Ingestion of IH636 grape seed proanthocyanidin extract to prevent selenite-induced oxidative stress in experimental cataract

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PURPOSE: To investigate whether dietary supplementation with IH636 grape seed proanthocyanidin extract (GSPE) prevents selenite-induced cataract.

SETTING: Department of Ophthalmology, Gulhane Military Medical Academy, Ankara, Turkey.

METHODS: Thirty Spraque-Dawley rat litters were put randomly into 3 groups. In group 1 (n = 10), sodium selenite (30 nmol/g body weight) was injected subcutaneously on postpartum day 10. In group 2 (n = 10), sodium selenite (30 nmol/g body weight) was injected on postpartum day 10 and oral GSPE (100 mg/kg body weight) was given for 1 week after sodium selenite injection. Only subcutaneous saline was injected in group 3 (control, n = 10). The development of cataract was assessed for 3 weeks, and its density was graded and photographed with a slitlamp. Removed rat lenses were analyzed for glutathione (GSH) and malondialdehyde (MDA).

RESULTS: All of the rats in group 1 had cataract between stage 6 and stage 3. In group 2, only 5 of 10 eyes had cataract between stage 3 and stage 2 and no cataract occurred in the remaining 5 rats. The difference between mean cataract stages in group 1 and group 2 was significant ($P<.05$). The mean GSH level in group 1 was significantly lower than in group 2 and controls ($P<.05$). The mean MDA level in group 1 was significantly higher than in group 2 and controls ($P<.05$).

CONCLUSIONS: IH636 grape seed proanthocyanidin extract effectively suppressed cataract formation in rats. Routine consumption of grape seed proanthocyanidin extract in the form of food or dietary supplement may offer a prophylactic measure against onset and progression of cataract.

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Selenite cataract is a rapid and convenient model of nuclear cataract produced in young rats by an overdose of the essential trace mineral selenium. It has been used in many experimental studies as a useful in vivo rodent model since 1978.[1] Selenite induces bilateral nuclear cataract within 4 to 6 days when administered to suckling rat pups before completion of the critical maturation period of the lens. Most pronounced biochemical mechanisms occur during development of selenite-induced cataract are altered epithelial metabolism, calcium accumulation, calpain-induced proteolysis, crystalline precipitation, phase transition, and cytoskeletal loss.[2] Although selenite cataract shows no high molecular weight covalent aggregates or increased disulfide formation, it has many similarities to human cataract such as vesicle formation, increased calcium, insoluble protein, proteolysis, decreased water soluble proteins, and glutathione (GSH).[2]

Reduced GSH has been implicated to play a significant role in the maintenance of the reduced state in the lens. The GSH decreases with cataract formation after selenite injection, and loss of reduced GSH from the nuclear region of the lens is supposed to be the crucial feature that precedes age-related cataract formation.[3] In addition, administration of selenite has been shown to increase peroxidation of lens lipids as well as formation of hydrogen peroxide in the aqueous.[4] Furthermore, physiological antioxidants such as pyruvate and nutritional antioxidants such as ascorbate, vitamin E, and carotenoids were found to delay the development of experimental cataract.[5,6] Thus, despite several possible modes of biochemical effects, selenite is considered

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as possibly acting as an oxidant, thereby contributing to the cataract formation.

IH636 grape seed proanthocyanidin extract (GSPE) has been reported to be a highly potent, safe, and bioavailable free radical scavenger that possesses a broad spectrum of pharmacological and medicinal properties against oxidative stress and provides excellent protection against free radicals in both in vitro and in vivo models.[7,8] Furthermore, GSPE has significantly superior scavenging ability to vitamins C, E, and β-carotene.[9] In this study, we assessed whether oral administration of a grape seed proanthocyanidin extract prevents cataract formation in a selenite-induced experimental cataract model as well as the status of GSH and malondialdehyde (MDA) as a marker of lipid peroxidation in removed rat lenses.

MATERIALS AND METHODS

The Ethics Committee of Gülhane Military Medical Academy reviewed the study, and the experiments were conducted in accordance with the ARVO Statement for Use of Animals in Ophthalmic and Vision Research and Guiding Principles in the Care and Use of Animals.

Spraque-Dawley rat litters, housed with their mothers, were divided into 3 equal groups (2 experimental and 1 control). In group 1 (n = 10), sodium selenite (30 nmol/g body weight (Sigma Chem Co.) was injected subcutaneously on postpartum day 10. In group 2 (n = 10), subcutaneous sodium selenite (30 nmol/g body weight) was injected on postpartum day 10 and oral IH636 grape seed proanthocyanidin extract (100 mg/kg body weight; ActiVin, InterHealth Nutraceuticals Inc.) was given. The GSPE dose was continued orally for 1 week after sodium selenite injection. Group 3 (control, n = 10) received only subcutaneous saline injections. The development of cataract was assessed for 3 weeks, and its density was graded and photographed with a Nikon F3-3V zoom-photograph slitlamp (Figure 1).

At the final examination, the pupils were dilated and staging of the selenite cataract was performed by slitlamp biomicroscopy on a scale of 6 to 0. Stage 6 was mature dense opacity involving the entire lens. Stage 5 was nuclear opacity not involving the lens cortex. Stage 4 was partial nuclear opacity. Stage 3 was diffuse nuclear opacity with cortical scatters. Stage 2 was slight nuclear opacity with swollen fibers or posterior subcapsular scatterings. Stage 1 was initial sign of posterior subcapsular or nuclear opacity involving tiny scatters, and stage 0 was a normal transparent lens.[10]

Reduced Glutathione and Malondialdehyde Analysis

The litters were killed, and their eyes were enucleated at the end of the study. The lenses were removed intracapsularly using a posterior approach. Removed rat lenses were analyzed for GSH and MDA.

After all lenses were rinsed thoroughly with physiological saline, 4 lenses at a time from each group were homogenized in 1mL of distilled water. Clear supernatant was obtained by centrifugation in glass tubes at 6000*g* for 30 minutes and used for the measurements.

Reduced glutathione was estimated by Ellman's method.[11] One half milliliter of 10% trichloroacetic acid solution was added to 0.5 mL of aqueous lens extract. A protein-free supernatant was obtained by centrifugation. Four milliliters of 0.3 mol/L Na_2HPO_4 and 0.5 mL of a 5,5"-dithiobis (2-nitrobenzoic acid) (DTNB) were added to 0.5 mL of this supernatant. The intensity of the resulting yellow color was read spectrophotometrically at 410 nm. The DTNB reagent was prepared by dissolving 4 mg DTNB in 10 mL of a 1% trisodium citrate solution. The GSH content was calculated by comparing the absorption of the sample treated as above with that of the standard run simultaneously. The GSH concentrations in the lenses were expressed as micromoles per gram of wet weight (μmol/g wet weight).

The MDA content was determined by the method of Ohkawa et al.[12] Then, 0.2 mL of sodium dodecyl sulfate (SDS) 8.1%, 1.5 mL of 3 mol/L acetate buffer (pH 3.5), and 1.5 mL of aqueous thiobarbituric acid 0.82% were added in succession to 0.5 mL of lens homogenate. The mixture was heated in a boiling water bath for 45 minutes. After the mixture was cooled to room temperature, 0.5 mL of SDS 35% was added and the tubes were heated as above for an additional 10 minutes. The mixture was then cooled and centrifuged, and the optical density of the supernatant was read at 532 nm. The MDA content was calculated by reference to the optical density of the standard prepared from 1,1",3,3"-tetramethoxy propane. The MDA concentrations in lenses were expressed as nanomoles per gram of wet weight (nmol/g wet weight).

Statistical Analysis

The analyses of the results were carried out with SPSS statistical software. All data were analyzed statistically using the Kruskal-Wallis 1-way analysis of variance or the Mann-Whitney U test as indicated. The results are expressed as mean ± SD, and a *P* value less than 0.05 was considered statistically significant.

RESULTS

All rats in group 1 had cataract between stage 6 and stage 3. In group 2, only 5 of 10 eyes had cataract between stage 3 and stage 2 and no cataract occurred in the remaining 5 rats. The mean cataract stage in group 1 and group 2 was 4.50 ± 1.35 and 2 ± 1.25, respectively, and the difference was significant ($P < .05$; Figure 2). All control lenses (group 3) were clear.

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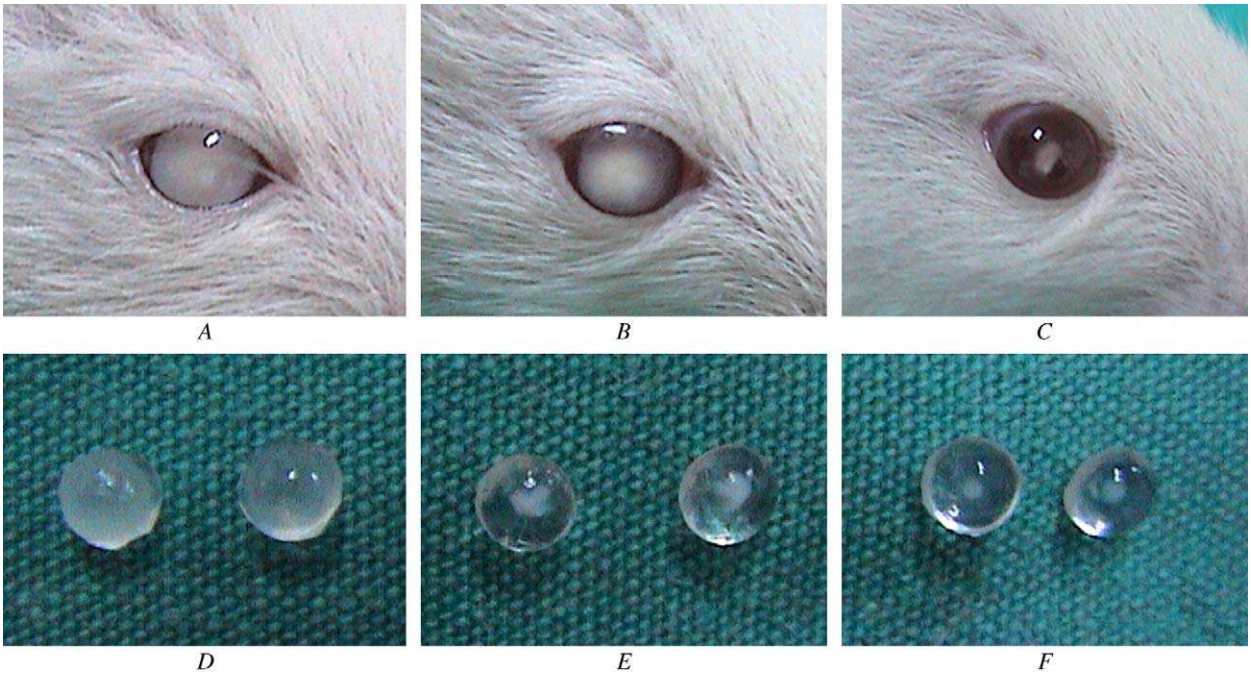

Figure 1. Selenite-induced cataract stages. *A*: Stage 6, mature dense opacity involving the entire lens. *B*: Stage 5, nuclear opacity not involving the lens cortex. *C*: Stage 4, partial nuclear opacity. *D*: Stage 3, diffuse nuclear opacity with cortical scatters. *E*: Stage 2, slight nuclear opacity with swollen fibers or posterior subcapsular scatterings. *F*: Stage 1, initial sign of posterior subcapsular or nuclear opacity involving tiny scatters.

The mean GSH level in group 1 (4.38 ± 0.14 µmol/g wet weight) was significantly lower than that in group 2 (8.72 ± 0.87 µmol/g wet weight; $P<.05$) and in controls (11.93 ± 0.54 µmol/g wet weight; $P<.05$; Figure 3). The mean MDA level in group 1 (9.39 ± 1.12 nmol/g wet weight) was significantly higher than that in group 2 (6.12 ± 0.96 nmol/g wet weight; $P<.05$) and in controls (5.63 ± 0.92 nmol/g wet weight; $P<.05$; Figure 4).

DISCUSSION

Age-related cataract is a significant problem worldwide. Yet, a pharmacological treatment against cataract has not been achieved. Although it is a multifactorial disease associated with several risk factors, oxidative stress has been suggested as a common underlying mechanism of cataractogenesis, and augmentation of the antioxidant defenses of the lens has been shown to prevent or delay

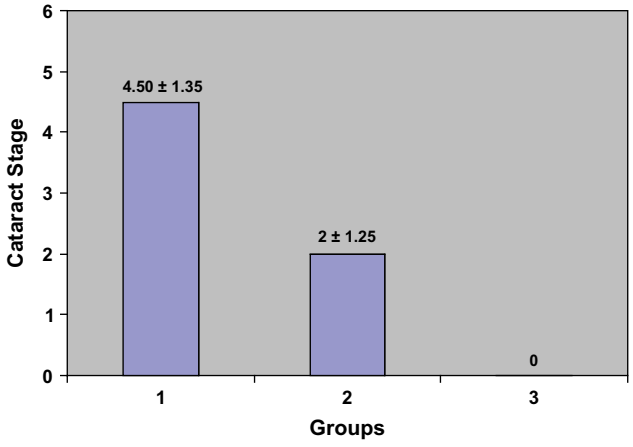

Figure 2. Mean cataract stages in group 1 (selenite-treated rats), group 2 (selenite- and IH636-treated rats), and group 3 (saline as control).

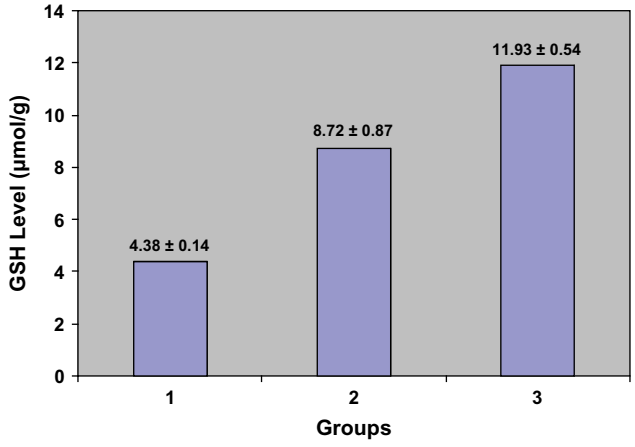

Figure 3. Mean GSH level in group 1 (selenite-treated rats), group 2 (selenite- and IH636-treated rats), and group 3 (saline as control).

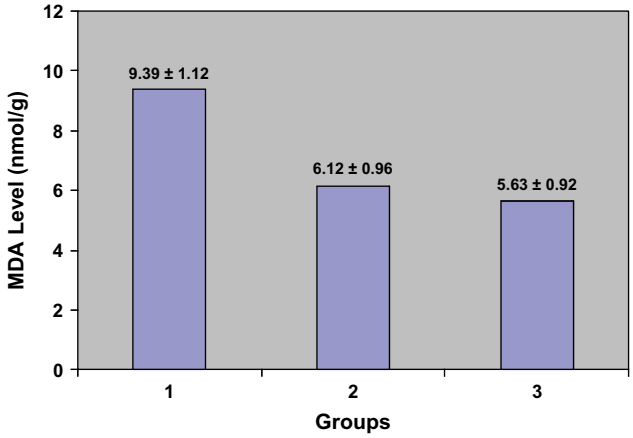

Figure 4. Mean MDA level in group 1 (selenite-treated rats), group 2 (selenite- and IH636-treated rats), and group 3 (saline as control).

experimental cataract.[13] In addition, superoxide dismutase, GSH peroxidase, and catalase were found to act as defense enzymes to the oxidative stress in cataract.[14] The crystalline lens contains a high concentration of GSH that protects the lens from oxidant damage and toxic chemicals.[15] Human senile or experimental cataract formation is associated with progressive GSH decrease in the lens.[15,16] Therefore, it is suggested that GSH plays an important role in maintaining the lens function and transparency by protecting the sulfhydryl groups from oxidation.[16,17]

Various experimental models have been developed to delineate the mechanisms of cataractogenesis and focus the identification of the crucial targets. Selenite cataract was found to be the most reliable and reproducible animal model compared with radiation, galactose, streptozotocin, and Royal College of Surgeons models, especially for advanced cataract evaluation.[18]

Selenite-induced cataractogenesis in young rats has been shown to mimic human senile cataract with respect to several morphological and biochemical changes in the lens.[19] Although various biochemical changes associated with selenite-induced cataract have been reported, the mode of selenite action is not well understood. It is hypothesized that the early changes in the lens epithelium may result from oxidative damage caused by selenite, possibly to critical sulfhydryl groups on molecules such as Ca-ATPase or ion channels. Thus, increased lens calcium may be caused by oxidation of sulfhydryls and other changes in the membranes caused by selenite, leading to inhibition of the Ca-ATPase pump and selective calcium permeability. It is proposed that the loss of calcium homeostasis could be prevented by antioxidants.[2]

Recently, great emphasis has been placed on the possible roles of physiological and nutritional antioxidants in cataract. Various pharmacological agents including antioxidants, thiophosphates, disulfides, and chelators have been used against cataract development,[20] and efforts are still ongoing to find an antioxidant that can be consumed daily as a part of our diet.

Oligomeric proanthocyanidins, naturally occurring antioxidants widely available in fruits, vegetables, nuts, seeds, flowers and bark, have been reported to possess a broad spectrum of biological, pharmacological, and therapeutic activities against free radicals and oxidative stress.[7] IH636 grape seed proanthocyanidin extract has been reported to be a highly potent, safe, and bioavailable free radical scavenger and is widely used as nutritional support and a food additive.[7] The GSPE exhibits free radical scavenging abilities toward biologically generated free radicals, such as superoxide anion, hydroxyl radicals, and peroxyl radicals, and exhibits superior performance compared with vitamins C, E, and β-carotene.[9] Besides the free radical scavenging and antioxidant activity, proanthocyanidins modulate immune function and platelet activity and produce vasorelaxation by inducing NO release from endothelium.[21] It has been found that proanthocyanidin content in the plasma can be maintained after regular intake of sufficient quantity of fresh fruits and vegetables or supplementation of bioavailable proanthocyanidins.[22] The GSPE was also examined for acute and subchronic oral toxicity and for mutagenic potential. The results indicated a lack of toxicity and supported use of proanthocyanidin-rich extracts from grape seeds in various foods.[23,24]

It is known that selenite induces a significant depletion of GSH and increases membrane damage as indicated by the levels of MDA.[25] We observed that grape seed proanthocyanidin (IH636) extract prevented cataract development in at least half the eyes and reduced the amount of opacity in the rest. This effect was associated with higher GSH levels and lower MDA concentrations. Restoration of GSH and MDA levels and maintenance of lens clarity without a doubt establish the protective action of GSPE. Possibly, GSPE may be preventing inhibition of the Ca-ATPase pump and selective calcium permeability by means of scavenging reactive oxygen radicals.[26,27]

In a recent study, proanthocyanidins derived from cacao (CLP) were tested for their preventive effect on cataract development in rats with diabetes induced by streptozotocin.[28] The authors found that dietary supplementation with CLP did not affect hyperglycemia but made a significant change in production of hydroxynonenal, a marker of oxidative stress. Hydroxynonenal was only detected when rats were fed a normal diet without CLP. These observations suggested that the CLP may prevent cataract formation by decreasing oxidative stress induced by hyperglycemia.[28]

An IH636 grape seed proanthocyanidin extract effectively suppressed cataract formation in rats. The

protective effect was supported by lower GSH and higher MDA levels. The data clearly showed that grape seed proanthocyanidin significantly improves the antioxidant defense mechanisms of the lens. Because proanthocyanidin extract has no known harmful effect, routine consumption of grape seed proanthocyanidin extract in the form of food or a dietary supplement may offer a prophylactic measure against onset and progression of cataract.

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