Aldose Reductase Inhibitory Activity of Scrophularia Species

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Abstract – Effects of the species Scrophularia takesimensis, S. kakudensis, S. boreali-koreana, and S. buergeriana on rat lens aldose reductase inhibition have been investigated. Among them, the extracts of S. kakudensis and S. boreali-koreana were exhibited good inhibitory potencies compared with those of S. takesimensis and S. buergeriana. The IC\textsubscript{50} values of the aerial part extracts from S. kakudensis and S. boreali-koreana were demonstrated 0.46 and 0.35 mg/ml, respectively.

Keywords – Scrophularia species, 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 \textit{et al.}, 1984; Beyer-Mears and Cruz, 1985), and retinopathy (Engerman and Kern, 1984). AR inhibitors can prevent or reverse early abnormalities in diabetic complications. Among the AR inhibitors such as zopolrestat, ponalrestat, sorbinil, tolrestat, epalrestat, and ranirestat \textit{etc.}, which have been developed with promising results in the past years (Constantino \textit{et al.}, 1999; Sun \textit{et al.}, 2006; Drel \textit{et al.}, 2008; Hotta \textit{et al.}, 2006; Matsumoto \textit{et al.}, 2008). These AR inhibitors, however, almost all have several problems such as side effects and decrease of effects \textit{etc.} during human clinical trials causing hindrance to development of research (Ziegler, 2004; Chalk \textit{et al.}, 2007). Therefore, recently natural sources for AR inhibitors potential are spotlight for the treatment and prevention of diabetic complications due to safer and more effective phytochemicals (Jesús Ángel and Sonia, 2003; Kawanishi \textit{et al.}, 2003).

The genus Scrophularia of the family Scrophulariaceae comprises about 300 species of herbaceous flowering plants and these are found throughout the Northern Hemisphere, but concentrated in Asia with only a few species in Europe and North America (Chung and Shin, 1990). The dried roots of Scrophularia species have been used in Asian medicine as a treatment for fever, laryngitis, swelling, constipation, neuritis, and pharyngitis (Qian \textit{et al.}, 1992; Park \textit{et al.}, 2003). Five types of Scrophularia species such as S. takesimensis, S. kakudensis, S. koraiensis, S. boreali-koreana, and S. buergeriana naturally grow in Korea (Ahn, 2005). Among these, S. buergeriana has been cultivated and used as a medicinal plant for diverse purposes while other species have been grown wild.

In present study, as a preliminary step for the evaluations potential of naturally occurring AR inhibitors, we tested the effects of Scrophularia species (S. takesimensis, S. kakudensis, S. boreali-koreana, and S. buergeriana) on rat lens AR inhibition.

Experimental

\textbf{Plant materials –} The extracts of S. kakudensis and S. boreali-koreana were purchased from PEB (Plant Extract Bank) of KRIBB in Daejeon, Korea. S. takesimensis was collected at Ulleung Island, Korea and S. buergeriana was purchased from Kyungdong market, Seoul, Korea. The specimens of S. takesimensis and S. buergeriana were

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botanically authenticated by Prof. Y. H. Ahn, Chung-Ang University, Korea.

**General instruments and Reagents**—Fluorescence analysis 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 MeOH extracts of *S. takakinesis* and *S. boreal-koreanana* were purchased from PEB of KRIBB. *S. takakinesis* and *S. buergeriana* were extracted with MeOH under reflux (3 h × 5 times). Each sample of the MeOH extract (1.0 mg) was dissolved in DMSO (1 ml).

**Measurement of AR activity**—Rat lenses were removed from Sprague-Dawley rats (weighing 250-280 g) and preserved 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 measuring the decrease in absorption of NADPH at 340 nm for a 4 min period at room temperature with DL-glyceraldehyde 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 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.

### Results and Discussion

The MeOH extracts of *Serophularia* species were tested for their inhibitory effects on rat lens AR activity, and the results were summarized in Table 1. The rat lens AR inhibition percentages of the root extracts of *S. takakinesis*, *S. kakadensis*, *S. boreal-koreanana*, and *S. buergeriana* were 39.50, 58.75, 24.79 and 11.41%, respectively. Except for *S. kakadensis*, however, the root extracts of other species showed below 50% degree of inhibition on rat lens AR that are supposed to be far less deserving of further consideration. The aerial part extracts of *S. takakinesis*, *S. kakadensis*, and *S. boreal-koreanana* were subjected to test for rat lens AR activity, exclusive of *S. buergeriana*, which is generally used for medicinal purpose. As shown in Table 1, the aerial part extracts of *S. kakadensis* and *S. boreal-koreanana* were exhibited good inhibitory potencies on rat lens AR compared with those of the root extracts. The aerial part extracts of *S. kakadensis* and *S. boreal-koreanana* were exhibited highest rat lens AR inhibition (72.88 and 77.85%, respectively).

To evaluate the rat lens AR inhibitory activity potencies between active species extracts, *S. kakadensis* and *S. boreal-koreanana*, more precisely, their inhibitory percentage and IC$_{50}$ values were tested. Quercetin known as a very strong AR inhibitor (IC$_{50}$ value, 0.19 mg/ml) in natural constituents was used as a positive control and the results were indicated in Table 2. The IC$_{50}$ values of the aerial part extracts of *S. kakadensis* and *S. boreali-