Arch Pharm Res Vol 25, No 3, 306-312, 2002

Archives of Pharmacal Research
http://apr.psk.or.kr

Isoflavonoids from the Rhizomes of *Belamcanda chinensis* and Their Effects on Aldose Reductase and Sorbitol Accumulation in Streptozotocin Induced Diabetic Rat Tissues

Sang Hoon Jung, Yeon Sil Lee, Sanghyun Lee, Soon Sung Lim, Yeong Shik Kim, and Kuk Hyun Shin
Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea

(Received April 2, 2002)

Aldose reductase, the key enzyme of the polyol pathway, is known to play important roles in the diabetic complication. The inhibitors of aldose reductase, therefore, would be potential agents for the prevention of diabetic complications. To evaluate active principles for the inhibition of aldose reductase from the rhizomes of *Belamcanda chinensis*, twelve phenolic compounds were isolated and tested for their effects on rat lens aldose reductase. As a result, isoflavones such as tectorigenin, irigenin and their glucosides were found to show a strong aldose reductase inhibition. Tectoridin and tectorigenin, exhibited the highest aldose reductase inhibitory potency, their IC_{50} values, being 1.08×10^{-6} M and 1.12×10^{-6} M, respectively, for DL-glyceraldehyde as a substrate. Both compounds, when administered orally at 100 mg/kg for 10 consecutive days to streptozotocin-induced diabetic rats, caused a significant inhibition of sorbitol accumulation in the tissues such as lens, sciatic nerves and red blood cells. Tectorigenin showed a stronger inhibitory activity than tectoridin. From these results, it is suggested that tectorigenin is attributed to be a promising compound for the prevention and/or treatment of diabetic complications.

Key words : Aldose reductase, *Belamcanda chinensis*, Tectorigenin, Streptozotocin, Sorbitol, Diabetic complications

INTRODUCTION

Aldose reductase (AR), the key enzyme in the polyol pathway, has been demonstrated to play important roles not only in the cataract formation in the lens (Van Heyningen, 1959) but also in the pathogenesis of diabetic complications such as neuropathy (Ward, 1973), nephropathy (Beyer-Mears *et al.*, 1984) and retinopathy (Engerman and Kern, 1984). Evidence suggests that compounds which inhibit AR could be effective for the prevention of diabetic complications. A number of structurally diverse naturally occurring and synthetic AR inhi bitors have been studied *in vivo* to clarify their effectiveness for prevention of cataract formation as well as diabetic complications in experimental animals (Beyer-Mears and Cruz, 1985) as well as in clinical trials (Handelsman and Turtle, 1981).

Correspondence to: Kuk Hyun Shin, Natural Products Research Institute, Seoul National University, Seoul 110-460, Korea
E-mail: khshin@plaza.snu.ac.kr

In a series of investigations to evaluate potential AR inhibitors from medicinal plants, we have shown that some hot water extracts from herbal medicines exhibited a significant inhibition of bovine lens AR (BLAR) *in vitro* (Shin *et al.*, 1993), and a number of flavonoidal compounds were isolated and characterized as AR inhibitors from plants (Shin *et al.*, 1994; 1995).

Belamcanda chinensis (Iridaceae), is a perennial shrub growing on the hill sides in the east Asia including Korean peninsula, and have been used as Chinese traditional medicine for the treatment of throat ailment such as asthma and tonsillitis and a number of isoflavonoids have already been isolated (Yamaki *et al.*, 1990). Among the active plants, the rhizomes of *Belamcanda chinensis* were shown to have a significant AR inhibitory activity of > 50% at 50 μg/ml.

The present study was carried out to find out constituents with AR inhibitory activity from this plant in search of potential compounds for prevention and/or treatment of diabetic complications. Systematic fractionation of the methanol extract led to the isolation of 12 phenolic compounds.

Among various phenolic compounds isolated from this plant, we found that isoflavones such as tectorigenin, irigenin and their glucosides exhibited a strong inhibition of RLAR *in vitro*.

The effects of tectorigenin and tectoridin on intracellular sorbitol accumulation in discrete tissues including rat lens, red blood cells and sciatic nerves in streptozotocin-induced diabetic rats were also evaluated.

MATERIALS AND METHODS

Plant materials

The rhizomes of *Belamcanda chinensis* were collected in the vicinity of Seoul in Korea and the voucher specimen (Voucher No. NPRI-97-037) were deposited at the Herbarium of Natural Products Research Institutes, Seoul National University, Seoul, Korea.

Preparation of rat lens AR (RLAR)

Crude RLAR was prepared as follows: rat lenses were removed from Sprague-Dawley rats weighing 250~280 g and frozen until use. The supernatant fraction of the rat lens homogenate was prepared according to Hayman and Kinoshita (1965) and then partially purified according to Inagaki *et al.* Partially purified enzyme with a specific activity of 6.5 mU/mg were routinely used to test enzyme inhibition. The partially purified material was separated into 1.0 ml aliquots and stored at -40°C.

Animals

Male Sprague-Dawley rats weighing 240 ± 20 g were supplied from Natural Products Research Institutes of Seoul National University. They were fed on lab. chows. and water *ad lib.* and were housed at 23°C and 10% humidity in a 12-h light-dark cycle.

Measurements of RLAR activity

RLAR activities were assayed spectrophotometrically by measuring the decrease in absorption of NADPH at 340 nm over a 4 min period with DL-glyceraldehyde as a substrate (Sato and Kador, 1990). Each 1.0 ml cuvette contained equal units of enzyme, 0.10 M sodium phosphate buffer (pH 6.2), 0.3 mM NADPH with or without 10 mM substrate and inhibitor. The concentration of inhibitors giving 50% inhibition of enzyme activity (IC_{50}) was calculated from the least-squares regression line of the logarithmic con-centrations plotted against the remaining activity.

In vivo experiments (Sorbitol Accumulation in STZ-induced Diabetic Rat Tissues)

Diabetes was induced in male Sprague-Dawley rats (240-270 g) by a single intraperitoneal injection of streptozotocin (65 mg/kg) in sodium citrate buffer (pH 4.5). Control rats were injected with the vehicle only. The animals were fed standard rat food and water *ad libitum* throughout the study. Seven days after the induction of diabetes, the animals were commenced on either tectoridin, tectorigenin, epalrestat (ONO Co.), or vehicle alone via an intragastric tube, per a day at a dose of 100 mg/kg/day for 10 days. Tectoridin, tectorigenin and Epalrestat were suspended in saline contained 0.5% carboxymethyl cellulose. The animals were then sacrificed under ether anesthesia. The contents of sorbitol in the rat RBC, sciatic nerves, and lenses were determined enzymatically (Aida *et al.*, 1988).

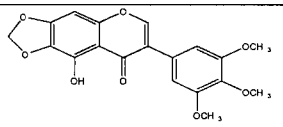
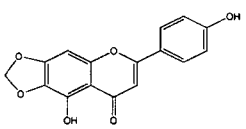
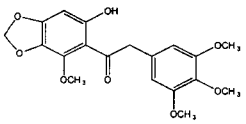
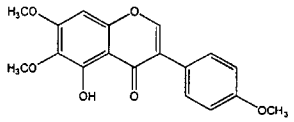
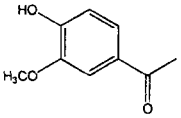
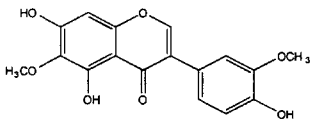
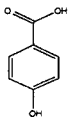
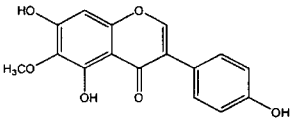
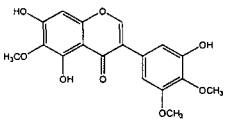
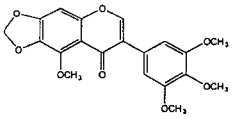
Isolation and identification of phenolic compounds

The dried rhizome was extracted four times with methanol by refluxing for 5 h. After removal of the solvent in vacuo, the residue was suspended in water and then extracted with n-hexane, methylene chloride, ethyl acetate and *n*-butanol to obtain an n-hexane, methylene chloride, ethyl acetate and *n*-butanol soluble fraction after evaporation. A portion of the methylene chloride fraction was purified by chromatography on silica gel eluted with hexane and increasing proportion ethyl acetate (8 : 2 → 5 : 5), and recrystallization in methanol gave ten phenolic compounds. A portion of the *n*-butanol fraction was purified by chromatography on silica gel eluted with chloroform and increasing proportion methanol (9 : 1→6 : 4), and recrystallization in methanol gave two isoflavone glycoside. All compounds identified by EI-Mass, IR and NMR spectra and direct comparison with authentic compounds. Their chemical structure are shown in Table I.

Noririsflorentin: crystallized from MeOH as needles; mp 239–240°C; EI-MS *m/z* (rel. int.): 372 (M^+, 69.1), 357 (31.7), 271 (8.9), 192 (1.6, retro-Diels-Alder fragment), 181 (5.3), 180 (3.0, retro-Diels-Alder fragment), 149 (19.9); ^{1}H NMR (300 MHz, $CDCl_3$) : δ 12.67 (s, 5-OH), 7.86 (s, H-2), 6.46 (s, H-8), 6.67 (s, H-2', 6'), 3.88 (s, 9H, OCH_3), 6.05 (2H, s, methylenedioxy) ^{13}C NMR (75 MHz, $CDCl_3$) : δ 153.1 (C-2), 123.7 (C-3), 181.1 (C-4), 153.1 (C-5), 142.6 (C-6), 153.3 (C-7), 89.3 (C-8), 154.2 (C-9), 106.4 (C-10), 125.9 (C-1'), 106.3 (C-2', 6'), 153.1 (C-3', 5'), 142.6 (C-4'), 102.8 (methylenedioxy).

Kanzakiflavone-2: crystallized from MeOH as pale yellow needles; mp > 310°C; EI-MS *m/z* (rel. int.): 298 (M^+, 100), 180 (RDA with A ring, 11.2), 118 (RDA with B ring, 7.5); ^{1}H NMR (300 MHz MeOH-d_4): δ 6.66 (s, H-3), 6.76 (s, H-8), 7.87 (d, *J*=9.0 Hz, H-2',6'), 6.93 (d, *J*=9.0 Hz, H-3', 5'), 6.10 (s, methylenedioxy).

Table 1. Effects of isolated compounds from *Belamcanda chinensis* on rat lens aldose reductase

Compound name	Structure	Inhibition (%)
Noririsflorentin		54.1
Kanzakiflavone-2		60.4
sheganone		37.6
4', 7-Di-O-methyltectorigenin		45.3
apocynin		33.7
Iristectorene B		69.8
p-hydroxybenzoic acid		32.8
tectorigenin		83.5
irigenin		70.9
irisflorentine		40.9
iridin	Irigenin-7-glucoside	46.8
tectoridin	Tectorigenin-7-glucoside	83.1

Sample concentration was 10 μM.
Inhibition rate was calculated as percentage with respect to the control value

Sheganone: crystallized from MeOH as yellow needles; EI-MS *m/z* (rel. int.): 376 (M^+, 42), 195 (M^+-$C_{10}H_{13}O_3$, 100), 182 (M^+-$C_9H_7O_3$, 12), 180 ($C_9H_7O_5$-CH_3, 31); ^{1}H NMR (300 MHz, $CDCl_3$) : δ 6.21 (s, H-5), 6.44 (s, H-2'), 5.93 (s, methylene dioxy), 4.26 (s, α-methylene), 4.09, 3.86, 3.85 (s, OCH_3).

4', 7-Di-*O*-methyltectorigenin: crystallized from MeOH as pale yellow needles; mp 180–182°C; IR ν_{max} (KBr)(cm^{-1}): 3447 (OH), 1655 (conjugated C=O), 1516, 1458 (aromatic); EI-MS *m/z* (rel. int.): 328 (M^+, 100), 196 (RDA with A ring, 2.0), 132 (RDA with B ring); ^{1}H NMR (300 MHz, Acetone-d_6) : δ 8.29 (s, H-2), 6.72 (s, H-8), 7.01 (d, *J*=8.7 Hz, H-2',

6'), 7.57 (d, *J*=8.7Hz, H-3', 5'), 12.94 (s, 5-OH), 3.81, 3.85, 3.99 (s, OCH_3).

Apocynin: crystallized from MeOH as yellow powder; ^{1}H NMR (300 MHz, DMSO-d_6) : δ 7.50 (dd, *J*=2.1, 8.4 Hz, H-6) 7.43 (d, *J*=2.1 Hz, H-2), 6.86 (d, *J*=8.4 Hz, H-5), 9.97 (brs, OH), 3.82 (s, OCH_3), 2.50 (s, CH_3); ^{13}C NMR (75 MHz, DMSO-d_6) : δ 129.2 (C-1), 111.4 (C-2), 152.0 (C-3), 147.8 (C-4), 115.2 (C-5), 123.7 (C-6), 55.8 (OCH_3), 26.4 (CH_3), 196.4 (C=O).

Iristectorene B: crystallized from MeOH as yellow needles; mp 186–188°C; IR ν_{max} (KBr)(cm^{-1}) : 3460 (OH), 1624 (conjugated C=O), 1583, 1521, 1462 (aromatic); EI-MS *m/z* (rel. int.): 330 (M^+, 100), 182 (RDA with A ring, 0.9), 148 (RDA with B ring, 9.0); ^{1}H NMR (300 MHz, DMSO-d_6) : δ 8.35 (s, H-2), 6.49 (s, H-8), 7.12 (d, *J*=1.8 Hz, H-2'), 6.81 (d, *J*=8.1, H-5), 6.98 (dd, *J*=1.8, 8.1 Hz, H-6), 13.06 (s, 5-OH), 10.75 (brs, OH), 9.14 (brs, OH), 3.74, 3.78 (s, OCH_3); ^{13}C NMR (75 MHz, DMSO-d_6) : δ 154.5 (C-2), 121.9 (C-3), 180.7 (C-4), 152.9 (C-5), 131.8 (C-6), 157.6 (C-7), 94.0 (C-8), 153.5 (C-9), 105.1 (C-10), 122.0 (C-1'), 113.5 (C-2'), 147.4 (C-3'), 146.9 (C-4'), 115.4 (C-5'), 121.8 (C-6'), 60.1, 55.9 (OCH_3).

4-Hydroxybenzoic acid: crystallized from MeOH as white needle; ^{1}H NMR (300 MHz, MeOH-d_4) : δ 7.88 (d, H-2, 6, *J*=8.7Hz), 6.82 (d, H-3, 5, *J*=8.7Hz); ^{13}C NMR (75 MHz, MeOH-d_4) : δ 170.2 (C=O), 163.2 (C-4), 133.0 (C-2,6) 122.6 (C-1), 115.3 (C-3,5).

Tectorigenin: crystallized from MeOH as pale yellow needles; mp 230°C; ^{1}H NMR (300 MHz, Acetone-d_6) : δ 8.32 (s, H-2), 6.49 (s, H-8), 7.36 (d, *J*=8.7 Hz, H-2'), 6.81 (d, *J*=8.7 Hz, H-3',5'), 13.05 (s, 5-OH), 10.75 (7-OH), 9.57 (s, 4'-OH), 3.74 (s, OCH_3); ^{13}C NMR (75 MHz, Acetone-d_6) : δ 154.3 (C-2), 122.0 (C-3), 180.7 (C-4), 152.9 (C-5), 131.6 (C-6), 153.4 (C-7), 94.0 (C-8), 157.6 (C-9), 105.0 (C-10), 121.4 (C-1'), 130.3 (C-2'), 115.2 (C-3'), 157.6 (C-4'), 115.2 (C-5'), 130.3 (C-6'), 60.1 (6-OCH_3).

Irigenin: crystallized from MeOH as pale yellow needles; mp 185°C; IR ν_{max} (KBr)(cm^{-1}) : 3427 (OH), 1662 (conjugated C=O), 1600, 1464, 1429 (aromatic); EI-MS *m/z* (rel. int.): 360 (M^+, 100), 183 (RDA+H, 7.7); ^{1}H NMR (300 MHz, Acetone-d_6 : δ 8.24 (s, H-2), 6.50 (s, H-8), 6.79 (d, *J*=2.1 Hz, H-2'), 6.78 (d, *J*=2.1 Hz, H-6'), 13.22 (s, 5-OH) 9.24 (s, 3'-OH), 3.80(s, OCH_3), 3.87 (s, OCH_3); ^{13}C NMR (75 MHz, Acetone-d_6) : δ 154.5 (C-2), 123.4 (C-3), 181.3 (C-4), 153.8 (C-5), 127.6 (C-6), 154.1 (C-7), 94.5 (C-8), 157.8 (C-9), 105.9 (C-10), 127.5 (C-1'), 110.7 (C-2'), 151.1 (C-3'), 136.0 (C-4'), 153.9 (C-5'), 105.9 (C-6'), 60.7 (6-OCH_3), 60.6 (4'-OCH_3), 56.3 (5'-OCH_3).

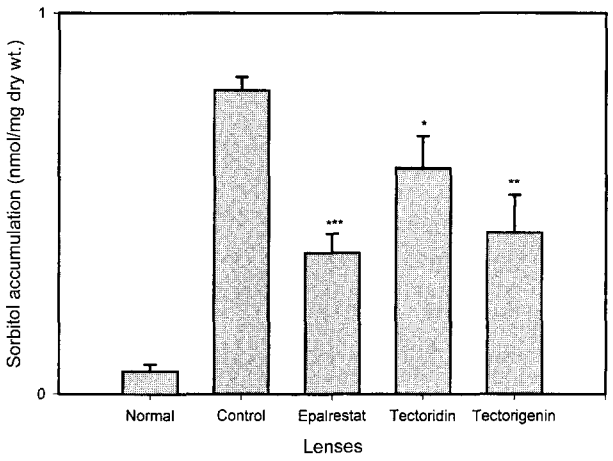


Fig. 1. The effect of tectoridin, tectorigenin and epalrestat on the accumulation of sorbitol in the lenses of diabetic rats. Animals were given the vehicle alone, tectoridin, tectorigenin or epalrestat via intragastric tubing per a day at a dose of 100 mg/kg/day for 10 days. The standard error of the mean is illustrated by the verticle line.
$^*p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$ vs control.

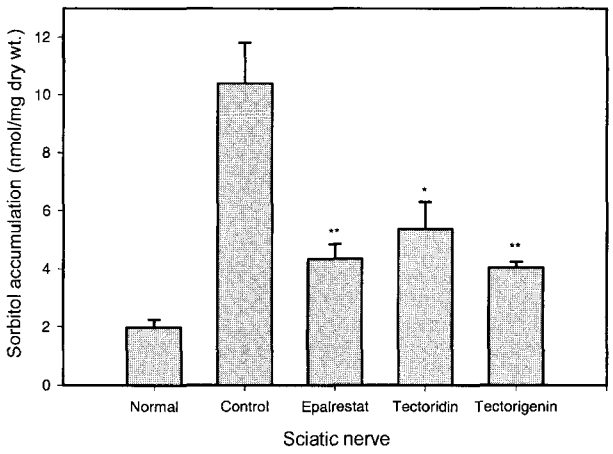


Fig. 2. The effect of tectoridin, tectorigenin and epalrestat on the accumulation of sorbitol in the sciatic nerve of diabetic rats. Animals were given the vehicle alone, tectoridin, tectorigenin or epalrestat via intragastric tubing per a day at a dose of 100 mg/kg/day for 10 days. The standard error of the mean is illustrated by the verticle line.
$^*p < 0.05$, $^{**}p < 0.01$ vs control.

Irisflorentine: crystallized from MeOH as needles; mp 166–167°C; IR ν_{max} (KBr)(cm^{-1}) : 1657 (conjugated C=O), 930 (methylenedioxy); EI-MS *m/z* (rel. int.): 386 (M^+, 100), 194 (RDA with A ring, 5.2), 195 (RDA with B ring, 4.0); ^{1}H NMR (300 MHz, $CDCl_3$) : δ 7.81 (s, H-2), 6.65 (s, H-8), 6.76 (s, H-2', 6'), 3.87 (s, 9H, OCH_3), 4.09 (3H, s), 6.08 (2H, s, methylenedioxy); ^{13}C NMR (75 MHz, Acetone-d_6) : δ 153.8 (C-2), 125.8 (C-3), 174.7 (C-4), 142.3 (C-5), 137.3 (C-6), 155.3 (C-7), 94.1 (C-8), 152.3 (C-9), 114.8 (C-10),

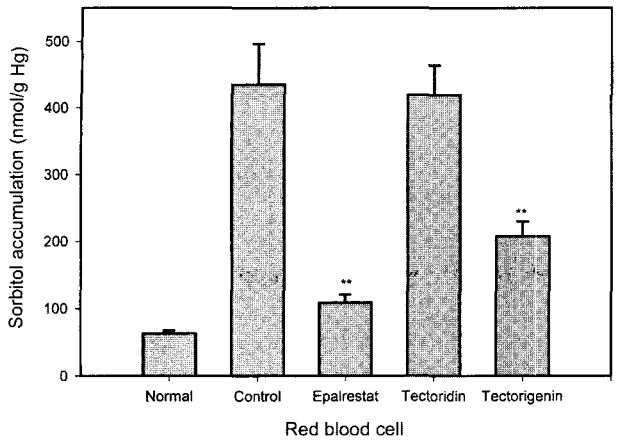


Fig. 3. The effect of tectoridin, tectorigenin and epalrestat on the accumulation of sorbitol in the sciatic nerve of diabetic rats. Animals were given the vehicle alone, tectoridin, tectorigenin or epalrestat via intragastric tubing per a day at a dose of 100 mg/kg/day for 10 days. The standard error of the mean is illustrated by the verticle line.
$^{*}p < 0.05$, $^{**}p < 0.01$ vs control.

128.7 (C-1'), 107.8 (C-2'), 153.9 (C-3'), 138.6 (C-4'), 153.9 (C-5'), 107.8 (C-6'), 61.3, 60.5, 56.5, 56.5 (OCH_3), 103.6 (methylenedioxy).

Iridin: crystallized from MeOH as pale yellow needles; mp 208°C; ^{1}H NMR (300 MHz, DMSO-d_6) : δ 8.48 (s, H-2), 6.89 (s, H-8), 6.72 (d, *J*=1.8 Hz, H-2'), 6.68 (d, *J*=1.8 Hz, H-6'), 12.90 (s, 5-OH), 9.27 (s, 3'-OH), 3.69, 3.76, 3.78 (s, OCH_3), 5.09 (H-1"), 4.3-5.0 (H-2"-6"); ^{13}C NMR (75 MHz, DMSO-d_6) : δ 155.6 (C-2), 122.1 (C-3), 180.7 (C-4), 153.1 (C-5), 132.7 (C-6), 153.0 (C-7), 94.3 (C-8), 156.9 (C-9), 106.6 (C-10), 126.1 (C-1'), 110.6 (C-2'), 150.5 (C-3'), 136.7 (C-4'), 152.6 (C-5'), 104.8 (C-6'), 60.5 (6-OCH_3), 60.1(4'-OCH_3), 56.0 (5'-OCH_3), 100.3 (H-1"), 73.3 (H-2"), 76.9 (H-3"), 69.8 (H-4"), 77.5 (H-5"), 60.9 (H-6").

Tectoridin: crystallized from MeOH as pale yellow needles; mp 257–258°C; ^{1}H NMR (300 MHz, DMSO-d_6) : δ 12.92 (s, 5-OH), 9.59 (s, 3'-OH), 8.44 (s, H-2), 6.88 (s, H-8), 7.39 (d, *J*=8.7 Hz, H-2'), 6.82 (d, *J*=8.7 Hz, H-3',5'), 3.77 (s, OCH_3), 5.11 (H-1"), 4.3-5.0 (H-2"-6"); ^{13}C NMR (75 MHz, DMSO-d_6) : δ 154.9 (C-2), 122.2 (C-3), 180.9 (C-4), 152.6 (C-5), 132.6 (C-6), 153.1 (C-7), 94.2 (C-8), 156.8 (C-9), 106.6 (C-10), 121.2 (C-1'), 130.3 (C-2'), 115.3 (C-3'), 157.6 (C-4'), 115.3 (C-5'), 130.3 (C-6'), 60.5 (6-OCH_3), 100.3 (H-1"), 73.3 (H-2"), 76.9 (H-3"), 69.8 (H-4"), 77.5 (H-5"), 61.0 (H-6").

Statistical analysis

The data are shown as the mean ± S. E. M. Significance of difference was calculated by Students *t*-test and linear regression was analyzed by the least square method.

Table 2. AR inhibitory potencies of isoflavonoids from *Belamcanda chinensis* on rat lens aldose reductase

Sample	Concentration (μM)	Inhibition (%)	IC_{50} (μM)
TMG*	10	82.1	0.63
	1	53.7	
	0.1	29.8	
Iristectorene B	10	69.8	2.33
	5	63.5	
	1	36.9	
irigenin	10	70.9	2.42
	5	61.2	
	1	36.7	
tectorigenin	10	83.5	1.12
	1	44.5	
	0.5	40.9	
tectoridin	10	83.1	1.08
	5	77.0	
	1	47.8	

Inhibition rate was calculated as percentage with respect to the control value
*TMG : tetramethylene glutaric acid

RESULTS AND DISCUSSION

The chemical structures of eight isoflavonoids, one flavonoid, and three phenolic compounds isolated were identified, and their inhibitory effects on RLAR using DL-glyceraldehyde as a substrate are shown in Table I.

Isoflavones such as tectorigenin, irigenin, and their glycosides were found to exhibit much stronger AR inhibition (more than 70% inhibition at 10 μM) than other isoflavones, flavone or phenolic compounds. The aglycones were a little stronger than the corresponding glycosides.

The presence of a hydroxyl and a methoxyl group in ring A seem to be essential for the AR inhibitory activity, but the presence of substituents in ring C appeared to have almost no influence on the inhibitory effects.

Substitution with a methylenedioxy group in ring A markedly reduced the inhibitory activity.

Varma and Kinoshita (1976) have indicated some possible relationships of structure to the inhibiting potencies of flavones. Inhibition is greater in trihydroxy- than dihydroxyflavones, the hydroxylation in the 4-position has beneficial effects, and the abolition of the double bond between C-2 and C-3 leads to a decrease of inhibition. In support of this hypothesis, trihydroxy isoflavone (iristectorene B, tectorigenin and irigenin) was much strong inhibitory activity than dihydroxy isoflavone, and the inhibition was greater in 4-hydroxyl group (iristectorene B and tectorigenin).

To evaluate the AR inhibitory activities between active iso flavonoids more precisely, their inhibitory potencies and IC_{50} values were estimated and indicated in Table II.

Although slightly less potent than tetramethylene glutaric acid (TMG), known as one of typical AR inhibitors

(IC_{50} value, 0.63×10^{-6} M), the inhibitory potencies of tectorigenin and tectoridin as expressed by IC_{50} values, were 1.12×10^{-6} M and 1.08×10^{-6} M, respectively.

In diabetes, the high extracellular levels of glucose disturb the cellular osmoregulation and sorbitol is formed intracellularly due to the intracellular polyol pathway, which is suspected to be one of the key processes in the development of diabetic complications and associated cellular dysfunctions.

Evidence for a causal relationship between increased flux through the polyol pathway and diabetic complications has come from studies on cataractogenesis and impaired peripheral nerve function in diabetic and galactosemic animals (Kinoshita *et al.*, 1979). The development of lenticular cataracts and defects in nerve conduction velocity are associated with increases in lens, RBC, and peripheral nerve sorbitol and fructose level.

To confirm the effectiveness of tectorigenin and tectoridin *in vivo*, we also studied the effects of both compounds on sorbitol accumulation in STZ-induced diabetic rat tissues.

The mean sorbitol contents of the red blood cells (RBC), sciatic nerves and lenses were observed to alter significantly as shown in Fig. 1-3. In diabetic control rats, the sorbitol contents of the RBC (434.6 ± 122.5 nmol/g Hb, $P < 0.001$ *vs* normal) was shown to be markedly elevated compared to those of the normal rats (62.2 ± 3.9 nmol/g Hb). In diabetic rats treated with tectorigenin or epalrestat orally for 10 consecutive days at 100 mg/kg, the accumulation of sorbitol in the RBC was significantly inhibited by 77.4% ($P < 0.01$ *vs* control), and 87.2% ($P < 0.01$), respectively. There was no significant differences in the content of RBC between rats treated with tectoridin and diabetic control rats. The sorbitol accumulation in the sciatic nerves in the vehicle treated diabetic rats was also shown to be 10.4 nmol/mg dry wt. ($P < 0.001$ *vs* normal) which was significantly higher than that of the normal rats (1.98 nmol/mg wet wt.). Treatment with tectoridin and tectorigenin, the accumulation of sorbitol in the sciatic nerves was significantly inhibited by 59.6% ($P < 0.05$ *vs* diabetic contol), 75.5% ($P < 0.01$ *vs* diabetic control), respectively. Tectorigenin suppressed the accumulation of sorbitol in sciatic nerves a little stronger than Epalrestat (71.9%, $P < 0.01$ *vs* control). In the lenses of diabetic rats, treatments of tectoridin, tectorigenin and epalrestat decreased sorbitol contents by 27.7, 50.5 and 57.7%, respectively.

Although tectorigenin, a aglycone of tectoridin had a similar inhibitory activity *in vitro*, tectorigenin was much greater inhibition of sorbitol accumulation in diabetic rat tissues.

It has been reported that lens AR plays a central role in the reduction of aldose to polyol and cataract formation and other complications in diabetes and is triggered by the accumulation in the tissues of excessive sorbitol synthesized by the action of AR (Kinoshita, 1974).

AR inhibitors thus have been shown to prevent or delay significantly diabetic complications, and synthetic AR inhibitors are currently available and many have been tested for their clinical use, albeit with limited success (Raskin and Rosenstock, 1987) i. e.; Synthetic compounds with diverse structures such as sorbinil (Beyer-Mears and Cruz, 1985), epalrestat (Terashima *et al.*, 1984), other hydantoin derivatives (Inagaki *et al.*, 1982) etc., and flavonoids (Shimizu, 1984), isoliquiritigenin (Aida *et al.*, 1990) and luteolin (Shin *et al.*, 1994; 1995) from natural origin have been extensively studied and reported to inhibit AR.

The present study was carried out in a search for a new potential AR inhibitors useful for the treatment of diabetic complications from the rhizomes of *Belamcanda chinensis*, and we found that tectorigenin was the most promising active principle utilizable as a lead compound, because this compound not only inhibited AR *in vitro* but also prevented sorbitol accumulation in the diabetic rat tissues.

It is concluded, therefore, that tectorigenin, an isoflavone of this plant, could be offered as a leading compound for further study as a new drug for diabetic complications.

REFERENCES

Aida, K., Tawata, M., Shindo, H., Tsukahara, S. and Onaya, T., Effects of anti-inflammatory drugs and ONO-2235 on lens aldose reductase and on sorbitol accumulation in red blood cells. *Yamanashi Med,*. 3, 47-56 (1988).

Aida, K., Tawata, M., Shindo, H., Onaya, T., Sasaki, H., Yamaguchi, T., Chin, M. and Mitsuhashi, H., Isoliquiriti genin: A new aldose reductase inhibitor from Glycyrrhizae Radix. *Planta Med.*, 57, 254-258 (1990).

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

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

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

Handelsman D. J. and Turtle J.R., Clinical trial of an aldose reductase inhibitor in diabetic neuropathy. *Diabetes,* 30, 459-464 (1981).

Hayman, S. and Kinoshita, J. H., Isolation and properties of lens aldose reductase. *J. Biol. Chem.*, 240, 877-882 (1965).

Inagaki, K., Miwa, I. and Okuda, J., Affinity purification and glucose specificity of aldose reductase from bovine lens. *Arch. Biochem. Biophys.*, 216, 337-344 (1982).

Kinoshita, J. H., Mechanism initiating cataract formation. *Invest. Ophthalmol.*, 13, 713-723 (1974).

Kinoshita, J. H., Fukushi, S., Kador, P. and Merola, L. O., Aldose reductase in diabetic complications of the eye. *Metabolism,* 28, 462-469 (1979).

Malone, J. I., Knox, G., Benford, S., Tedesco, T. A., Red cell sorbitol: an indicator of diabetic control. *Diabetes,* 29, 861-864 (1980).

Raskin, P. and Rosentstock, J., Aldose reductase inhibitors and diabetic complications. *Am. J. Med.*, 83, 298-306 (1987).

Yamaki, M., Kato, T., Kashihara, M. and Takagi, S., Isoflavones from *Belamcanda chinensis. Planta Med.*, 56, 335 (1990).

Okuda, J., Miwa, I., Inagaki, K., Horie, T. and Nakayama, M., Inhibition of aldose reductases from rat and bovine lenses by flavonoids. *Biochem. Pharm.,* 31, 3807-3822 (1982).

Sato, S. and Kador, P. F. Inhibition of aldehyde reductase by aldose reductase inhibitors. *Biochem. Pharmacol.*, 40, 1033-1042 (1990).

Shimizu, M., Ito, T., Terashima, S., Hayashi, T., Arisawa, M., Morita, N., Kurokawa, S., Ito, K. and Hashimoto, Y., Inhibition of lens aldose reductase by flavonoids. *Phytochemistry,* 23, 1885-1888 (1984).

Shin, K. H., Chung, M. S., Chae, Y. J., Yoon, K. Y. and Cho, T. S., A survey for aldose reductase inhibition of some herbal medicines. *Fitoterapia,* 14, 130 (1993).

Shin, K. H., Kang, S. S., Kim, H. J. and Shin, S. W., Isolation of an aldose reductase inhibitor from the fruits of *Vitex rotundifolia. Phytomed.*, 1, 145-147 (1994).

Shin, K. H., Kang, S. S., Seo, E. A. and Shin, S. W., Isolation of aldose reductase inhibitors from the flowers of *Chrysanthemum boreale. Arch. Pharm. Res.*, 18, 65-68 (1995).

Terashima, H., Hama, K., Yamamoto, R., Tsuboshima, M., Kikkawa, R., Hatanaka, J. and Shigeta, Y., Effects of a new aldose reductase inhibitor on various tissues *in vitro. J. Pharm. Exp. Ther.*, 229, 226-230 (1984).

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

Van Heyningen, R., The polyol pathway in the neuropathy of early diabetes. In "dvances in Metabolic Disorders (Suppl. 2)" ed. By R. A. Camerini-Davalos and H. S. Cole, Academic Press, New York, pp. 425 (1973).

Varma, S. D. and Kinoshita, J. H., Inhibition of lens aldose reductase by flavonoids--their possible role in the prevention of diabetic cataracts. *Biochem. Pharm.*, 25, 2505-2513 (1976).

Ward, J. D., The polyol pathway in the neuropathy of early diabetes. In "dvance in Metabolic Disorders (Suppl. 2)" ed. By R. A. Camerini-Davalos and H. S. Cole, Academic Press, New York, pp. 425 (1973).