sample preparation – Tartary buckwheat, yangjul-maemil and daesan-maemil were extracted with MeOH under reflux (3 h × 5 times). Tartary buckwheat extract was suspended in distilled water and partitioned with *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH, successively. Each sample (1.0 mg) of MeOH extract, *n*-hexane, CH_2Cl_2, EtOAc, and *n*-BuOH fractions was dissolved in DMSO (1 ml).

Isolation and identification of rutin – Tartary buckwheat seeds (3 kg) were dried and finely powdered, then extracted with MeOH for 3 h (4 L × 8) under reflux at 65 °C - 75 °C. The solvent was evaporated *in vacuo* to produce the MeOH extract (147.2 g). This extract was suspended in distilled water and partitioned with *n*-hexane (14.9 g), CH_2Cl_2 (9.7 g), EtOAc (3.0 g), and *n*-BuOH (10.3 g), successively. Among them, compound **1** was isolated from the EtOAc fraction by recrystalization with MeOH.

Compound **1**: FAB-MS *m/z*: 611 $[M+H]^+$; ^{1}H-NMR (500 MHz, DMSO-d_6): δ 1.00 (3H, d, J = 6.0 Hz, Rha CH_3), 3.07-3.69 (12H, m, J = 2 Hz, sugar H), 4.38 (1H, s, Rha H-1), 5.34 (1H, d, J = 7.5 Hz, Glc H-1), 6.19 (1H, d, J = 1.2 Hz, H-6), 6.38 (1H, d, J = 1.2 Hz, H-8), 6.84 (1H, d, J = 8.5 Hz, H-5'), 7.54 (1H, d, J = 2.5 Hz, H-2'), 7.55 (1H, dd, J = 2.5, 8.5 Hz, H-6'), 12.58 (1H, s, 5-OH); ^{13}C-NMR (125 MHz, DMSO-d_6): δ 156.5 (C-2), 133.2 (C-3), 177.3 (C-4), 161.1 (C-5), 98.6 (C-6), 164.1 (C-7), 93.5 (C-8), 156.5 (C-9), 103.9 (C-10), 121.5 (C-1'), 115.2 (C-2'), 133.2 (C-3'), 148.4 (C-4'), 116.2 (C-5'), 121.1 (C-6'), 101.1 (Glc C-1), 74.0 (Glc C-2), 76.4 (Glc C-3), 70.5 (Glc C-4), 75.9 (Glc C-5), 67.0 (Glc C-6), 100.7 (Rha C-1), 70.3 (Rha C-2), 70.0 (Rha C-3), 72.0 (Rha C-4), 68.2 (Rha C-5), 17.7 (Rha C-6).

Measurement of AR activity – Lenses were removed from the eyes of Sprague-Dawley rats (weighing 250 - 280 g) and preserved by freezing 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 β-NADPH at 340 nm for a 4 min period at room temperature, with DL-glyceraldehydes as a substrate (Sato and Kador, 1990). 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 the test extract sample (in DMSO) with 0.025 M DL-glyceraldehyde as the substrate in the quartz cell. Total volume of assay mixture is 1 ml for the test. The IC_{50} values, which are the concentrations of the test sample that produced 50% inhibition of enzyme activity, were calculated from the least-squares regression line of the logarithmic concentrations plotted against the residual activity. Quercetin which is a known ARI was used as a positive control.



1, R = rutinose

2, R = glucose

3, R = galactose

4, R = H

Fig. 1. Structures of flavonoids used in this study.

Table 1. Rat lens AR inhibitory activity of MeOH extracts from buckwheat species

Sample	AR inhibition[a] (%)
Tartary Buckwheat	38.21
Yangjul-Maemil	33.23
Daesan-Maemil	29.01

Each sample concentration was 1 mg/ml DMSO.
[a] Inhibition rate was calculated as a percentage with respect to the control value.

Results and Discussion

The MeOH extracts of tartary buckwheat and common buckwheat cultivars (yangjul-maemil and daesan-maemil) were tested for their inhibitory effects on rat lens AR activity, and the results were summarized in Table 1. The AR inhibition of extracts from tartary buckwheat, yangjul-maemil and daesan-maemil in rat lens were 38.23, 33.23, and 29.01%, respectively. As shown in

Table 2. IC_{50} of the fractions of tartary buckwheat on AR in rat lens

Sample	Fraction tested	Concentration (μg/ml)	AR inhibition[a] (%)	IC_{50}[b] (μg/ml)
Tartary Buckwheat	*n*-hexane	10	16.78	–
	CH_2Cl_2	10	23.69	–
	EtOAc	10	57.36	8.19
		5	37.64	
		1	14.62	
	n-BuOH	10	36.47	–
		1	73.32	
Quercetin*	–	0.5	47.91	0.47
		0.1	35.68	

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

Table 3. IC_{50} of flavonoids on AR in rat lens

Compounds	Concentration (μg/ml)	AR inhibition[a] (%)	IC_{50}[b] (μM)
Rutin (**1**)	10	64.25	9.28
	5	46.01	
	1	9.19	
Isoquercitrin (**2**)[c]	5	78.60	1.79
	1	53.28	
	0.5	41.63	
Hyperin (**3**)[d]	1	62.20	1.12
	0.5	51.72	
	0.1	14.46	
Quercetin (**4**)*	1	61.10	1.56
	0.5	56.66	
	0.1	18.33	

[a] Inhibition rate was calculated as a percentage with respect to the control value.
[b] IC_{50} value was calculated from the least-squares regression equations in the plot of the logarithm at three graded concentrations *vs* % inhibition.
[c] Isoquercitrin from *Vaccinium koreanum* (Joo *et al.*, 1999)
[d] Hyperin from *Acanthopanax chiisanensis* (Lee *et al.*, 2004)
*Quercetin was used as a positive control.

Table 1, tartary buckwheat extract exhibited inhibitory potencies on rat lens AR compared to those of the other extracts. Thus, stepwise polarity fractions of the methanol extract from tartary buckwheat were investigated with an *in vitro* evaluation system using AR inhibitory activities. The EtOAc fraction of tartary buckwheat exhibited significant rat lens AR inhibition (IC_{50} value, 8.19 μg/ml). Quercetin, known as a very strong ARI in natural constituents, was used as the positive control. The results are indicated in Table 2.

Further fractionation of the EtOAc from tartary buckwheat led to the isolation of **1** by MeOH recrystalization. The typical flavonoid signals of **1** were observed in the ^{1}H- and ^{13}C-NMR spectra. The ^{1}H-NMR spectra revealed that it had an ABX system (H-2', -5' and -6'), as demonstrated by the coupling constant signal at δ 7.54 (d, H-2'), 7.55 (dd, H-6'), and 6.84 (d, H-5') in the B-ring structure. Compound **1** is a glycoside of the flavonoid. The ^{13}C-NMR of **1** can be correlated with the flavonoid moiety of quercetin with rutinose, with the latter comprising glucose and rhamnose units. The FAB-MS spectrum of **1** showed a quasimolecular ion peak at *m/z* 611, corresponding to a molecular formula of $C_{27}H_{30}O_{16}$. Accordingly, the structure of **1** was elucidated as rutin (5,7,3',4'-tetrahydroxyflavone-3-rutinose) (Yu *et al.*, 1996).

This compound was subjected to a test for AR inhibitory activity at three graded concentrations, and the results are shown in Table 3. Rutin (**1**) improves glucose homeostasis and recovery of retinal function, and may

consequently reduce the symptoms of diabetes (Kamalakkannan *et al.*, 2006). Flavonoids and phenol constituents have strong AR inhibitory activity (Collins *et al.*, 1977; De la Fuente and Manzanaro, 2003; Kawanishi *et al.*, 2003; Jung *et al.*, 2004; Lee *et al.*, 2008; Yawadio *et al.*, 2007). The AR inhibition of rutin (**1**) from tartary buckwheat was 64.25% at 1.0 mg/ml. Quercetin (**4**), a strong ARI (IC_{50} value, 1.56 μM) in natural constituents, was used as a positive control. The IC_{50} value of rutin (**1**) was 9.28 μM. In addition, flavonoid monoglycosides, such as isoquercitrin (**2**) and hyperin (**3**) showed stronger inhibitory activities than the flavonoid diglycoside, rutin (**1**). The IC_{50} values of isoquercitrin (**2**) and hyperin (**3**) were 1.79 and 1.12 μM, respectively.

In this paper, the EtOAc fraction of tartary buckwheat was found to demonstrate good inhibitory activities from *in vitro* data. It is postulated that rutin (**1**) from buckwheat can also be effective in preventing and/or retarding cataractogenic or diabetic complications. The results of the present study demonstrate that tartary buckwheat contains rutin (**1**) and has potential AR inhibitory effects for the preventive effect of diabetic complications.

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