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Protective Effects of Different Doses of Ginsenoside-Rb1 Experimental Cataract Model That in Chick Embryos

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ABSTRACT

Purpose: There has been increased interest in phytochemical antioxidants to prevent protein damage and aggregate formation in cataract treatment. In this study, the protective effect of different doses of Rb1 (GRb1), one of the ginsenosides of Panax Ginseng, in the experimental cataract model formed in chick embryos was investigated.

Methods: Five different experimental groups were formed with 100 SPF fertilized eggs: Control (0.9% NaCl to physiological saline), hydrocortisone hemisuccinate sodium (HC), low dose (HC + L-GRb1 (1 mg/kg)), medium dose (HC+). M-GRb1 (2.5 mg/kg)), and high dose (HC + H-GRb1 (5 mg/kg)). All solutions were given to air sack at 15 days of incubation. On the 17th day, the bulbous oculi of the chick embryos were dissected. Cataract formations of the lenses, glutathione (GSH), malondialdehyde (MDA), total antioxidant (TAS), total oxidant (TOS) levels, Caspase-3 H-score, and TUNEL index were determined. In addition, crystalline alpha A (CRYAA) gene expression was evaluated.

Results: Cataracts were observed in the control, HC, HC + L-GRb1, HC + M-GRb1, and HC + H-GRb1 groups with a frequency of 0%, 100%, 75%, 56.25%, and 100%, respectively. There were statistically significant differences between the control and HC groups in terms of TAS, TOS, MDA, GSH, Caspase-3 H-score, and TUNEL index ($p < .05$). When the therapeutic effect of the GRb1 groups was evaluated, the HC group showed significant differences with the HC + L-GRb1 and HC + M-GRb1 groups in almost all parameters ($p < .05$), while there was no statistical difference with the HC + H-GRb1 group ($p > .05$). In addition, gene expression levels differed between the groups, although not statistically significant ($p > .05$).

Conclusion: 1 mg/kg and 2.5 mg/kg GRb1 applications show therapeutic properties on the HC-induced cataract model. This effect is more pronounced at 2.5 mg/kg.

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Cataract; chicken embryo; Ginsenoside-Rb1 (GRb1); lens; Panax Ginseng

Introduction

The lens is responsible for refracting the light coming into the eye and focusing it on the retina. The loss of transparency of the lens with age results in cataracts characterized by opacity and blurred vision. The prevalence of cataract increases after middle-aged adults and is considered one of the most common causes of vision loss worldwide, especially in low-income countries.[1–3] Of the 1 billion preventable visual impairments worldwide, 65.2 million are caused by cataracts.[4]

Lens transparency is vital to visual acuity. Therefore, many patients need cataract treatment. Surgical methods are widely used to prevent cataracts. Thus, patients can gain a significant portion of their visual impairment.[5] As in many diseases, alternatives to surgical methods are being investigated in the treatment of cataracts. In recent years, with the understanding of the protective roles of antioxidant enzymes against oxidative stress-related cell damage, interest in phytochemical agents has increased in the fight against cataracts after oxidative damage.[6,7] Current evidence on the efficacy of herbal compounds containing powerful antioxidants has highlighted that consumption of these herbs can reduce the effects of cataracts.[8,9]

The biotransformation of ginsenosides in the roots or extracts of Panax Ginseng, which has a high consumption market, has been of interest in recent years due to its high pharmacological activity and pharmacokinetics. Recent research is based on the fact that the active ingredients responsible for ginseng's wide spectrum of pharmacological activities are provided by ginsenosides (saponins).[10,11]

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Ginsenosides also increase antioxidant enzyme activity.[12,13] Therefore, extensive clinical practice-based evidence in the scientific literature worldwide has demonstrated its broad therapeutic and pharmacological effects in different diseases. It also has therapeutic properties in various eye diseases such as macular degeneration and glaucoma.[14] On the other hand, studies on the efficacy of ginseng in cataract treatment are limited. Although previous studies contain available evidence that ginseng has a protective role in cataracts, they have reported conflicting findings due to Ginseng's structural properties.[15,16] It is unclear whether or which of the saponin and non-saponin compounds of ginseng have therapeutic properties. At this point, the literature on the phytochemical properties of ginseng states that determining the properties of a single ginseng compound with cataract-preventing activity may contribute to the development of therapeutic agents in the future.[17,18]

Ginsenoside Rb1 (panax ginseng saponin) is a protopanaxadiol.[19] This ginsenoside and its metabolites have a variety of *in vitro* and *in vivo* effects, including neuroprotective, anti-arthritic, antioxidant, anti-inflammatory, and anti-obesity effects.[19–22] No detailed studies have been conducted to evaluate the protective effects and underlying mechanisms of how GRb1 combats cataracts and their complications. The glucocorticoid-induced chick embryo cataract model is an easy, feasible, and ideal experimental model to screen for parameters associated with the antioxidant activity of some phytochemical agents.[9,23,24] This cataract model is often associated with posterior subcapsular cataract (PSC). Since oxidative stress is a significant risk factor in the early stages of developing PSC opacities, it is possible to reduce the incidence of PSC by various means, including antioxidants.[25] In this study, the protective and therapeutic effects of GRb1 were investigated in the chick embryo cataract model induced by hydrocortisone hemisuccinate sodium (HC).

Materials and methods

Animals

In this experiment, 100 SPF (Specific Pathogen-Free) eggs (each fertile 60 ± 5 g, 0-day-old fertile White Leghorn) taken by Turkey's Ministery of Agriculture and Farming, Bornova Veterinary Control and Research Institute Administration, have been used. All animal procedures were approved by the Local Ethics Committee of Afyon Kocatepe University Animal Experiments (Number: 49533702/95, Date: 08/07/2021).

Experimental design

This experimental study was carried out in accordance with the rules specified in the experimental animals ethical committee guidelines. Experimental methods recommended in reference studies were used for the formation of steroid-induced cataract models in chick embryos.[8,9,23,24,26] The eggs were placed in the incubator with the pointed ends down, by adjusting the incubator at 37 °C and 70% relative humidity. The day the SPF eggs were incubated was considered day 0. Study groups were formed on the 15th day of incubation. 100 SPF eggs were randomly assigned to five groups ($n = 20$). SPF eggs were cleaned with 70% ethanol on day 15. Pre-prepared HC (Sigma-Aldrich, St. Louis, MO, USA) (0.50 millimoles in 100 µL) and physiological saline (0.9% NaCl) were applied to the air sack with an insulin injector (26 gauge) through the hole made from the blunt end of the egg. GRb1 dissolved in physiological saline as in the reference study.[27] The dosage of GRb1 was chosen based on previous studies and preliminary experiments.[28,29] GRb1 treatment started in 3 h after HC application. 1, 2, and 5 mg/kg doses of GRb1 solutions were injected through previously drilled holes. After the injection, the holes opened in the eggs were closed with a sterile drape and placed back in the incubator. Experimental groups were designed as follows:

1. Chick embryo group (Control) exposed to serum physiology (100 microL)
2. Chick embryo group exposed to HC (0.50 millimoles in 100 microL)
3. Chick embryo group (HC + L-GRb1) exposed to HC (0.50 millimoles in 100 microL) and low dose GRb1 (1 mg/kg)
4. Chick Embryo group (HC + M-GRb1) exposed to HC (0.50 millimoles in 100 microL) and medium dose GRb1 (2.5 mg/kg)
5. Chick Embryo group (HC + H-GRb1) exposed to HC (0.50 millimoles in 100 microL) and high dose GRb1 (5 mg/kg)

On the 17th day of the study (48 h after the injection), all the eggs were taken from the incubator separately according to the experimental groups and the embryos were hatched. Under the dissection microscope (Carl Zeiss, Stemi 2000-C), the bulbous oculi of the embryos were removed and the lenses of each embryo were examined under a light microscope (Olympus, CX21) for morphological analysis. The bulbous oculi of 13 embryos from each group were dissected and their lenses were removed. The lenses of 5 of these 13 embryos were stored in Eppendorf at −80° for genetic analysis. The lenses of the remaining eight embryos were photographed first. It was then stored in Eppendorf at −80° for biochemical analysis. The integrity of the bulbous oculi of the remaining seven embryos was preserved and included in the fixative prepared beforehand for histological tissue follow-up.

Cataract formation

A five-point scoring system was used; grade I: no lens opacity; grade II: pale white ring around the lens core; grade III: clear white ring around the lens core; grade IV: the opacity of the lens core does not spread to the center of the core; grade V: opacity of the lens core radiating to the center of the core[26] (Figure 1).

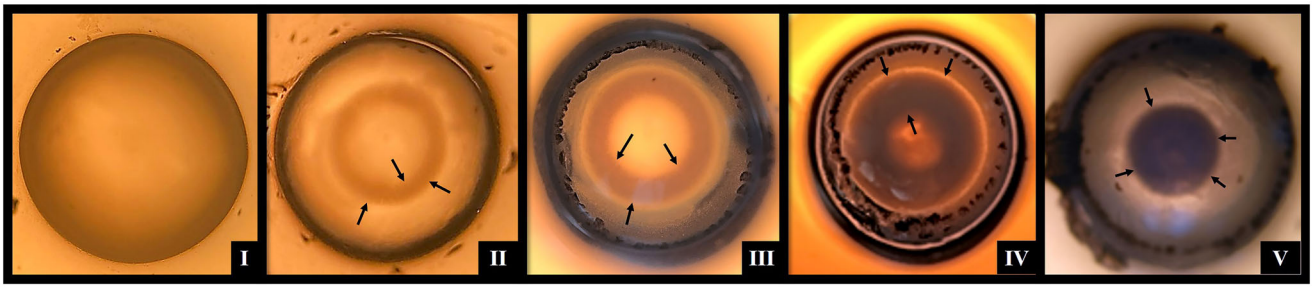

Figure 1. Images of the cataract grades of the groups. a: Grade I, b: Grade II, c: Grade III, d: Grade IV, e: Grade V. The arrows show lens opacity. GRb1: Ginsenoside-Rb1; HC: hydrocortisone hemisuccinate sodium.

Biochemical examination

Lens samples were carefully dissected from embryos. Lens samples were added to 0.4 ml distilled water followed by 1 min ultrasonication at 20 000 rpm with a Hielscher (Germany) sonicator. The lens homogenates were then centrifuged at 10.000 g for 15 min and the supernatant was removed and stored at −20 °C. GSH and MDA levels from lens samples were determined according to the manufacturer's instructions and using the ELISA kit (BT LAB, Shanghai/China). TAS was measured by colorimetric method using a commercial kit (Mega Medicine/Gaziantep/TURKEY) for TOS values. A microanalyzer was used for absorbance experiments (ChemWell 2910). TOS, TAS, GSH, MDA levels were expressed as µmol Trolox Equiv./Lens, µmol H2O2 Equiv./Lens, mg/Lens, nmol/Lens, respectively.

Histochemistry examination

Caspase-3 immunohistochemistry: After histological tissue processing of the lenses, paraffin blocks were formed and 5 μ thick sections were taken. Sections were prepared for IHC Staining. Endogenous peroxidase activity was suppressed with 3% hydrogen peroxide after antigen retrieval application. After protein block solution, incubated with anti-Caspase 3 antibody (1/200, NB100-567608, Novusbio) at +4 °C for 1 night. After using the avidin with biotin method, DAB was made visible with chromogen. Photographs were taken with a light microscope (NIKON Eclipse E600) using the Image Analysis Program (NIS elements, Japan). H-Score evaluation was performed after immunohistochemical staining (Figure 2).

TUNEL method: *In situ* apoptosis detection kit (Abcam, ab206386) was used. All analysis procedures were performed according to the Manufacturer's instructions. The number of TUNEL positive cells was determined by counting 100 cells in randomly selected areas from each sample (Figure 2).[30]

Genetic examination

Assembly of total RNA isolation from lenses with PureZOL RNA isolation kit (Biorad, USA, Cat. No: 732-6890). The temperature of isolated RNAs for analysis of *crystalline alpha A (CRYAA)* gene mRNA expression elevation. Server using cDNA synthesis kit (Biorad, USA, Cat. No: 1708841), which contains cDNA for 1 µg total RNA tests as part of real-time PCR. Monitoring the cDNA sample Intensify the quantitative Real-Time PCR method in determining *CRYAA* gene expression. The region of interest using primer pairs with the *CRYAA* gene was maintained in the Real-Time PCR instrument. The *GAPDH* mRNA level was taken as a reference to normalize *CRYAA* genes.

Statistical analysis

Statistical analyses were performed with SPSS Version 25 (IBM Corporation, Armonk, NY) software. In comparisons between groups, TAS, TOS, MDA, GSH levels and Caspase-3 H scores and TUNEL index were analyzed using the Kruskal–Wallis test. Dunn's Test was used for pairwise comparisons between each independent group. The differences between categorical variables (cataract stages and groups) were analyzed by the Chi-Square test and Fisher Exact analysis. $p < .05$ was accepted as the significant value.

Results

Within the scope of the study, a total of 100 SPF eggs were used in five groups, 20 in each group.

Cataract formation

Statistically significant differences were found between the groups in terms of the degree of cataract ($p < .001$). While cataract was not observed in any lens in the control group, cataract was observed in all of the HC and HC + H-GRb1 groups. In the HC + L-GRb1 and HC + M-GRb1 groups, there were cataracts at rates of 75% and 56.25%, respectively (Table 1).

Biochemical analysis

When the TAS, TOS, MDA, and GSH levels of the lenses were compared between the study groups, statistically significant differences were found ($p < .001$). Supplementary Table 1 contains detailed p values between control and HC groups and treatment groups. The highest and lowest mean values for all parameters varied between the control and HC groups, and there were statistically significant differences between the

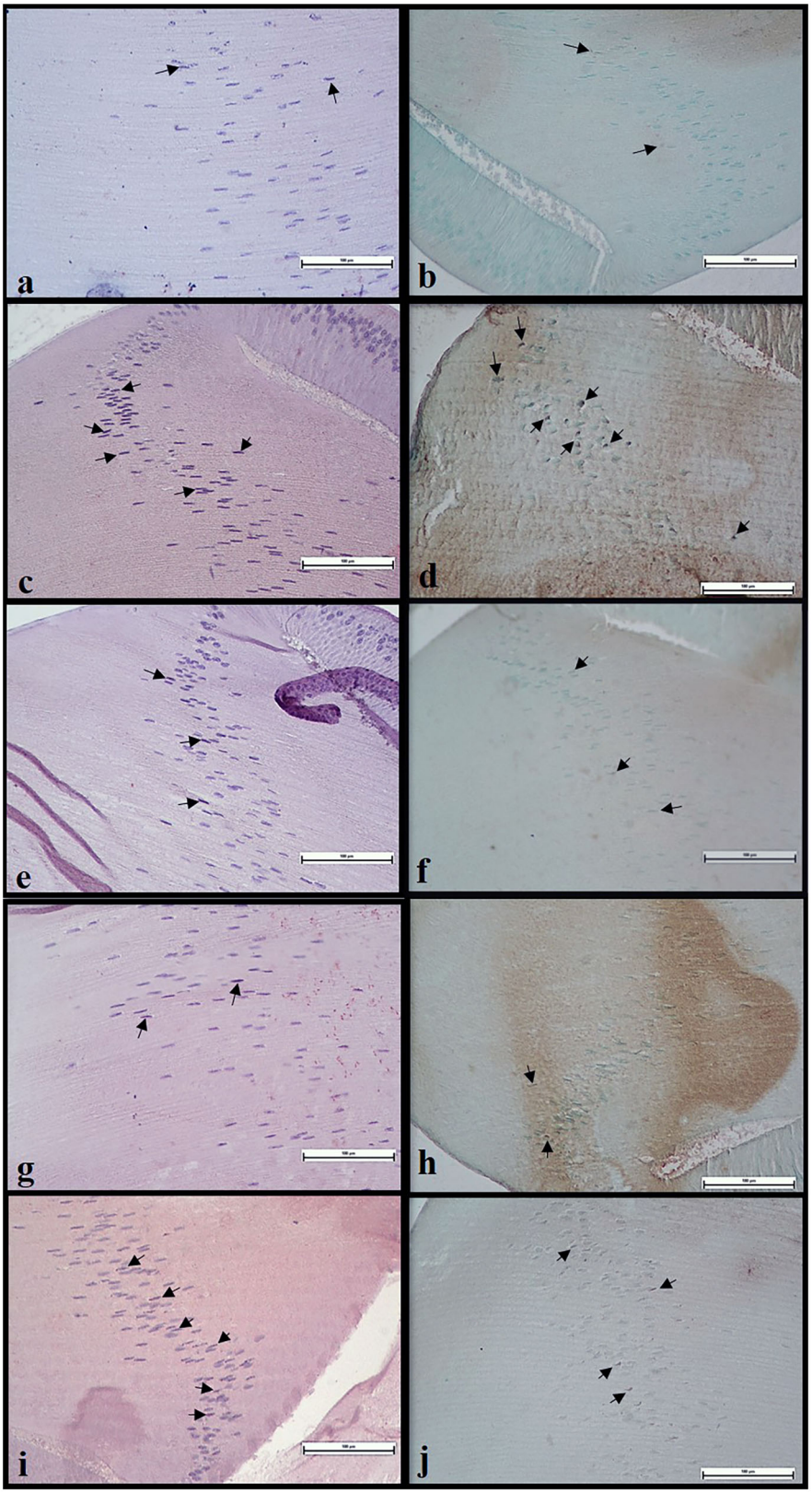

Figure 2. The detection of TUNEL and Caspase-3. In the micrografts in the left column, arrows show cells with dark nuclei staining Caspase-3 positive, while in the micrografts in the right column, the arrows indicate cell nuclei stained positive in TUNEL (original magnification, ×20). a and b: control; c and d: HC; e and f: HC + L-GRb1; g and h: HC + M-GRb1; i and j: HC + H-GRb1 groups; GRb1: Ginsenoside-Rb1; HC: hydrocortisone hemisuccinate sodium.

two groups ($p < .001$). The results of multiple comparisons of the mean values of the treatment groups and the control and HC groups varied. The mean and statistical differences between the study groups are presented in Table 2.

Immunohistochemical analysis

When the TUNEL indexes and Caspase-3 H scores in the lenses of each embryo were compared between the groups, a

statistically significant difference was found ($p < .001$). Supplementary Table 1 contains detailed p values between control and HC groups and treatment groups. TUNEL index and Caspase-3 H score were highest in the HC group and lowest in the control group ($p < .001$). Multiple comparison analyses of the mean of the study groups are shown in Table 2.

Genetic analysis

CRYAA gene expression level in embryo lenses of the HC group was downregulated (0.86-fold) compared to the control group. *CRYAA* gene expression was upregulated in the embryo lenses of the 1 mg/kg and 2.5 mg/kgGRb1 groups compared to the control group (1.23-fold and 1.69-fold, respectively). *CRYAA* gene expression was downregulated in the 5 mg/kg GRb1 group embryo lenses compared to the control group (0.49-fold) ($p > .05$) (Figure 3).

Discussion

Reactive oxygen species derived from oxidative stress appear to be an important factor in cataract formation by causing tissue damage.[31] With aging, because crystalline proteins are damaged by oxidative stress, insoluble protein aggregates lead to opacity of the lens and deterioration of vision. This causes a decrease in color and shape sensitivity and blurred vision, as the lens cannot focus on near or far objects and is damaged.[2,3,14,32] Glucocorticoid-induced chick embryo cataract models are a valuable model for the study of anti-

Table 1. Distribution of cataract degrees of lenses between groups.

	Cataract grades, n (%)						
Groups	I	II	III	IV	V	Total	*p*
Control	16 (100)	–	–	–	–	16	<.001[a]
HC	–	1 (6.25)	5 (31.25)	7 (43.75)	3 (18.75)	16	
HC + H-GRb1	–	3 (18.75)	5 (31.25)	6 (37.5)	2 (12.5)	16	
HC + M-GRb1	7 (43.75)	5 (31.25)	3 (18.75)	1 (6.25)	–	16	
HC + L-GRb1	4 (25)	3 (18.75)	9 (56.25)	–	–	16	
Total	27 (33.75)	12 (15)	22 (27.5)	14 (17.5)	5 (6.25)	80	

GRb1: Ginsenoside-Rb1; HC: hydrocortisone hemisuccinate sodium.
[a]Fisher's Exact Test was used.

Table 2. Comparison of oxidant and antioxidant levels of lenses between groups.

Parameters and groups	N	Control Mean ± SD	HC Mean ± SD	HC + H-GRb1 Mean ± SD	HC + M-GRb1 Mean ± SD	HC + L-GRb1 Mean ± SD	*p* Value
TAS (U/lens)	8	$5.03 \pm 1.09^{b,c,e}$	$2.28 \pm 0.71^{a,d}$	$2.37 \pm 0.95^{a,d}$	$4.09 \pm 1.52^{b,e}$	3.38 ± 0.96^{a}	<.001
TOS (pg/lens)	8	$8.09 \pm 1.23^{b,c,e}$	$17.47 \pm 6.63^{a,c,d}$	12.84 ± 4.34^{a}	10.10 ± 1.28^{b}	$10.83 \pm 2.46^{a,b}$	<.001
GSH (nmol/lens)	8	$609.48 \pm 68.38^{b,c}$	$430.34 \pm 46.73^{a,c,d}$	$450.84 \pm 40.23^{a,c,d}$	$542.76 \pm 60.54^{b,e}$	$526.01 \pm 66.85^{b,e}$	<.001
MDA (μmo/lens)	8	$2.11 \pm 0.92^{b,c}$	$3.19 \pm 0.21^{a,c,d}$	$2.89 \pm 0.22^{a,d}$	$2.37 \pm 0.44^{b,e}$	2.39 ± 0.50^{b}	<.001
TUNEL (index)	7	$0.05 \pm 0.02^{b,c,e}$	$0.57 \pm 0.05^{a,c,d}$	$0.48 \pm 0.05^{a,c,d}$	$0.35 \pm 0.02^{b,e}$	$0.34 \pm 0.05^{a,b,e}$	<.001
CASPASE 3 (H score)	7	$189.00 \pm 4.00^{b,c,d}$	$345.00 \pm 20.55^{a,c,d}$	$287.00 \pm 19.07^{a,d}$	$234.14 \pm 7.71^{a,b}$	$233.43 \pm 14.89^{b,e}$	<.001

Notes: Results of Kruskal–Wallis test and the post hoc Dunn's multiple comparisons test. GRb1: Ginsenoside-Rb1; HC: hydrocortisone hemisuccinate sodium; SD: Standard deviation. Explanation of symbols: [a]Statistically significant difference compared with the control group, [b]Statistically significant difference compared with the HC group, [c]Statistically significant difference compared with the HC + L-GRb1, [d]Statistically significant difference compared to the HC + M-GRb1, [e]Statistically significant difference compared to the HC + H-GRb1.

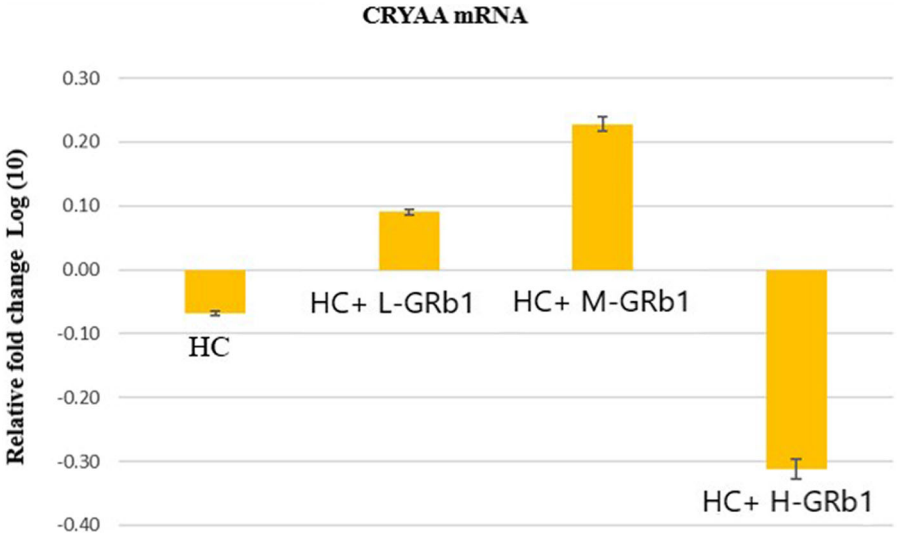

Figure 3. Logarithmic display of *CRYAA* gene mRNA fold changes in embryo lenses treated with HC and different doses of GRb1. The GAPDH gene was used for normalization. GRb1: Ginsenoside-Rb1; HC: hydrocortisone hemisuccinate sodium.

cataract agents due to their ease of administration and high incidence of reproducibility.[26] In the current study, the protective effects of GRb1 at three different doses were investigated in the cataract model formed in HC-induced chick embryo lenses. TAS and GSH levels were the lowest in the HC group, while TOS, MDA levels, TUNEL index, and Caspase-3 H-score were at the highest levels. Contrary to this situation, TAS and GSH levels were at the highest levels in the control group, while TOS, MDA, TUNEL index, and Caspase-3 H-score were at the lowest levels. There were statistically significant differences between the control group and the HC group in all of these parameters evaluated in terms of oxidant and antioxidant levels. Therefore, it is thought that changes due to oxidative stress are observed in HC group lenses. The increase in the MDA level, which is used as a biomarker for oxidative stress in the lenses of HC-induced embryos, supports this idea. In addition, when considered together with the findings on cataract grades, it provides evidence that the oxidative stress-induced cataract model can be realized experimentally, as specified in reference studies.[8,9,23]

Findings from the HC + M-Rb1 and HC + L-Rb1 groups provided new preclinical evidence against GRb1 cataracts and their complications, and it was determined that 1 and 2.5 mg/kg GRb1 application doses had protective effects on cataracts. Although therapeutic changes were obtained in terms of GSH and MDA levels in both of these groups, quantitatively closer values were obtained in the HC + M-Rb1 group to the control group. These results indicate that 2.5 mg/kg GRb1 administration may be more effective in the treatment of cataracts. In parallel with the existing biochemical analyses, TUNEL index and Caspase-3 H-scores of the lenses belonging to the HC + M-Rb1 and HC + L-Rb1 groups were statistically significantly lower than the lenses of the HC group in IHC analyses. These findings support our previous comments. On the other hand, in all parameters examined, the therapeutic effects of the HC + H-Rb1 group were very weak compared to the other two doses and were similar to the HC group.

In the current study, the doses of 1 mg/kg and 2.5 mg/kg selected in the HC + M-Rb1 and HC + L-Rb1 groups are thought to exert an effort to treat the cataract in the HC group. At this point, immunohistochemical and biochemical parameters also support this assumption. In addition, we think that the 5 mg/kg dose applied in the HC + H-Rb1 group has a toxic effect on the lenses. However, we emphasize that it would be more accurate to consider our findings together with the results of previous studies on GRb1 in the literature, instead of making definite inferences about the toxic effect. GRb1 may inhibit apoptosis, while its metabolites may support this response.[20,33] In addition, the apoptotic effect may vary depending on the dose. It is stated that the antiapoptotic effects of GRb1 on apoptosis are mediated by the inhibition of mitogen-activated protein kinases and caspase activation.[34] In our study, the toxic effect at high doses may have developed due to the fact that metabolites in excess in the environment facilitated apoptosis. Our findings were in line with previous experimental studies in which similar conditions were established. A previous *in vitro* study examined the effects of GRbl on cell viability in relation to the mitochondrial activity of chondrocytes treated with H2O2. In this study, after administration of 50, 100, and 400 μM GRb1 together with 500 μM H2O2, higher cell viability was obtained in the 100 μM GRb1 group, which was determined only as a medium dose, compared to the H2O2 group.[35]

In a previous study, Sun-ginseng extract was investigated in a rat model of selenite-induced cataracts and it was stated that the saponin fraction of ginseng did not have a protective effect on cataracts.[15] On the other hand, in a similar experimental model, it was stated that the application of Ginseng extract increased the activity of antioxidant enzymes in the lens and this may be indirectly related to the effect of preventing cataracts.[36] In another study, it was stated that GRg1 prevented lens opacity caused by H2O2. It was emphasized that after GRg1 treatment, water-soluble protein content, SOD level, total GSH and reduced GSH increased while MDA and oxidized GSH levels decreased.[16] Our study provides new evidence for research in the literature regarding which or which of the Ginseng saponin and nonsaponin compounds are effective in the treatment of cataracts. The results of our study also suggest that GRb1 may have a protective capacity against cataracts caused by oxidative stress. In addition, in our study, it was shown that GRb1 may be effective in the treatment of cataracts, similar to GRg1, by emphasizing the therapeutic role of ginsenosides through their antioxidant effect. In previous studies, it was emphasized that GRb1 has antioxidant activity[37] and may alleviate diabetic retinopathy by regulating antioxidant function in rat retinas.[38]

Crystalline genes are important in that they encode the main soluble proteins of the lens, and especially *CRYAA* gene plays an important role in the development of the lens.[39,40] Mutations in the CRYAA protein structure can cause congenital or age-related cataracts.[41–43] Our findings appear to affect the transcription and expression of these genes, as suggested in previous studies where CRYAA is down-regulated in lenses with age-related nuclear cataracts.[44,45] In the presented study, there was a negligible decrease in CRYAA gene expression with HC exposure. Although this decrease in gene expression seems compatible with the literature, it needs to be interpreted with caution as it is not based on statistically significant findings. The association between deficiencies in the CRYAA gene and increased lens opacity was associated with nuclear cataract type, one of the age-related cataract types, and was based on studies on mouse and human lenses.[44–46] Whereas, our study was an experimental design involving chick embryo lenses, which is usually associated with PSC. The mechanism of age-related cataract formation may differ in morphological subtypes. It is possible that CRYAA gene deficiencies are more involved in nuclear cataract than in PSC. The level of CRYAA gene expression in chickens is lower than in other animals.[47] Finally, many different variants and mechanisms other than CRYAA contribute to congenital or age-related cataracts.[48] All of these may explain our findings in

our current study involving minor changes in the CRYAA gene due to lens opacity.

Lens epithelial cells and lens fiber cells are very sensitive to changes in oxidative stress.[25] CRYAA gene contributes to the clarity and refractive properties of the lens, can prevent protein damage and protect against oxidative stress.[40,49,50] A previous study emphasized ginsenosides can reduce cataractogenesis by inhibiting H2O2-induced expression of apoptosis-related genes in lens epithelial cells.[51] Specifically, GRb1 was shown to protect retinal ganglion cells against apoptosis induced by H2O2-induced oxidative stress.[21] The quantitative effects of increasing CRYAA expression in the current study groups seem to indicate that GRb1 also plays a role in the treatment mechanism by which it reduces oxidative stress and apoptosis. Our study shows that the contribution of CRYAA alone is not sufficient on this therapeutic effect of GRb1. The antioxidant properties of GRb1 are associated with several different mechanisms. Recent evidence has reported the main protective properties of GRb1 and other major ginsenosides against oxidative stress damage are through the activation of the Nuclear Factor Erythroid 2-Related Factor 2 (Nrf2).[38,52] It was stated that the increase in gene expression induced by Nrf2 partially plays a role in the anti-oxidative properties of GRb1 treatment associated with increase in GSH level and decrease in MDA level.[38] Similarly, studies on potential therapeutic mechanisms of GRb1 suggested that Keap1/Nrf2 activation ameliorates various diseases by showing inhibitory effects on oxidative stress.[38,53–55] It has also been reported that GRb1 protects DNA from damage caused by these acids by directly scavenging hydroxyl radical and hypochlorous acid.[56] In our study, we think that GRb1 plays a therapeutic role by reducing oxidative damage in lenses with cataracts in a similar way. Although various transcriptional links have been reported between Nrf2 and CRYAB, the links between CRYAA and Nrf2 are not yet clear.[57] Considering the results of this previous study and the current study, future studies may explore the mechanistic relationship between lens tissue-specific regulation of CRYAA for the protective effect of GRb1 and oxidative stress from Nrf2 depletion.

This study is the first to show that GRb1, the main active ingredient of ginseng, may be an anti-cataract agent with antiapoptotic and antioxidant properties in a dose-dependent manner. As the growing interest in ginseng continues, so will the evidence from animal experiments or previous clinical studies of GRb1 for ocular diseases. Our findings can provide a theoretical basis for new research in drug discovery for further pharmacological studies and clinical treatments. As with many studies, it has some limitations. The study did not show a significant dose-response relationship between GRb1 and CRYAA expression. This may be related to the selected doses. In addition, the results of CRYAA gene expression and therapeutic mechanisms in lens opacification were not significant. These limitations preclude drawing firm conclusions about the meaning of CRYAA results. In addition, the current findings may relate to PSC, possibly as an effect of experimental design. Therefore, it may not be reproducible in all experimental cataract models, and therefore different results specific to cataract types may occur.

Conclusion and recommendations

In this study, 1 mg/kg and 2.5 mg/kg GRb1 had therapeutic effects on HC-induced cataracts. Especially in 2.5 mg/kg GRb1 treatment, results were quantitatively closer to the control group parameters. The application of 5 mg/kg GRb1 did not cause a therapeutic effect on cataracts, possibly by reducing cell viability.

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Disclosure statement

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Data availability statement

The data that support the findings of this study are available from the corresponding author, [YEK], upon reasonable request.

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