**Letter**

# Antioxidative and aldose reductase-inhibitory effects of a fermentation filtrate of *Rubus coreanus*

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Antioxidative and aldose reductase (AR)-inhibitory effects of a fermentation filtrate of *Rubus coreanus* (FRC) were investigated using corneal/retinal homogenate and lens cytosol, respectively. Rat corneal/retinal homogenate was treated with 50 $\mu$M $FeCl_3$ in the presence of FRC (3.2-100 $\mu$g/mL) for 30 min at 37ºC, and thiobarbituric acid-reactive substances (TBARS) was quantified as a lipid peroxidation parameter. FRC markedly suppressed the TBARS production in a concentration-dependent manner, leading to 50% ($IC_{50}$) and 100% ($IC_{100}$) inhibitory concentrations of 20 and 95 $\mu$g/mL, respectively, which was similar to the effect of butylated hydroxyanisole. Activity of AR from rat lens was assayed in the presence of FRC (1-31.6 $\mu$g/mL) at 25ºC using glyceraldehyde as a substrate. FRC inhibited lens AR by 50% ($IC_{50}$) and 90% ($IC_{90}$) at approximately 2 and 31.6 $\mu$g/mL, respectively, comparable to the effect of quercetin. The results indicate that ERC could be a promising candidate for the improvement of eye injury and visual dysfunction of dry eye and diabetic patients.

Key words: *Rubus coreanus*, antioxidative activity, aldose reductase inhibition

Dry eye comes from injuries of lacrimal functional unit (lacrimal glands, cornea and eyelids and/or impairments of sensory and motor nerves connecting the unit lacrimal functional unit [1]. The disease is a complex symptom with rapid breakdown of tear film, resulting in corneal damage hindering eye ball movement and convenient seeing [2-4]. The major causes of dry eye are 1) environmental factors such as reduced blinking frequency, aging, low humidity, and antimuscarinic agents including atropine, 2) secondary effect from immune dysfunction in Sjogren syndrome (or sicca syndrome) and rheumatoid arthritis, and 3) excessive evaporation of tears [5-8].

Diabetic retinopathy (DR), one of the most serious complications of diabetes mellitus, frequently leads to blindness [9,10]. Proliferative diabetic retinopathy (PDR) is the advanced stage of DR characterized by new vessel formation accompanied by fibrous tissue accumulation leading to vitreous hemorrhage and subsequent tractional retinal detachment.

Among several biochemical changes, increased oxidative stress [11-13] and accumulation of advanced glycation end products (AGE) [14,15] may underlie the development of PDR. Since excess accumulation of sorbitol produced from high glucose by the catalytic activity of aldose reductase (AR) also contribute to DR, some new drugs targeting these biochemical changes, such as antioxidants [11-13], aldose reductase inhibitors [16] and AGE inhibitors [17,18], have been studied as candidates for the prevention of corneal injury and improvement of AR.

*Rubus coreanus*, which is well known as its Korean name "Bokbunja", has antiinflammatory, antifatigue, antioxidative and male reproductive function-recovering properties [19-22]. Since focal blood flow is one of the key factors of diabetic foot ulcers [23], it is suggested that vasodilatating polyphenols in *Rubus coreanus*, ginsenosides and vitamin C should be beneficial for the improvement of visual function [22,24,25]. It is of interest to note that they are also strong antioxidants [21,26], and especially ginseng components and vitamin C

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were found to inhibit AR [27,28]. Such results led us to investigate the antioxidative and AR-inhibitory activities of a fermentation filtrate of *Rubus coreanus* (FRC) in the eye tissues, to confirm its potential effectiveness for the improvement of visual function.

Ripe (reddish purple) fruits of the *Rubus coreanus* plant were purchased from an organic farm at Hamyang, Korea, in 2008. The fruits were minced, macerated, and adjusted to 25-30% precipitates by adding purified water, to produce a 20% starch content mixture. Into the homogenate, cultivated *Saccharomyces* (strain HM 2104) was added up to 4% and fermented at 45°C and pH 5.5 for 30-40 h. The crude culture precipitates were removed by centrifugation and microfiltration (pore size, 0.05 μm), and the alcohol produced during fermentation was fully removed in a vacuum evaporator.

Cornea and retina were obtained from the eye balls of Sprague-Dawley (SD) rats by removing the lens and vitreous body on an ice block. The tissues were homogenized in 19 volumes of 10 mM sodium phosphate-buffered saline (PBS, pH7.4) to make a 5% homogenate at 4°C. To induce lipid peroxidation, the homogenate (225 μL) was mixed with 12.5 μL ferric chloride ($FeCl_3$, final 50 μM) in the presence of FRC (final 3.2-100 μg/mL) or butylated hydroxyanisole (BHA, final 100 μM), and incubated for 30 min at 37°C. The reaction was then stopped by adding 250 μL sodium dodecyl sulphate (SDS, 8.1% solution) and 500 μL 20% acetic acid (adjusted to pH 3.5). After adding 250 μL 2-thiobarbituric acid (TBA, 0.75% solution), the mixture was boiled in a glass tube capped with aluminum foil for 30 min. Samples were cooled on ice, centrifuged at 13,000 *g* for 10 min, and absorbance of the supernatant was read at 532 nm for the quantification of thiobarbituric acid-reactive substances (TBARS).

Lenses of SD rats were vigorously homogenized in 9 volumes of homogenization buffer (100 mM sodium phosphate, 0.5 mM phynylmethylsulfonylfluoride, 10 mM 2-mercaptoethanol, pH7.0) to make a 5% homogenate at 4°C. The homogenate was centrifuged at 105,000 *g* for 60 min, and the supernatant (cytosol) was used as AR enzyme source. Into 417.5 μL sodium phosphate buffer (100 mM, pH7.0), 50 μL lens cytosol), 10 μL β-nicotinamide adenine dinucleotide phosphate (reduced form of NADPH, final 0.16 mM) and FRC (final 1-31.6 μg/mL), vitamin C (final 10-100 μg/mL) or quercetin (final 0.32-31.6 μg/mL) were added, and mixed well. After adding 12.5 μL glyceraldehyde (10 mM in 2% $NaCO_3$ in 0.1 N NaOH, pH 7.0), absorbance change at 340 nm was recorded for 5 min at 25°C.

Data were expressed as the mean±SEM. Statistical analysis was performed using an analysis of variance (ANOVA) with the aid of SPSS for Windows v.10.0 (Chicago, Illinois, USA).

**Table 1.** Antioxidative effects of a fermentation filtrate of *Rubus coreanus* (FRC) and butylated hydroxyanisole (BHA) on $FeCl_3$-induced lipid peroxidation of cornea and retina

| Treatment | Concentration (μg/mL) | TBARS (nM) | % of control |
|---|---|---|---|
| Vehicle | - | 1.58±0.92 | 100.0±7.7 |
| $FeCl_3$ alone | (50 μM) | 2.76±0.87 | 174.7±9.2* |
| FRC | 3.2 | 2.55±0.60 | 161.4±5.5 |
| | 10 | 2.28±0.57 | 144.3±8.2# |
| | 31.6 | 1.97±0.54 | 124.7±5.8# |
| | 100 | 1.54±0.47 | 97.5±5.4# |
| BHA | 100 | 1.61±0.41 | 101.9±6.4# |

TBARS: thiobarbituric acid-reactive substances. *Significantly different from vehicle control ($P<0.05$). #Significantly different from $FeCl_3$ alone ($P<0.05$).

A *P* value <0.05 was considered statistically significant.

Fifty μM $FeCl_3$ significantly increased TBARS concentration, lipid peroxidation products, by 74.7% (Table 1). Oxidative stress is an important factor for damage of eye components including cornea and retina in dry eye and diabetic patients [4,12]. However, FRC treatment markedly attenuated the TBARS production in a concentration-dependent manner (40.7% at 10 μg/mL and 66.9% at 31.6 μg/mL), leading to 50% ($IC_{50}$) and 100% ($IC_{100}$) inhibitory concentrations of 20 μg/mL and 95 μg/mL, respectively, which was comparable to the effect of a well-known antioxidant BHA. In fact, antioxidative activities of *Rubus coreanus* and its ingredients has been reported in other oxidation-inducing systems (lipopolysaccharide or copper) in different tissues (liver and lipoproteins) [21,26]. Notably, therefore, it was confirmed that *Rubus coreanus* exerts antioxidative activity in diverse oxidative systems including hepatic inflammation, atherosclerosis and ocular injury.

FRC remarkably inhibited lens AR in a concentration-dependent manner (37.4 and 67.7% at 1 and at 3.16 μg/mL), leading to $IC_{50}$ and $IC_{90}$ of 2 and 31.6 μg/mL, respectively (Table 2). Notably, vitamin C did not significantly inhibit lens AR activity, in contrast to the strong inhibition of erythrocyte AR [28]. Such a discrepancy in the inhibition of AR might be due to the differences in tissues, implying that vitamin C can exert negligible inhibitory effect on AR in the target tissue (lens), rather than in blood erythrocytes with limited access to eye tissues. In contrast, quercetin strongly inhibited AR activity, leading to 50 and 90% inhibition at 0.4 and 20 μg/mL, respectively. The AR-inhibitory activity of quercetin was higher than FRC. Unfortunately, however, it was reported that quercetin has genotoxicity [29], implying that safety assessment on quercetin is required for human trials. By comparison, *Rubus coreanus* is being widely consumed as diverse types of wine or juice in Korea. Although ginseng

**Table 2.** Inhibitory effects of a fermentation filtrate of *Rubus coreanus* (FRC), vitamin C and quercetin on lens aldose reductase

| Treatment | Concentration (μg/mL) | ΔOD/min | % of control |
|---|---|---|---|
| Vehicle | - | 0.0215±0.0028 | 100.0±11.8 |
| FRC | 1 | 0.0134±0.0021 | 62.3±6.6* |
| | 3.2 | 0.0076±0.0033 | 35.3±10.1* |
| | 10 | 0.0045±0.0011 | 20.9±4.1* |
| | 31.6 | 0.0023±0.0008 | 10.7±4.6* |
| Vitamin C | 10 | 0.0230±0.0031 | 107.0±13.2 |
| | 31.6 | 0.0223±0.0033 | 103.7±12.7 |
| | 100 | 0.0204±0.0021 | 94.9±5.3 |
| Quercetin | 0.32 | 0.0112±0.0019 | 52.1±8.8* |
| | 1 | 0.0057±0.0018 | 26.5±6.4* |
| | 3.2 | 0.0046±0.0024 | 21.4±7.0* |
| | 10 | 0.0030±0.0011 | 14.0±2.8* |
| | 31.6 | 0.0015±0.0005 | 7.0±1.6* |

TBARS: thiobarbituric acid-reactive substances. *Significantly different from vehicle control ($P<0.05$).

was demonstrated to inhibit lens AR [27], FRC was superior to ginseng extract in our study (data not shown).

Taken together, our results demonstrated that FRC exhibits strong antioxidative and AR-inhibitory activities in eye components, in addition to its vasodilatating effect. Although the analysis of active ingredients remains to be clarified, it is suggested that FRC could be a promising candidate for the improvement of eye injury and visual dysfunction of dry eye and diabetic patients.

# Acknowledgment

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