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Research Article
Protective Effects of Quercetin on Selenium-Induced Cataracts via Modulation of Heat Shock Protein 70 Expression

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Abstract

Background and Aim: Cataracts have the highest incidence among eye diseases, particularly blindness. Quercetin as a major flavonoid in the diet, can prevent cataracts. This study was aimed at investigating the molecular mechanism of the protective effects of quercetin in selenium-induced cataracts in Wistar rat. **Materials and Methods:** Ten-day old Wistar rats were randomly divided into four groups, all treated by intraperitoneal injection with 20 μM kg^{-1} sodium selenite to induce cataracts. The groups were a model group with no further treatment (A), a heated bath group (B), an Alanine-Glutamine treatment group (1.125 g kg^{-1}; group, C) and a quercetin treatment group (400 mg kg^{-1}, i.p., D). Expression level of HSP70 was determined by quantitative real-time PCR and immunohistochemistry. 8-Hydroxy-2-deoxyguanosine (8-OHdG), superoxide dismutase (SOD) and catalase (CAT) were assessed by ELISA assay. **Results:** The selenium-induced cataract model in rats was established successfully. The expression level of HSP70 in the groups B and C was significantly higher than that in group A. In contrast, the expression level of HSP70 in group D was significantly lower than that in group A ($p<0.05$). 8-OHdG levels in lens epithelial cells of group D were significantly lower than those in the cells of group A ($p<0.05$). CAT and SOD activities were enhanced to a much greater extent in group D than in group A ($p<0.05$). **Conclusion:** Quercetin has a protective effect on selenium-induced cataracts and probably modulates the expression of HSP70 to maintain homeostasis of oxidative stress.

Key words: Selenium-induced cataract, HSP70, quercetin, oxidative stress, Wistar rat

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Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Cataracts have the highest incidence among the eye diseases and can result in blindness[1]. Almost half the cases of blindness around the world are caused by cataracts. Moreover, new cases of cataracts and blindness increase annually[2,3]. At present, the cataract is predominantly classified into two categories: congenital and acquired. However, the acquired cataract accounts for more than 95% of all cataract cases[4]. Investigating the pathogenesis of cataracts and approaches to prevent or delay their occurrence is an important issue for clinical and scientific researchers[5].

Oxidative stress is implicated in the initiation of maturity-onset cataracts[6,7]. Stress injury is caused by active oxygen free radicals that are generated in the lens and cannot be eliminated in time by antioxidant enzymes[8]. Studies have shown that antioxidants from phytochemical materials can prevent cataracts[9]. Quercetin is the major flavonoid in the diet, thus, quercetin and its metabolites may have a significant protective effect on cataracts. These molecules inhibit opacification by reducing oxidative damage in the lens[10]. Therefore, it is possible that quercetin could remove free radicals and produce beneficial outcomes with regard to lens opacities.

In addition, extensive research has demonstrated that oxidative damage occurs in cellular membranes, proteins and DNA[11,12]. 8-Hydroxy-2-deoxyguanosine (8-OHdG) has been broadly used as a biomarker of elevated DNA oxidative damage or oxidative stress[13] and levels of 8-OHdG in cataract patients are higher than those in healthy individuals[14]. When oxidative stress levels change, the homeostasis of reactive oxygen species (ROS) and the antioxidant system is disturbed and ROS cannot be effectively eliminated by antioxidant enzymes such as catalase (CAT) and superoxide dismutase (SOD)[15]. Heat shock proteins (HSPs) are a family of stress-responsive proteins involved in processes such as hyperthermia, oxidative stress and aging[16]. HSP70, a major member of the HSP family, exhibits high expression levels in the lenses of cataract patients[17], thus it is presumed to be involved in cataract pathogenicity, although the mechanisms of this involvement remain unknown.

The mechanism of quercetin's effects on cataracts are also undefined and the purpose of this study was to use the selenium-induced cataract model to investigate its effects on antioxidant enzyme activities such as CAT and SOD as well as on oxidative damage as evaluated by the changes in 8-OHdG. The potential mechanisms of the protective effect of quercetin in this model also discussed.

MATERIALS AND METHODS

Animals: Ten-day-old male specific pathogen-free Wistar rats were purchased from Harbin Medical University in September, 2016. Experimental animals were housed at 28-30°C and 45-65% relative humidity under a 12 h light/12 h dark cycle in the animal laboratory. The rats were fed with formula milk by stomach intubation. Experiments were approved by the Animal Procedures Committee of Harbin Medical University before initiation. The rats were handled and euthanized according to ethical guidelines.

Animal cataract model: Rats were randomly divided into either control or experimental groups (8 rats each). After acclimatization for 3 days, rats in the control group were given normal saline by intraperitoneal injection (i.p.) without any other treatment. Rats in experimental groups were given a one-time administration of sodium selenite (Sinopharm Chemical Reagent Co., Ltd, China, dissolved in saline, 20 μmol kg^{-1} b.wt.) by intraperitoneal injection when they were 14 days old. Eye lenses in all rats were observed daily and the degree of lens opacity was examined with a slit-lamp biomicroscope (STXO-JC, ZEISS, Germany)[18]. After 1 week, rats were sacrificed by cervical rupture and their lens opacities were graded according to Table 1. At the start of the experiment, all lenses were defined as having no cataract (Grade 0).

Experimental design: To explore the effect of quercetin on selenium-induced cataracts, four experimental groups were used (8 rats in each group): The model group (A), the heating bath group (B), the Alanine-Glutamine (Ala-Gln) group (C) and the quercetin (3, 3, 4, 5, 7-pentahydroxyflavone, Sinopharm Chemical Reagent Co., Ltd, China) group (D). Rats in all groups were given a one-time administration of sodium selenite (20 μM kg^{-1} body weight, i.p.) at the start of the experiment. In addition, rats in group B were given a heat shock pretreatment by immersion in a hot water bath (maintaining an anal temperature of 42°C for 15 min) following sedation with 10% chloral hydrate (Sinopharm Chemical Reagent Co.,Ltd, China) and this treatment was continued every 2 days throughout the duration of the experiment. Rats in group C were administered Ala-Gln (1.125 g kg^{-1}, i.p.) daily and rats in group D were treated with quercetin (400 mg kg^{-1}, i.p.) both 24 h prior to the sodium selenite administration and daily thereafter.

Table 1: Grade of lens opacities

Grades	Degree of lens opacity
0	Normal clear lens
1	Initial sign of posterior subcapsular or nuclear opacity involving tiny scatter
2	Slight nuclear opacity involving tiny scatter
3	Moderate opacity between slight and diffuse nuclear opacity involving tiny scatter
4	Diffuse nuclear opacity with cortical scattering
5	Partial nuclear opacity
6	Heavy nuclear opacity

Lens isolation: One week after treatment, rats were sacrificed and their eyes were removed. The eye lens tissues were immediately dissected out, marked and fixed in 4% paraformaldehyde (Wuhan Google biological science and Technology Co., Ltd. China) and frozen at -80°C[19].

Hematoxylin-eosin (H and E) staining: Fixed lenses were dehydrated using a serial alcohol gradient (70-100%) and embedded in paraffin wax. Before staining, lens tissue sections (4 μm thick) were dewaxed in xylene, rehydrated from 100% ethanol (analytically pure) to 70% ethanol (analytically pure), washed in double distilled water and stained with H and E[20]. After staining, sections were dehydrated and cleared through increasing concentrations of ethanol and xylene, respectively.

Quantitative real-time PCR (qPCR): Total RNA was extracted from lenses using Trizol reagent following the manufacturer's instructions (TAKARA, Dalian, China). First-strand cDNA was prepared from total lens RNA using the TaKaRa PrimeScript II 1st Strand cDNA Synthesis Kit according to the manufacturer's instructions (D6210A, TAKARA, Dalian, China). The PCR was performed with gene-specific primers designed using Primer Expression 3.0 software (ABI). Rat glyceraldehydes-3-phosphate dehydrogenase (GAPDH) was used as an endogenous control. The primers used for HSP70 and GAPDH were based on the rat sequences 5-CGGTAGAGGCCCGTCTTTTT-3 (forward) and 5-GCAGCGGTCGCTATACTCAT-3 (reverse) and 5-TGCCACTCAGAAGACTGTGG-3 (forward) and 5-TTCAGCTCTGGGATGACCTT-3 (reverse), respectively. The following cycling conditions were used: 120 sec of initial denaturation at 50°C, followed by 10 min at 95°C, then 40 cycles of 15 sec at 95°C and 60 sec at 60°C. The relative amounts of mRNAs obtained were determined using the Relative Expression Software Tool (REST).

Immunohistochemical staining: Fixed eye lens tissues were paraffin embedded, sectioned and processed for immunohistochemical staining. For immunohistochemistry, sections were incubated with mouse monoclonal antibodies (1:500, SC-24, Santa Cruz Biotechnology, Santa Cruz, Canada), followed by incubation with peroxidase-conjugated secondary antibodies (1:5000, UltraSensitive™ S-P Mouse/Rabbit IHC Kit, MXB Biotechnology, Fuzhou, China). HSP70-positive cells from HSP70 immunohistochemical staining were counted. The number of positive cells and the level of HSP70 expression were measured for statistical analysis.

Enzyme-linked immunosorbent assay (ELISA) for 8-OhdG: Precooled physiological saline was added to lens tissues at a volume ratio of 9:1. Subsequently, samples were homogenized with a high-speed tissue homogenizer (TIANGEN, Beijing, China) at 12000 rpm for 2 min at 4°C. The homogenate was centrifuged at 2000 rpm for 8 min. The supernatant was used to detect 8-OHdG levels by ELISA following the recommendations of the manufacturer of the 8-OHdG ELISA kit (KANGLANG, Shanghai, China). Absorbance values were measured at 450 nm.

Measurement of CAT and SOD activities: Lenses were rinsed twice using cold phosphate-buffered saline (PBS, 10 mM, PH 7.0-7.2) without calcium and magnesium. Lenses were homogenized and centrifuged using the same method used for detecting 8-OHdG levels. The sediment was washed once with PBS and split with the lysate solution in an ice-bath for 30 min. Subsequently, mixtures were centrifuged at 12000 rpm for 30 min. The supernatant was tested with CAT and SOD assay kits according to the manufacturers' instructions (Nanjing Jiancheng Bioengineering Institute, China). Optical density (OD) was measured at 405 nm (CAT) and 550 nm (SOD). CAT activity (U mg^{-1} protein) = $(OD_{control}- OD_{sample}) \times 271 \times (1/60\ m_{sample})/C_{sample}$, SOD activity = $(OD_{control}-OD_{sample})/OD_{control}/50\% \times V_{total}/V_{sample}/C_{sample}$.

Statistical analysis: Statistical analysis was performed with the Statistical Package for Social Sciences software package for Windows (Version 19.0, Chicago, IL, USA). Differences between the control and experimental groups were compared with an independent *t*-test. Differences among all experimental groups were subjected to one-way analysis of variance and the Least-Significant difference test. Experiments were performed at least three times with each sample. The $p<0.05$ was considered statistically significant.

RESULTS

Establishment of selenium-induced cataracts in rats: To establish the animal cataract model, rats were randomly

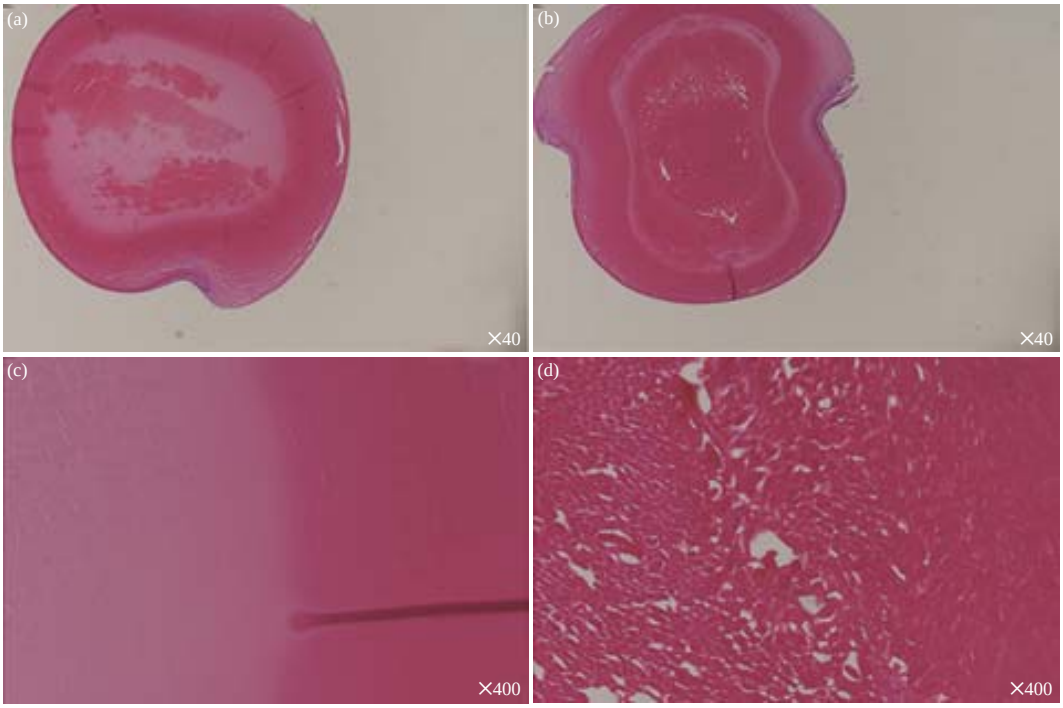

Fig. 1(a-d): HE staining of crystalline lens (a) The normal lens from the control group (b) An abnormal lens from the experimental group, (c) A normal lens from the control group and (d) An abnormal lens from the experimental group
Magnification: A and B = ×40 and C and D=×400

divided into a control group and experimental groups. In this study, a lens opacity classification system was used for classifying cataracts. In the model experimental group (group A), lens opacities were measured on the 3rd day after intraperitoneal injection of sodium selenite. On the 6th day, lens opacity was present in all rats and the average degree of opacity was 4.67±0.59.

Both normal and abnormal epithelial cells were found by H and E staining in the lenses of rats from group A (Fig. 1b) when compared with the control group (Fig. 1a and c). Abnormal epithelial cells contained large numbers of intermediate filaments, fewer organelles and nuclei of varying size while cell arrangements were disordered and the spacing between cells was larger (Fig. 1d). Thus, the rat model of selenium-induced cataracts by sodium selenite intraperitoneal injection was established successfully.

Expression level of HSP70: The expression level of HSP70 was measured by qPCR and immunohistochemistry. qPCR showed that HSP70 was more highly expressed in the heating bath (B) and the Ala-Gln (C) groups than in the model group (A, Fig. 2, $p<0.05$). In contrast, the expression level of HSP70 in the quercetin group (D) was significantly reduced when compared with that in group A ($p<0.05$). This was consistent with the results of immunohistochemical staining (Fig. 3), which showed that HSP70 in groups B and C was more widely distributed than in groups A and D. The average OD in groups B and C increased significantly compared with that in group A (Fig. 3e, $p<0.05$), whereas the OD of group D decreased ($p<0.01$). There was no significant difference between the ODs of groups B and C ($p>0.05$).

8-OHdG content: The contents of 8-OHdG were detected with an ELISA assay following the recommendations of the manufacturer for 8-OHdG ELISA kit. It was found that the contents of 8-OHdG increased significantly in lens epithelial cells in groups B and C but decreased significantly in group D when compared with group A ($p<0.05$, Fig. 4).

Antioxidative enzyme activities: Both the CAT activity and SOD activity were measured with the CAT Assay Kit and SOD Assay kit respectively. The results showed that the CAT and SOD activities decreased significantly in groups B and C and increased significantly in group D when compared with group A ($p<0.05$, Fig. 5). However, the CAT activity enhanced much more in the quercetin group than that in the model group ($p<0.05$). In addition, the SOD activity was down-expressed in group B and group C compared with group A ($p<0.05$, Fig. 5).

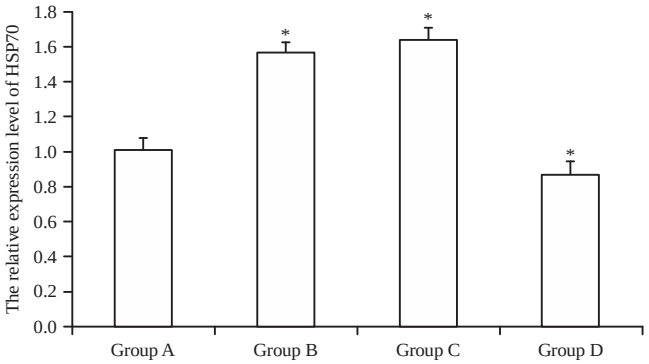

Fig. 2: Relative expression of HSP70 detected by qPCR, Group A: Intraperitoneal injection of sodium selenite, Group B: Heating bath and intraperitoneal injection of sodium selenite, Group C: Ala-Gln and intraperitoneal injection of sodium selenite and Group D: Quercetin and intraperitoneal injection of sodium selenite. Three repeats with different cDNA template in RT-PCR and three repeats of each sample. The relative expression level of HSP70 was analyzed by REST. *p<0.05

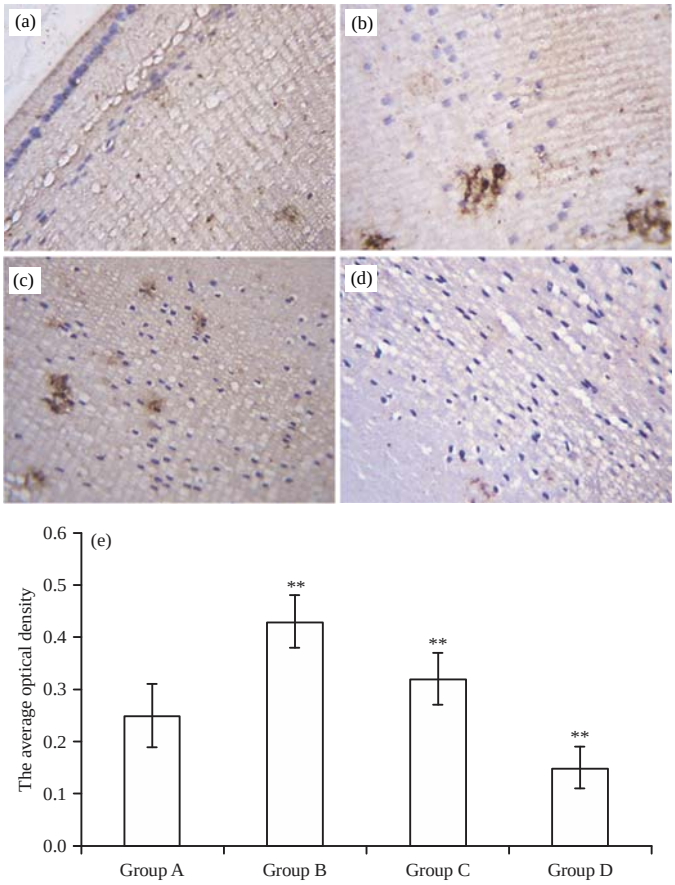

Fig. 3(a-e): Immunohistochemical staining of HSP70 (A-D) and optical density analysis (E). The immunohistochemical image of group A (A), group B (B), group C (C) and group D (D); E, the quantified value of HSP70 based on optical density. Group A: Intraperitoneal injection of sodium selenite. Group B: Heating bath and intraperitoneal injection of sodium selenite. Group C: Ala-Gln and intraperitoneal injection of sodium selenite. Group D: Quercetin and intraperitoneal injection of sodium selenite. The brown granules are positive results for HSP70 staining. **p<0.01, compared with group A. Magnification: ×400

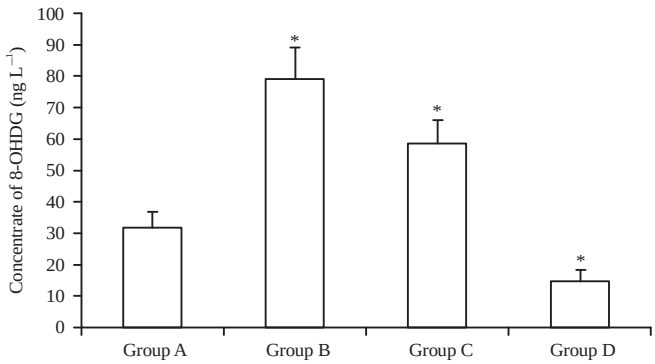

Fig. 4: Concentration of 8-hydroxy-2-deoxyguanosine (8-OHdG) in each group. Group A: Intraperitoneal injection of sodium selenite. Group B: Heating bath and intraperitoneal injection of sodium selenite. Group C: Ala-Gln and intraperitoneal injection of sodium selenite. Group D: Quercetin and intraperitoneal injection of sodium selenite. *p<0.05 compared with group A

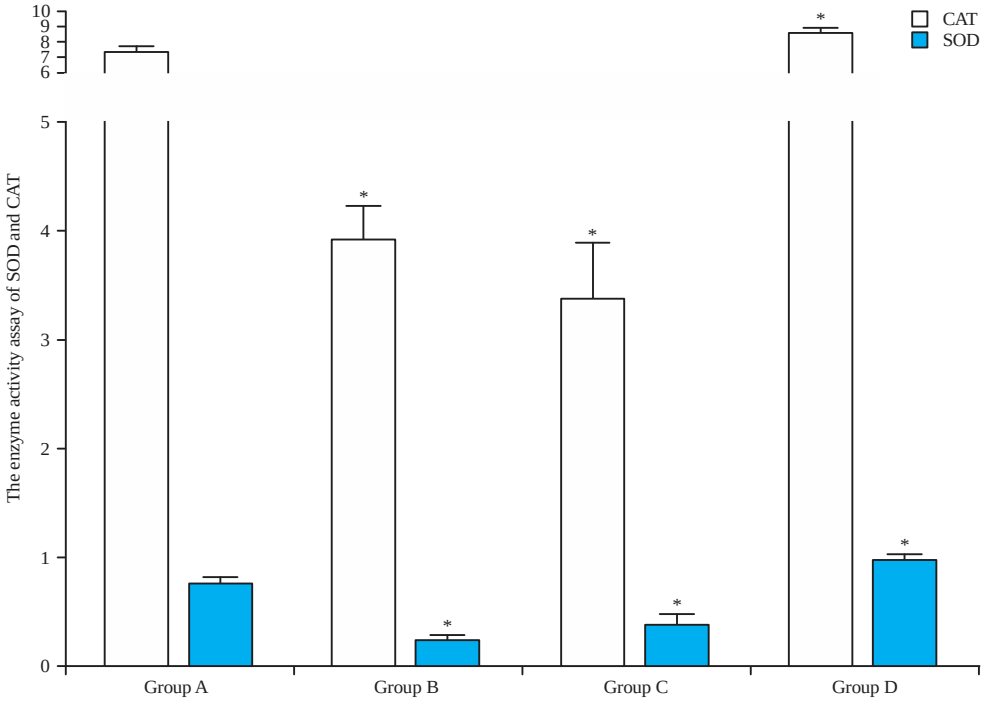

Fig. 5: Enzyme activities of SOD and CAT in each group. Group A: Intraperitoneal injection of sodium selenite. Group B: Heating bath and intraperitoneal injection of sodium selenite. Group C: Ala-Gln and intraperitoneal injection of sodium selenite. Group D: Quercetin and intraperitoneal injection of sodium selenite. *p<0.05 compared with group A

However, the SOD activity was much higher in the quercetin group than that in the model group (p<0.05).

DISCUSSION

The expression levels of HSP70 in the heating bath and Ala-Gln groups were significantly higher than that in the selenium-induced cataract model group. In contrast, the HSP70 expression in the quercetin group was significantly lower than that in the model group. The 8-OHdG levels in lens epithelial cells of the quercetin group were significantly lower than those of model group. CAT and SOD activities were enhanced to a much greater extent in the quercetin group than in the model group.

Some researchers have identified that quercetin and its metabolites are active in inhibiting oxidative damage in cataracts. In addition, previous investigations have shown that phytochemical antioxidants can prevent cataracts[21]. Selenium-based induction is the classical cataract model[22,23]. Its establishment is simple and convenient, thus, it is widely used in studying the pathogenesis of senile cataracts and the effects of anti-cataract drugs[24]. In view of the work described above, we established this model in rats and used it to explore the potential mechanism of the protective effects of quercetin. We subsequently showed that quercetin could prevent the progression of cataracts by enhancing the activities of SOD and CAT as well by reducing the level of 8-OHdG. Thus, quercetin might maintain the homeostasis of cellular oxidative stress by modulating the expression of HSP70 in selenium-induced cataracts.

Similar to the oxidative stress marker, the concentration of 8-OHdG in cataracts was greater than that in the healthy group[25]. Thus, oxidative DNA damage increased in rats with cataracts induced by sodium selenite and the level of 8-OHdG in the quercetin group decreased when compared with that in the model group, indicating that quercetin had reduced the oxidative DNA damage in cataracts. Quercetin is a natural antioxidant because of its ability to directly scavenge free radicals and is found in various plant-derived foods. Previous studies have also shown that quercetin can reduce oxidative stress by increasing the activities of CAT and SOD[26,27] and in this study, it also enhanced CAT and SOD activities in selenium-induced cataracts. Therefore, quercetin might also clear free radicals indirectly by elevating expression of antioxidant enzymes in cataracts to produce beneficial effects against oxidative DNA damage.

Currently, the mechanism of quercetin's effects on cataracts is not fully understood. The heat shock response is a potential protective mechanism to maintain a normal microenvironment in the lens[28,29]. It has been reported that a constitutive expression of HSP70 in the lens is caused by continual oxidative stress[30-32]. In addition, a high expression level of HSP70 has been reported the in lenses of cataract patients[29,33]. The heating bath provides heat stimulation and can upregulate the expression level of HSP70 in the lens[34] and the dipeptide Ala-Gln has also been shown to upregulate HSP70 expression[35]. Therefore, both these methods were used to induce the expression of HSP70 in this study and indeed, both these treatments did increase the expression when compared with the model group. Conversely, the expression of HSP70 in the quercetin group was lower than that in the other groups, suggesting that quercetin probably prevented the development lens opacity in cataracts by regulating and controlling the expression of HSP70.

However, there are some limitations in this study. For example, the pathways by which quercetin modulates HSP70 are unknown and more experiments in this regard are necessary. In addition, cataracts are a multifactorial disorder with both genetic and environmental factors. However, our findings provide insights into the modulating pathways in the pathogenesis of senile cataracts, although further research will still be necessary to understand the interactions between HSP70 and antioxidant enzymes. It would also be helpful to identify the apoptotic pathways involved in the pathogenesis of senile cataracts.

CONCLUSION

In conclusion, this study confirms that quercetin prevents development of lens opacity in cataracts because it can enhance the activities of antioxidant enzymes and reduce 8-OHdG oxidative damage. The probable mechanism is that quercetin prevents the formation of cataracts by modulating the expression of HSP70 to control oxidative stress. The study is significant in ongoing research on cataract prevention and treatment.

SIGNIFICANCE STATEMENT

This study demonstrates that quercetin prevents lens opacity in selenium-induced cataracts by enhancing the activities of antioxidant enzymes and reducing 8-OHdG oxidative damage. This study will help researchers to explore important aspects of the potential molecular mechanisms of quercetin activity in selenium-induced cataracts, thereby aiding in further research into cataract prevention and treatment. Thus, a new theory on quercetin and cataract prevention may be arrived at.

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