学 位 論 文 の 要 旨

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学 位 論 文 名　Protection of Rat Retina Against Light-induced Damage by Intraperitoneal and Oral Administration of a Polyphenol Fraction From Seed Shells of Japanese Horse Chestnuts (*Aesculus turbinata* BLUME)

発 表 雑 誌 名（巻，初頁～終頁，年）　Shimane Journal of Medical Science (in press)

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論 文 内 容 の 要 旨

INTRODUCTION

Age-related macular degeneration (AMD), progressive blinding disease with no cure, is a product of aging and long-term light exposure. Therefore, preventive medicine is required, especially in countries with an aging population. Because oxidized retinal phospholipids increase with aging, a cause of AMD might be oxidative stress. Retinal tissue is exposed to oxidative stress resulting from reactive oxygen species caused by strong visible light, too. A rat model of light damage used in AMD studies clarified that antioxidants, such as ascorbic acid, dimethylurea, N-acetylcysteine, and phenyl N-tert-butyl nitrone (PBN), decrease retinal light damage. In these studies, the drugs were administrated intraperitoneally. Especially, PBN has been used extensively as a positive control in the retinal light damage model, but PBN is not approved for human use because it cannot be administered orally.

The seeds of the Japanese horse chestnuts (*Aesculus turbinata* BLUME) have been used as emergency food since antiquity, and used as an ingredient in the rice cakes and rice balls today. The seed shells of Japanese horse chestnuts, i.e., waste products in preparation of edible seeds, contain polyphenolic compounds (flavonol *O*-glycosides and highly polymeric A-type proanthocyanidin) with high antioxidant activity.

In this study, we investigated to clarify the retinal protective effects of the polyphenol fraction from the seed shells of Japanese horse chestnuts in the light-induced retinal damage

model. To compare the polyphenol fraction and PBN, like PBN, we tested the polyphenol fraction by intraperitoneal administration. Moreover, we examined the protective effect of oral administration of the polyphenol fraction against light-induced retinal damage. We found that oral administration of the polyphenol fraction prevents retinas from light-induced damage.

MATERIALS AND METHODS

The seed shells of Japanese horse chestnuts were refluxed by boiling in distilled water. The polyphenol fraction was separated from the extract with column chromatography on the Diaion HP-20 and Chromatorex ODS 1024T. The antioxidant activities of the polyphenol fraction were measured by hydrophilic oxygen radical absorbance capacity (H-ORAC) and 1,1-diphenyl-2-picrylhydrazyl (DPPH) methods. To compare the effects of the polyphenol fraction with a positive control (phenyl N-tert-butyl nitrone [PBN]), received the polyphenol fraction was administrated intraperitoneally (IP) into the rats. We also evaluated oral administration (PO; per os) of the polyphenol fraction against light damage. To determine the retinal histologic damage, the outer nuclear layer (ONL) thickness was measured on hematoxylin and eosin-stained sections, and then, the ONL area under the curve (AUC) was compared. To determine the retinal functional damage, the electroretinograms (ERGs) were recorded.

All experiments with animals in this study were approved by the Ethics Committee for Animal Experimentation of Shimane University and they were handled according to our institutional guidelines.

RESULTS AND DISCUSSION

The polyphenol fraction (2 g) was obtained from the seed shells (16.2 g, dry weight [DW]). The antioxidant activity measurement indicate that the H-ORAC value of the polyphenol fraction was 6.90 mmol Torolox equivalent (TE) /g dry weight (DW) and DPPH radical-scavenging activity was 4.94 mmol TE /g DW. The H-ORAC value of PF was 3.2-fold higher than that of ascorbic acid which prevents light damage in retinas. IP of the polyphenol fraction (100 mg/kg body weight [BW]) and PBN (50 mg/kg BW) significantly ($P < 0.05$) suppressed the ERG amplitude decreases. The polyphenol fraction (25, 50, and 100 mg/kg BW) and PBN (50 mg/kg BW) suppressed decreases in the ONL thickness. PO of the polyphenol fraction (100 mg/kg BW) and IP of PBN (10 and 50 mg/kg BW) protected the rat retina from the damaging effects of light stress morphologically and functionally. Therefore, the results suggested that oral administrated the polyphenol fraction protects eyes from light exposure. In summary, the polyphenol fraction of the seed shells in Japanese horse chestnuts protected the retina from light exposure damage by inhibiting oxidative stress.

CONCLUSION

Our results indicate that intraperitoneal and oral administration of the polyphenol fraction alleviates light-induced retinal damage by inhibiting oxidative stress. Consequently, ingestion of the polyphenols from the seed shells of Japanese horse chestnuts is potentially useful to prevent AMD caused by oxidative stress.