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RESEARCH ARTICLE

Aldose reductase inhibitors from *Litchi chinensis* Sonn

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Abstract
Diabetes is one of the major risk factors for cataract. Aldose reductase has been reported to play an important role in sugar-induced cataract. In this study, we conducted pharmacological investigations upon experimental rat lenses using extracts of the fruits of *Litchi chinensis* (Sapindaceae). Of the extracts and organic fractions of *L. chinensis* tested, a MeOH extract and an EtOAc fraction were found to be potent inhibitors of rat lens aldose reductase (RLAR) *in vitro* — their IC_{50} values being 3.6 and 0.3 μg/mL, respectively. From the active EtOAc fraction, four minor compounds with diverse structural moieties were isolated and identified as D-mannitol (**1**), 2,5-dihydroxybenzoic acid (**2**), delphinidin 3-*O*-β-galactopyranoside-3′,5′-di-*O*-β-glucopyranoside (**3**), and delphinidin 3-*O*-β- galactopyranoside-3′-*O*-β-glucopyranoside (**4**). Among these, **4** was found to be the most potent RLAR inhibitor (IC_{50} = 0.23 μg/mL), and may be useful in the prevention and/or treatment of diabetic complications.

Keywords: Litchi chinensis*; rat lens aldose reductase inhibitor; cataract; delphinidin 3-*O*-B-galactopyranoside-3′-*O*-β-*glucopyranoside; diabetes.*

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Introduction

Cataract, the leading cause of blindness worldwide, is associated with several risk factors, a major one of which is considered to be diabetes. Various pharmacological intervention strategies aimed at the prevention of diabetes are currently being investigated. Among these, aldose reductase inhibitors (ARIs) have received considerable attention owing to the proposed involvement of aldose reductase (AR) in the pathophysiology of diabetic complications, including cataract [1]. A vast literature exists demonstrating that cataract progression can be slowed or prevented by the use of natural therapies, and particularly by the use of plants having high flavonoid contents, which have exhibited considerable *in vivo* AR inhibitory effects [2].

Litchi (*Litchi chinensis Sonn.*, Sapindaceae) is a tropical to subtropical crop that originated in South-East Asia. With the gradual consumer acceptance of litchi fruits for their delicious taste and attractive red skin, there has been a steady increase in litchi production over recent decades, with increasing exports into Europe and North America from production areas in both the Southern and Northern hemispheres. Litchi fruit pericarp (LFP) accounts for approximately 15% by weight of the whole fresh fruit and comprises significant amounts of flavonoids. Consequently, litchi LFP tissues may be a potentially important source of AR-inhibiting compounds [3]. Previous pharmacological and phytochemical studies on litchi have revealed the fruit to be a rich source of anthocyanins [4] and several flavone glycosides [5]. Duan *et al*. (2007) reported that anthocyanins from LFP tissues strongly inhibited linoleic acid oxidation and exhibited a dose-dependent free-radical scavenging activity against diphenylpicrylhydrazyl (DPPH) radicals, superoxide anions, and hydroxyl radicals. Wang *et al*. [6] reported that an LFP extract exhibited potential *in vitro* and *in vivo* anticancer activity against hepatocellular carcinoma. Furthermore, the LFP extract exhibited a dose-dependent, time-inhibitory effect on cancer cell growth. The anticancer activity of LFP water-soluble crude ethanolic extract might result, at least in part, from the inhibition of DNA synthesis, proliferation, and apoptosis induction in cancer cells [7].

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However, the biological activity of LFP has not been investigated in detail. From this perspective, various inhibitors, such as the inhibitors of rat lens aldose reductase (RLAR), are presently being developed as novel potential therapeutic agents. Therefore, we have screened LFP for its ability to inhibit RLAR *in vitro*.

In our continuing search for the bioactive principles from crude plant extracts, we have evaluated the extracts and fractions of *L. chinensis*, particularly in relation to their inhibitory effects on RLAR. Here, the effects of compound isolated from *L. chinensis* on RLAR activity *in vitro* are described.

Materials and methods

Plant material

Fruits of *L. chinensis* were purchased at a local market in NamDaeMun Prefecture, Seoul, South Korea. Voucher specimens (WKU-00801) have been deposited in the Herbarium of the College of Medicine, Wonkwang University (Sanbon, South Korea).

Chemicals

DL-Glyceraldehyde, sodium phosphate, and the reduced form of adenine dinucleotide phosphate (NADPH) were purchased from Sigma Chemical Co. (St. Louis, MO, USA.). All other chemicals and reagents were of analytical grade.

Extraction and isolation

The air-dried fruit pericarp of *L. chinensis* (500 g) was extracted with methanol (MeOH) at 80°C (3 × 1 L for 4 h). The MeOH extract (60 g) was suspended in water and then successively partitioned with n-hexane (28 g), Chloroform ($CHCl_3$) (5 g), ethyl acetate (EtOAc) (9 g), and n-butanol (n-BuOH) (15 g). The EtOAc fraction (8 g) was fractionated by extensive column chromatography over silica gel using an n-hexane:EtOAc:MeOH gradient and this yielded five fractions (fr. 1~fr. 5). Silica gel column chromatography of the major fraction (fr.4) with a solvent gradient of MeOH in $CHCl_3$ yielded five subfractions (fr. 4-1~fr. 4-5). Among these, fr.4-3 was eluted by C_{18} reverse-phase (RP) column chromatography with 100% H_2O to 100% MeOH as the eluent. This yielded six subfractions (fr. 4-3-1~fr. 4-3-6). Among these subfractions, fr. 4-3-3 yielded **1** (4 mg) and **2** (3 mg) by an additional purification step using C_{18} RP high-performance liquid chromatography (HPLC) (AcCN:MeOH = 4:6). **3** (5 mg) was isolated by C_{18} RP HPLC (AcCN:MeOH:H_2O = 45:10:45) from fr. 4-3-4. **4** (4 mg) was purified by an additional purification step on C_{18} RP HPLC (AcCN:MeOH:H_2O = 3:1:6) from fr. 4-3-5.

Rat lens aldose reductase activity in vitro

Rat lenses were removed from Sprague-Dawley rats (weighing 250–280 g, Jail animal Co, South Korea) under anesthesia and frozen until use. The supernatant fraction of the rat lens homogenate was prepared according to the procedures of Hayman and Kinoshita [8]. Partially purified enzyme was routinely used to test for enzyme inhibition. The partially purified material was separated into 1.0-mL aliquots and stored at –68°C. Rat lenses were homogenized and centrifuged at 12000 × *g* and the supernatant was used as an enzyme source. AR activity was assayed spectrophotometrically by measuring the decrease in the absorption of NADPH (16 mM) at 340 nm over a 5-min period, with DL-glyceraldehyde as the substrate [9]. The inhibitor concentrations producing 50% inhibition of the enzyme activity (IC_{50}) were calculated from the least-squares regression line of the logarithmic concentrations plotted against the remaining activity.

Results and discussion

The effects of the MeOH extract and the organic solvent fractions from *L. chinensis* on the RLAR enzyme were assessed, using DL-glyceraldehyde as a substrate. The inhibitory potencies and IC_{50} values against the RLAR enzyme were determined. As summarized in Table 1, the aldose redutase inhibitions were discerned with the methanol extracts from the fruit pericarp parts of *L. chinensis*, such as tetramethylene glutaric acid. (TMG), a known aldose redutase inhibitor (*10*) was 0.4 μg/mL. We investigated the inhibitory effects of hexane, $CHCL_3$, EtOAc, and BuOH fractions from the LFP, on the activity of RLAR. The EtOAc fraction from the MeOH extract was found to exhibit the strongest RLAR inhibitory activity, its IC_{50} being 0.3 μg/mL. Column chromatographic isolation of the EtOAc-soluble fraction from the LFP yielded four minor compounds (**1**~**4**) (Figure 1). These were identified as D-mannitol [**1**; [11]], 2,5-dihydroxybenzoic acid [**2**; [12]], delphinidin 3-*O*-β-galactopyranoside-3′,5′-di-*O*-β-glucopyranoside [**3**; [13]], and delphinidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside [**4**; [13]] by spectral analysis and comparison of the spectroscopic data with those previously reported. All of these compounds were isolated for the first time from the LFP. These observations

Table 1. Inhibitory Effects of the MeOH extract, organic solvent fractions, and isolated compounds on rat lens aldose reductase from *Litchi chinensis* Sonn.

MeOH extract, organic solvent fractions, and compounds	Inhibitory Concentration (IC_{50}, μg/mL)
MeOH extract	3.6*
n-Hexane fraction	N.E#
Chloroform fraction	4.8
EtOAc fraction	0.3
EtOAc subfraction - E1	N.E#
E2	N.E#
E3	2.3
E4	1.2
E5	5.3
n-BuOH fraction	12.3
Compound 1	>100
Compound 2	35.9
Compound 3	1.23
Compound 4	0.23
TMG (tetramethylene glutaric acid.)	0.48

*Inhibition rates were calculated as percentages with respect to the control value.

No Effects.

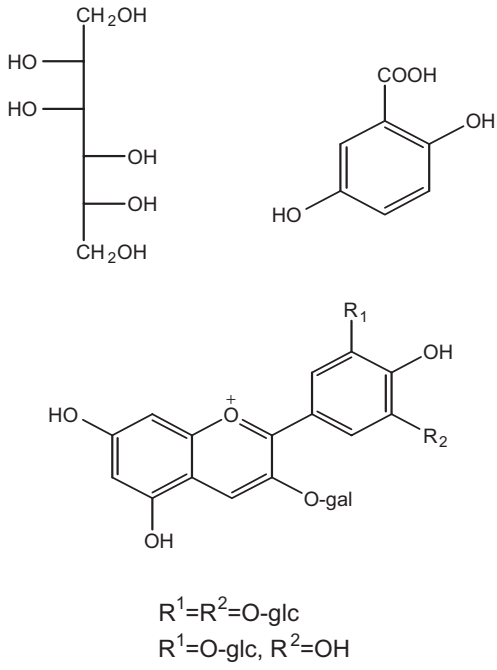

Figure 1. Structure of compounds **1–4**.

led us to investigate the ability of **1–4**, to regulate AR activity in rat lens, the results of which are summarized in Table 1. The results show that **4** exhibited significant inhibitory activity toward RLAR, with an IC_{50} of 0.23 μg/mL, which is equivalent to that of the positive control TMG (IC_{50} = 0.48 μg/mL). We concluded that the observed activity in the crude EtOAc extract (IC_{50} = 0.3 μg/mL) was probably attributable to the contribution of the inhibitory effects of known as well as unknown polyphenols, or other compounds present, and/or the synergistic effect of individual RLAR-inhibitory constituents when tested in the form of a complex mixture.

It is acknowledged that plant-derived extracts and phytochemicals are potential alternatives to synthetic inhibitors against RLAR. Currently, the compounds isolated from plants that have been characterized as RLAR inhibitors are classified as flavonoids [14], stilbenes [15], terpenoids [16], ellagic acid and its derivatives [17], and alkaloids [18]. In conclusion, the present study was carried out in an effort to identify new potential AR inhibitors from the LFP. Delphinidin 3-*O*-β-galactopyranoside-3′-*O*-β-glucopyranoside was isolated as an active principle. This compound may find future application in the treatment and prevention of diabetic complications.

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Hyung-In Moon and Won-Hwan Park equally contributed to this work.

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