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Effects of *Rubus coreanus* extract on visual processes in bullfrog's eye

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ABSTRACT

Ethnopharmacological relevance: The fruit of *Rubus coreanus* (Rosaceae) is traditionally used as an aphrodisiac, astringent, restorative and tonic in Asian countries. It is advised for treating diseases related to liver, kidney and urinary dysfunction, premature greying, blurred vision, infertility, impotence and premature ejaculation. Additionally, there is a long history of different parts of the plants being used in the treatment of ophthalmic diseases. However, no scientific studies have been undertaken to determine the effects of *Rubus coreanus* in visual processes of the vertebrate retina.
Aim of study: The purpose of the present study was to investigate the positive effects of *Rubus coreanus* extracts on visual processes in the vertebrate's eye.
Materials and methods: Electroretinogram (ERG) techniques were used to record the responses from a bullfrog's eye cup preparations. Active pharmacological agents were used to block specific receptors in the retina and to leave others unaffected. Lipid peroxidation in the retina was generated by adding $FeSO_4$ + Na-ascorbate.
Results: It was observed that both dark- and light-adapted ERG b-wave peak amplitude significantly increases with *Rubus coreanus* treatment. It was found that *Rubus coreanus* acts as a retinal neural antagonist but not as GABA receptor antagonist. *Rubus coreanus* treatment lowered the duration of rhodopsin regeneration. The results obtained indicated that *Rubus coreanus* protects against lipid peroxidation drop off ERG amplitude in retina.
Conclusion: Based on results obtained, it is suggested that *Rubus coreanus* can potentially improve visual sensitivity and can be used to treat pathophysiological conditions of eye.
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1. Introduction

Rubus represents one of the most diverse genus of plants, and *Rubus coreanus* (Rosaceae) is an important member distributed in Far East Asian countries (Kua and Mun, 2008a). Its unripe fruit has been used as a folk medicine in China, Japan and Korea for centuries (Bae et al., 2007). The constituents found in *Rubus coreanus* include phenolic acids, linoleic acid, linolenic acid, oleic acid, palmitic acid, organic acids, triterpenosides, flavonoids, gallotannin, and ellagitannin (Kua and Mun, 2008b). The various biological effects of *Rubus coreanus* include anti-oxidants, anti-fatigue, anti-fertility, improvement of sexual desire, astringent, anti-osteoporosis, ophthalmic, and anti-diabetic (Bae et al., 2007; Yoon et al., 2010). The extract of this plant improves the osteoblast function by increasing the proliferation and differentiation of osteoblast like MC3T3-E1 cells (Lee and Lee, 2008). It also increases the forced swimming capacity and decreases plasma ammonia accumulation in mice (Jung et al., 2007). Moreover, it inhibits collagen degradation by blocking matrix metallo protease production (Bae et al., 2007). Other activities of this plant include anti-cancer, anti-inflammatory, and bone protecting properties (Do et al., 2008).

Electroretinogram (ERG) is the measure of electric responses generated by layers of retina in response to a flash of light. ERG is a common tool for the evaluation of retinal functions and is clinically used for the diagnosis of retinal diseases (Carr and Siegel, 1982). It is also widely used in eye research because it provides information about the function of the retina that is not otherwise available. The common ERG response consists of a-, b-, c-, and d-waves produced by specific types of cells. The a-wave is produced in response to light by photoreceptors. The corneal positive b-wave is considered to be the activity of bipolar and Muller cells (Brown, 1968; Stockton and Slaughter, 1989; Wen and Oakley, 1990; Conn, 2008). The pigment epithelium is a black layer of cells located on the back of the retina and generates a typical c-wave in an ERG. The d-wave is the reflection of OFF bipolar and/or horizontal cell depolarization

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(Steinberg et al., 1970; Stockton and Slaughter, 1989). Among the ERG components, b-wave is the most sensitive, prominent and important to clinical studies. Therefore, the b-wave is a common choice of interest for many researchers as it is affected by changes in physical and chemical parameters. Clinically, these changes may be directly related to visual functions and eye sight (Linsenmeier et al., 1983; Dong and Hare, 2000).

Rubus Corenus has a long ethnopharmacological history for use to improve eye health (http://herb.daegu.go.kr/kor/index.asp) which has also been well documented in a very famous old Korean book Dongui Bogam. However, no scientific data has been reported to date about the effects of this plant in visual processes. Therefore, in the present study, an attempt was made to investigate the possible role of *Rubus coreanus* in visual processes of a vertebrate's eye using modern electrophysiological, biochemical, and molecular techniques. For this purpose, the activity of the extract derived from *Rubus coreanus* was tested on ERG sensitivity, ERG b-wave, visual sensitivity, and protective effects against lipid peroxidation damage to the visual processes. It was found that *Rubus coreanus* extract improves the ERG b-wave; increases ERG sensitivity, reduces the regeneration time of rhodopsin, and protects against lipid peroxidation damage to the visual processes and the ERG b-wave.

2. Materials and methods

2.1. Preparation of plant material

The dried fruits of *Rubus coreanus* were purchased from a local pharmacy in Seoul, South Korea. The voucher specimen (RC-FR-Kim002) was deposited in the main herbarium at the Department of Biology, Kyungpook National University, South Korea. The plant material (0.5 kg) was mechanically grinded and extracted at 40 °C with 80% (v/v) ethanol (5 L) for 6 h. The crude extract was filtered, and the filtrate was concentrated using a vacuum rotary evaporator (Laborota 4000, Heidolph, Japan). The concentrate was lyophilized in a freeze-dryer (Neocool, Yamato, Japan) and stored at −80 °C. The extract was dissolved in dimethyl sulfoxide (DMSO) before experiments.

2.2. Experimental animal

All experiments using animals and their care were performed according to our institutional guidelines, which completely follow the international laws and policies (NIH Guide for the Care and Use of Laboratory Animals, NIH publication no. 85-23, 1985). In the present study, bullfrogs (*Rana castesbeiana*) were used as the experimental animals, and were purchased from a local supplier in Deagu (South Korea).

2.3. Chemicals preparation and application

All the chemicals for preparation of Ringer's solution were purchased from Sigma–Aldrich. Active pharmacological compounds, picrotoxin (purity ≥ 98%, Sigma–Aldrich), and kynurenic acid (KYN) (purity ≥ 98%, St. Louis, MO) were purchased from Sigma. The chemicals were dissolved in Ringer's solution. In this study, 50 μM picrotoxin and 5 mM KYN were used as an optimal concentration. *Rubus coreanus* extract was used after 20–30 min of treatment of these pharmacological agents.

2.4. Sample preparation

For each experiment the animals were dark adapted for at least 1 h. For eye cup preparation, the animals were killed and the eyes were enucleated and dissected. The interior portion of the hemisect eyeball was cut off and the posterior portion was quickly mounted on the sample holder and placed in the modified Ussing chamber, located in the Faraday cage. Through both sides of the sclera and vitreous humor, the chamber was perfused with the bullfrog's basic Ringer solution under a continuous supply of oxygen. All the experimental steps were completed at room temperature with dim red light to avoid retinal damage.

2.5. Electrophysiological recordings

The recording system has optical devices which contained computer control stimulus and background light pathways. The stimulus beam was estimated to deliver flashes of 200 ms, and a background beam was used to provide a steady background light. The Halogen lamps (12 V/100 V) were used as a light source and were set through filters to deliver 505 nm of monochromatic light. The calibrated neutral-density and interference filters were placed to control the intensity and color of the light beams. For all protocols, a fixed set of stimuli was used in which the stimulation started with a low intensity, and then gradually increased to about 0.5 log steps. For the sensitivity of eyecup to be restored, enough inter flash duration was provided throughout the experiments. The inter-stimuli intervals were approximately 1 min at the lowest stimulus intensity and were slowly increased up to 4 min at the highest stimulus intensity. Without the neutral density filter, the stimulus light intensity was 2.48 candela seconds/meter square (cd s/m^2) and delivered 1.95×10^{14} photons/cm^2/s, while the background light intensity was 2.15 cd s/m^2 which delivered 8×10^{12} photons/cm^2/s on the eye cup. The light intensity and flash duration used in these experiments were slightly modified version of the International Society for Clinical Electrophysiology of Vision (ISCEV) standards. The modification was made due to the fact that the current experiments involved the eyecup recordings (which cannot be exposed to short duration high intensity light) as in clinical ERG recordings for which original ISCEV recommendations are available.

ERG recordings were performed with a fine glass micropipette Ag–AgCl agar bridge electrode filled with 3 M KCl in 3% agar and placed at the front and rear of the sample holder. The signal was amplified with a pre-amplifier (AI 417, Axon Instruments), and D.C. main amplifier (CyberAmp380, Axon Instruments). Subsequently, signals were filtered through an AD/DA converter (Digidata 1200A interface, Axon Instruments) and then stored on a computer. The data were analyzed with AxoScope 10.1 software (Axon Instruments, Inc.) and plotted with Excel and Sigma Plot (version 2001) software. Total gain for amplitude in the axoscope was 5000.

2.6. Statistical analysis

The data presented are the mean values ± SD (standard deviation) from 3 to 6 independent experiments. The data were calculated by Statistical Package for the Social Sciences (SPSS) software using students' *t*-test and where possible (multigroup comparisons), one-way analysis of variance (ANOVA). The significance of the differences between the means was determined by the Tukey range test. Statistical significance was at $p < 0.05$.

3. Results

3.1. Determination of optimal concentration for Rubus coreanus extracts treatment and its effect on ERG wave form

We performed experiments to find out the optimal concentration of *Rubus coreanus* treatment. During our experiment, we started with a low dose treatment and then gradually increase. It was observed that *Rubus coreanus* have dose dependent effect on ERG amplitude. Therefore, we performed repeated experiments

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