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Protective effect of a purified polyphenolic extract from *Ecklonia cava* against noise-induced hearing loss: Prevention of temporary threshold shift

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ABSTRACT

Objective: Noise is one of the most common causes of hearing loss. Approximately 16% of American teenagers (12–19 years) have hearing loss caused by loud noise. The implication of noise-induced hearing loss (NIHL) in teenagers has received increasing attention. Although temporary threshold shift (TTS), a type of NIHL, is a transient hearing loss, it can accelerate age-related hearing loss. Reactive oxygen species are a primary cause of TTS. As the polyphenols from *Ecklonia cava* are known to have potent antioxidant effects, we investigated the protective effects of a purified polyphenolic extract of *Ecklonia cava* (PPEE) against TTS in mice.

Methods: The radical-scavenging activity of PPEE was evaluated using the 1,1-diphenyl-2-picrylhydrazyl assay. The PPEE + Noise and Saline + Noise groups were administered intraperitoneal PPEE (100 mg/kg) and saline, respectively, for 5 days before exposure to noise at 100 dB SPL for 60 min. Hearing ability was assessed following noise exposure using auditory brainstem responses and distortion product otoacoustic emissions.

Results: PPEE exhibited significant radical scavenging activity. The ABR threshold shifts 1 day after exposure to noise at 16 kHz and 1, 7, and 14 days after exposure to noise at 32 kHz, were significantly less in the PPEE + Noise than in the Saline + Noise group. One day after noise exposure, mice in the PPEE + Noise group showed a significant degree of protection in relation to their DPOAE level at f2, 17, and 28 kHz.

Conclusions: These findings suggest that PPEE may be a potential preventive agent against TTS. In addition, as a food ingredient approved by the United States Food and Drug Administration, PPEE may be administered to those who are exposed to noise inevitably with little likelihood of adverse effects, thereby contributing to the prevention of TTS.

1. Introduction

Noise is one of the most common causes of hearing loss [1]. Noise-induced hearing loss (NIHL) is one of the most prevalent occupational disorders in industrialized countries [2,3]. According to the National Institute on Deafness and other Communication Disorders, noise is estimated to cause high-frequency hearing loss in approximately 15% of Americans between the ages of 20 and 69

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years (26 million people) [4]. Noise is one of the most significant causes of hearing loss in adults. Moreover, the implication of NIHL in teenagers has received increasing attention. The Centers for Disease Control and Prevention estimates that approximately 16% of American teenagers (12–19 years) have hearing loss caused by loud noise [5]. Given the considerable projected medical costs, NIHL is a significant social, clinical, and economical issue [6]. There are two types of NIHL: permanent threshold shift (PTS) and temporary threshold shift (TTS). Permanent hearing loss occurs in PTS, whereas hearing loss is recovered after a period of time in TTS. Therefore TTS had been considered not significant traditionally. However, several recent studies have been reported that TTS can accelerate age-related hearing loss by inducing synaptopathy [7–9]. Sequentially, Prevention of TTS has been receiving increased attention.

Previously, NIHL was thought to be caused by mechanical trauma, and the recommended precautions involved reducing or avoiding exposure to prolonged or intense noise [10,11]. However, these preventative measures are not sufficient for individuals who cannot avoid or reduce their exposure to noise, such as soldiers and construction workers [2]. Recently, reactive oxygen species (ROS) were identified as a cause of NIHL, and the use of antioxidants to prevent the disorder has been investigated [12–14]. However preventative treatments must be administered before the development of NIHL. Given the potential adverse effects of drugs, it is risky to prescribe them as a preventative treatment without knowing whether NIHL will develop; however, a food ingredient presents fewer risks.

Brown algae have long been used in traditional foods and folk medicine in Asian countries. Among the many brown algae species, *Ecklonia cava* produces unique polyphenols called eckols. Although *Ecklonia cava* produces several potentially medicinal polysaccharides and lipids, such as fucoidan, laminarin, fucoxanthin, and fucosterol, an increasing number of reports have indicated that many of the medicinal properties of this brown alga are derived from eckol and its derivatives [15–18]. Recently, various *in vitro* and *in vivo* studies have demonstrated that eckols have a broad range of bioactivities including radical scavenging, matrix metalloproteinase inhibitory, protease inhibitory, cytoprotective, and anti-inflammatory effects [19–21]. In this study, we investigated the protective effect of the purified polyphenolic extract from *Ecklonia cava* (PPEE) against temporary threshold shift (TTS) in an animal model of NIHL.

2. Materials and methods

2.1. Preparation of PPEE

PPEE was supplied by Botamedi Inc (Jeju, Korea) as a light brown powder. It was prepared as follows. *Ecklonia cava* was washed in a copious amount of water to remove salt and water-soluble components, and was then extracted with 95% ethanol. The extract was separated and concentrated *in vacuo* into a dark brown powder, and was further extracted using diethyl ether. The resulting extract was concentrated *in vacuo* to yield a light brown powder (PPEE). The compounds in PPEE were analyzed by high performance liquid chromatography (Waters, CAPCELL PAK ODS column [4.6 × 250 mm]; eluent: gradients 15% → 70% aqueous MeOH; flow rate: 0.8 mL/min; UV detector at 254 nm).

2.2. 1,1-Diphenyl-2-picrylhydrazyl (DPPH) assay

PPEE was diluted in distilled water to obtain the experimental concentrations (0, 1, 5, 10, 50, 100, and 200 μg/mL). 1,1-Diphenyl-2-picrylhydrazyl (DPPH) powder (Sigma) was mixed in 95% ethanol to create a 1 M stock. PPEE (in 95% ethanol) and 50% ethanol were aliquoted into individual wells of a 96-well plate. The DPPH solution was then added to each well, and was allowed to react for 30 min at room temperature in the dark. Absorbance was measured at 540 nm using a microplate reader. Scavenging activity was calculated using the following formula: Scavenging activity = (1−[PPEE value−Blank]/Control) × 100. Two wells were used for each concentration. The experiment was repeated twice.

2.3. Animals

Six-week-old male C57BL/6 mice weighing 20 g–25 g were purchased from Orient Bio Inc. (Seongnam, Korea). The mice were fed a standard commercial diet, and housed in a facility with an ambient temperature of 20 °C–22 °C and a relative humidity of 50% ± 5% under a 12:12 h light/dark cycle. All of the animal studies were approved by the Institutional Animal Care and Use Committee of Soonchunhyang University School of Medicine (SCHBC_2010-09).

2.4. Noise exposure and development of a mouse model for TTS

The noise exposure protocol was as follows: six mice were individually placed in a sound isolation booth and exposed to 100 or 120 dB SPL (sound pressure level) of noise for 60 min. The specially designed noise exposure apparatus consisted of an acrylic frame (53 × 35 × 53 × 2 cm [length × width × height × thickness]; Seoul, Korea) with a speaker attached to the top. White noise (10 kHz) was generated using sine-random generators (Type 1027; Brüel & Kjær, Nærum, Denmark). The overall noise level was measured at the center of the cage using a Brüel & Kjær 4135 1/4-inch microphone in combination with a Brüel & Kjær 2144 frequency analyzer/sound level meter, and Type 2690, 2669, 4231, Tektronix AM700 audio measurement set to broadband (0.2–70.0 kHz). Following noise exposure, auditory brainstem response (ABR) to tone bursts at 16 and 32 kHz were recorded to measure the animals' hearing thresholds on day 1, 7, 14 and 21 post-noise exposure. These results were used to determine the optimal protocol to produce TTS.

2.5. Experimental groups

Nineteen mice were assigned to either the baseline (n = 3), PPEE + Noise (n = 8) or Saline + Noise (n = 8) group. The PPEE + Noise group underwent intraperitoneal injections of PPEE (100 mg/kg; BotaMedi Inc., Jeju, Korea) for 5 days prior to noise exposure, and the Saline + Noise group were administered intraperitoneal injections of saline for 5 days and served as the control group for the evaluation of hearing function. Following treatment, the mice from the PPEE + Noise and Saline + Noise groups were exposed to 100 dB SPL of noise for 60 min. The baseline group was not exposed to noise.

2.6. Hair cell staining and counts

To validate the TTS protocol, three mice from each group (baseline, Saline + Noise and PPEE + Noise groups) were sacrificed 1 day after exposure to 100 dB SPL of noise for 60 min, and perfused through the heart with 4% paraformaldehyde in phosphate-buffered saline (PBS). After decapitation, the entire temporal bone was removed, and the cochleae were dissected for morphological evaluation of the hair cells. The cochleae were immersed in 4% paraformaldehyde in PBS for 24 h at 4 °C, and were then rinsed in several changes in 1 × PBS. The bony wall was removed completely to expose the spiral lamina. Reissner's membrane was removed to