Molecular Vision 2023; 29:245-255 <http://www.molvis.org/molvis/v29/245>
Received 23 February 2023 | Accepted 1 November 2023 | Published 5 November 2023

Investigation of the antioxidant effect of Chrysin in an experimental cataract model created in chick embryos

Gülan Albaş Kurt,[1] Tolga Ertekin,[1] Emre Atay,[1] Abdülkadir Bilir,[1] Halit Buğra Koca,[2] Esra Aslan,[3] Alperen Sarıtaş[1]

[1]Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Anatomy, Afyonkarahisar, Turkey;
[2]Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Medical Biochemistry, Afyonkarahisar, Turkey;
[3]Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Histology and Embryology, Afyonkarahisar, Turkey

Purpose: Cataract, which occurs as a result of lens opacification, is one of the most common causes of vision loss. In the literature, deterioration of the antioxidant system due to the increase in reactive oxygen species and oxidant levels is shown among the causes of cataract formation. The aim of this study was to investigate the antioxidant effect of chrysin on steroid-induced cataract development in an experimental chick embryo model using morphological, histological and biochemical parameters.

Methods: Within the scope of the study, 150 specific pathogen free (SPF) fertilized eggs were used. Eggs were divided into 6 groups as control (group 1), corn oil (group 2), hydrocortisone hemisuccinate sodium (HC) (group 3), low dose chrysin (group 4), medium dose chrysin (group 5) and high dose chrysin (group 6). On the 15th day of incubation, Chrysin and HC were applicated to the air sac of the eggs with Hamilton and/or insulin injector. On day 17, the chick embryos were removed from the eggs and the bulbus oculi of the embryos were dissected. Lenses of 9 embryos were used for morpholigical cataract grading in each group, lens of 8 embryos for biochemical analysis and intact eyes of 7 embryos for histological evaluation (TUNEL method).

Results: No opacity was observed in any of the lenses in Group 1 and 2. Cataract was observed in all lenses in Group 3. The mean opacity grades in group 3 were statistically significantly higher when compared to group 1 and 2 ($p<0.05$). The difference between group 6 and group 3 was statistically significant ($p<0.05$). GSH and TAS levels in the lenses were statistically significantly decreased compared to the control group due to HC application ($p<0.05$). It was determined that the decreased GSH and TAS levels in the lenses increased in relation to the Chrysin application doses. The increased levels of MDA, TOS, caspase 3 and caspase 9 in the HC group decreased significantly depending to the chrysin doses ($p<0.05$). In addition, while the rate of apoptotic cells determined by the TUNEL method was statistically significantly higher in the HC administered group than in the control group ($p<0.05$), it was statistically significantly decreased in the chrysin-administered groups, in relation to the dose of chrysin ($p<0.05$).

Conclusions: We think that anti-cataract effect of crhysin may be due to the antioxidant and antiapoptotic properties of chrysin. However, more research is needed to clarify the anti-cataract effects of chrysin.

Cataract is the decrease in the transparency of the lens of the eye, its clouding and, as a result, the decrease in visual acuity [1]. Cataract is one of the visual defects that still continues all over the world despite the developing scientific and technological treatment methods [2]. Studies show that 36 million people in the world suffer from blindness and approximately 12 million of these cases are caused by cataracts [3,4]. Although the etiology of cataract is not known exactly, aging is thought to be the most important factor. Exposure to ultraviolet rays, smoking, oxidative stress, and insufficient vitamin E and C intake are other risk factors for cataract formation [5–9].

Correspondence to: Gülan Albaş Kurt, Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Anatomy, Afyonkarahisar, Turkey; Phone: +90506 437 03 87; FAX: +90 272 246 33 00; email: fzt.gulan@hotmail.com

At the same time, the complication of corticosteroid therapy has an important place in cataract formation [10]. Corticosteroids are effective anti-inflammatory drugs used in a variety of clinical conditions, including autoimmune, neurological, rheumatological, dermatological and nephrological disorders [11]. Despite the therapeutic clinical effects of corticosteroids, especially long-term and high-dose use may cause serious complications [12].

Different experimental animal models such as mouse, rat, rabbit and dog have been used in the literature to examine the etiopathogenesis of cataract [13–15]. The chick embryo cataract model is one of the most frequently used experimental method in recent years to investigate cataract formation and the anti-cataract mechanisms of antioxidants [16–19].

Flavonoids are polyphenols with antioxidant properties that are beneficial to human health due to their biological

Table 1. Experimental procedure in groups.

Group	Dose	Form of administration	Volume	Frequency of administration	Day
Group 1 (n=25)	100 µl PBS (pH:7.4)	AS	0.1 ml	1 time	15 day
Group 2 (n=25)	100 µl corn oil	AS	0.1 ml	1 time	15 day
Group 3 (n=25)	(100 µl in 0.50 µmol HC)	AS	0.1 ml	1 time	15 day
Group 4 (n=25)	(100 µl in 0.50 µmol HC) + Chrysin (100 µl in 25 mg/kg)	AS	0.2 ml	1 time	15 day
Group 5 (n=25)	(100 µl in 0.50 µmol HC) + Chrysin (100 µl in 50 mg/kg)	AS	0.2 ml	1 time	15 day
Group 6 (n=25)	(100 µl in 0.50 µmol HC) + Chrysin (100 µl in 100 mg/kg)	AS	0.2 ml	1 time	15 day

HC: Hydrocortisone Succinate Sodium, AS: Air Sac, PBS: Phosphate Buffer Saline

properties. In the literature, the antioxidant effect of various flavonoids related to prevention and treatment of cataract development has been investigated [7,17,20]. Chrysin (5,7-dihydroxyflavone) is one of the flavonoids with antioxidant properties. It is obtained from passion flowers *Passiflora Caerulea*, *Passiflora Incarnata* and *Oroxylum Indicum*. It is also found in propolis and honey. Chrysin has many biological activities, including anti-inflammatory, anticarcinogenic, antioxidant [21].

The antioxidant effect of chrysin on cataract has not yet been clarified, and as far as we have researched, there are limited studies in the literature. The aim of this study was to evaluate the protective effect (antioxidant) of chrysin on the steroid-induced experimental cataract model in chick embryos by morphological, biochemical and histopathological methods.

METHODS

This study was carried out in Afyonkarahisar Health Sciences University, Faculty of Medicine, Department of Anatomy. Specific Pathogen Free (SPF) eggs were obtained from İzmir/Bornova Veterinary Control Institute. All experiments were performed in accordance with the animal research protocol of the local Ethics Committee. Permission for the study was obtained from Afyon Kocatepe University Animal Experiments Local Ethics Committee (Number: 49533702/104; date: 24.08.2021).

In our study, a standardized automatic incubator was used. Standardization in the incubator was determined as a constant ambient temperature of 37.5±0.5 °C and a relative humidity of 70±5%. 150 fertilized SPF White Leg Horn eggs with an average weight of 65±5 g were used in the study. The day the eggs were laid in the incubator was considered as day 0. Eggs were randomly divided into six groups and applications were made as shown in Table 1. Considering the doses in which chrysin was effective, the chrysin doses applied in our study were determined as 25, 50 and 100 mg/kg, in line with the literature [22].

On the 15th day of incubation, the eggs were removed from the incubator, the eggshell was sterilized using 70% ethanol. Hydrocortisone succinate sodium HC (100 µl in 0.50 µmol HC)/phosphate buffer saline (PBS)/chrysin and corn oil were injected into the air sac (AS) with Hamilton and/or insulin injector. Chrysin solution was applied to groups 4, 5 and 6, three hours after HC injection. The punctures that used for injection were sealed with sterile cellophane tape and the eggs were further incubated for 48 h in the same incubator.

On the 17th day of incubation, all the eggs were removed from the device in groups, their shells were broken, and the embryos were removed from the eggs. Lenses of 9 embryos in each group were separated for cataract grading, and lenses of 8 embryos were separated for biochemical analysis. The bulbus oculi of the remaining 7 embryos were placed in the fixative that we prepared before for histological tissue follow-up. One embryo in each group was excluded because it showed developmental delay or had damaged lenses during dissection.

Cataract formation in the lenses were determined under the stereo microscope (Zeiss, Stemi 2000-C, Germany) and lenses were photographed. Glutathione (GSH), malondialdehyde (MDA), total antioxidant status (TAS), total oxidant status (TOS), caspase 3 and caspase 9 levels in all lens tissues

were measured by biochemical methods. Apoptotic rate in lenses was determined using TUNEL staining method.

Evaluation of lens opacity: The lenses of 9 embryos in each group were investigated for cataract grading under a stereo microscope. A 5-point scoring system was used: 1: no lens opacity; 2: faint white ring in the periphery of the lens nucleus; 3: clear white ring in the periphery of the lens nucleus; 4: opacity of the lens nucleus not spreading to the center of the nucleus; and 5: opacity of the lens nucleus spreading to the center of the nucleus [16,23].

Biochemical analysis: Eight embryos from each group were used and two lenses of one embryo were considered as a single sample. By adding 0.1 M pH: 7.4 phosphate buffer to the samples, they were homogenized with Ultra Turrax (IKA Works, Wilmington, NC) brand homogenizer in ice at 10,000 ×g for 1 min. Then, the homogenates were sonicated with a sonicator (Dr. Hielscher-Germany) for 1 min at 20,000 cycles/sec. Then, homogenates were centrifuged at 10,000 ×g for 15 min and stored at -20 degrees until they were studied.

GSH levels in homogenates were measured using the BT-Lab brand (BT-Lab, Zhejiang, China) Chicken Glutathione Elisa Kit (Catalog no: E0120Ch, Bioassay Technology, Shanghai, China). Results are given in nmol/lens. MDA (Catalog no: EK700097, AFG Bioscience®, Northbrook, IL), TAS (Catalog no: EK710683, AFG Bioscience®), TOS (Catalog no: EK710417, AFG Bioscience®), caspase 3 (Catalog no: EK710396, AFG Bioscience®) and caspase 9 (Catalog no: EK710400, AFG Bioscience®) levels were studied using AFG Bioscience brand Elisa kits. Research Elisa kits were used for biochemical analyzes. Measurements were made in accordance with the kit procedures. For all biochemical kits, Inter CV assays are less than 10% and Intra assay CVs are less than 8%. Results are given as µmol/Lens for MDA, µmol/lens for TAS, pg/lens for TOS, ng/lens for caspase-3 and caspase-9. Absorbance reading was performed on ChemWell 2910 elisa reader device (Awareness Technology, Inc. Martin Hwy. Palm City, FL). All measurements were made according to the manufacturer's instructions.

Histological analysis: Intact eyes of 7 embryos from each group were fixed in 10% neutral formalin. The fixed tissues were taken for routine histological tissue follow-up in an automatic tissue tracking device (Leica Biosystems TP1020, Germany) and paraffin blocks were obtained by sagittal embedding in paraffin. Sections of 4-5 µm thickness from these blocks were taken on positively charged slides and subjected to TUNEL (Millipore brand ApopTag® Plus Peroxidase In Situ Apoptosis Kit) staining to observe apoptotic cells.

In sections obtained from eye tissues taken from each embryo, the area where the lens is located was evaluated under a light microscope (Nikon Eclipse E600W, Japan) with camera (Nikon Digital Sight D5-Fi2, Japan). Nikon NIS Elements 4.2 Image Analysis program was used for the evaluations and dark brown stained cells were considered positive. Apoptotic rate (TUNEL positive cell count/Total cell) was obtained by counting at least 300 cells in the lenses of each embryo. In the experiment, one embryo from each group was excluded from the study because it showed developmental delay or its lenses were damaged during dissection.

Statistical analysis: All findings were analyzed using the Statistical Package for the Social Sciences (SPSS) 25.0 program. Cataract levels, protein contents (TAS, TOS, MDA, GSH), and apoptosis scores were analyzed using non-parametric Kruskal–Walli's test. The Dunn test was used as a post hoc test. Values with $p<0.05$ were considered statistically significant. The data are presented as the mean ± standard deviation (M ± SD).

RESULTS

Cataract grading results: The degrees of opacification in all embryo lenses in Groups 1 and 2 were compatible with stage 1 (Figure 1A). The mean cataract value in the HC group (Group 3) embryos was 3.5, and the results were statistically significantly higher than in Groups 1 and 2. In groups 4, 5 and 6, the mean degree of cataract decreased in related to the chrysin dose, and a statistically significant difference was determined only between group 6 and group 3 ($p<0.05$; Table 2).

Determination of GSH, MDA, TAS, and TOS levels in lenses: GSH levels in the groups were shown in Figure 2. As a result of the biochemical analyzes, the mean GSH level was determined as 7.57 nmol/lens in group 1, while it was determined as 7.24 nmol/lens in group 2. In Group 3, GSH level decreased due to HC application and the mean GSH value was calculated as 5.93 nmol/lens. The difference between group 1 and group 3 was determined to be statistically significant ($p<0.05$). GSH levels were determined to be significantly higher in groups 5 (6.91 nmol/lens) and 6 (7.22 nmol/lens) compared to group 3 due to Chrysin administration ($p<0.05$; Figure 2).

The mean MDA level was calculated as 4.07 µmol/lens in group 1 and 4.32 µmol/lens in group 2. The mean MDA levels in the groups were significantly higher in group 3 (9.47 µmol/lens) compared to group 1 ($p<0.05$). Compared to group 3, MDA levels were statistically significantly lower in groups 5 (5.67 µmol/lens) and 6 (4.52 µmol/lens) ($p<0.05$; Figure 2).

Table 2. Cataract grades of lenses in all groups.

Groups	N	Cataract grades							
		1	2	3	4	5	Mean	Min.	Max.
Group 1	9	9	0	0	0	0	1	1	1
Group 2	9	9	0	0	0	0	1	1	1
Group 3	9	0	1	4	2	2	3.5	2	5
Group 4	9	0	1	6	2	0	3.1	2	4
Group 5	9	1	4	4	0	0	2.3	1	3
Group 6	9	4	5	0	0	0	1.5	1	2

While the mean TAS value was 13.13 µmol/lens in group 1, it was found as 12.52 µmol/lens in group 2. Mean TAS levels were significantly lower in group 3 (9.62 µmol/lens) compared to group 1 and group 2 ($p<0.05$). When compared to group 3, TAS levels in groups 4 (11.50 µmol/lens), 5 (11.57 µmol/lens) and 6 (12.39 µmol/lens) increased due to Chrysin application and this increase was statistically significant ($p<0.05$; Figure 2).

TOS levels were shown in Figure 2. The mean TOS level in Group 1 was determined as 170.89 pg/lens. Compared to group 1, TOS levels in the lenses were significantly higher in group 3 (252.34 pg/lens). TOS levels of the lenses in group 6 (200.03 pg/lens) were statistically significantly lower when compared to group 3 ($p<0.05$; Figure 2).

Caspase 3 and Caspase 9 levels in lenses: The mean caspase 3 levels in the lenses were shown in Figure 2. The mean caspase 3 level in group 1 was determined as 9.01 ng/lens. Caspase 3 levels were statistically significantly higher in group 3 (13.81 ng/lens) compared to group 1 due to HC administration ($p<0.05$). Caspase 3 levels were statistically lower in groups

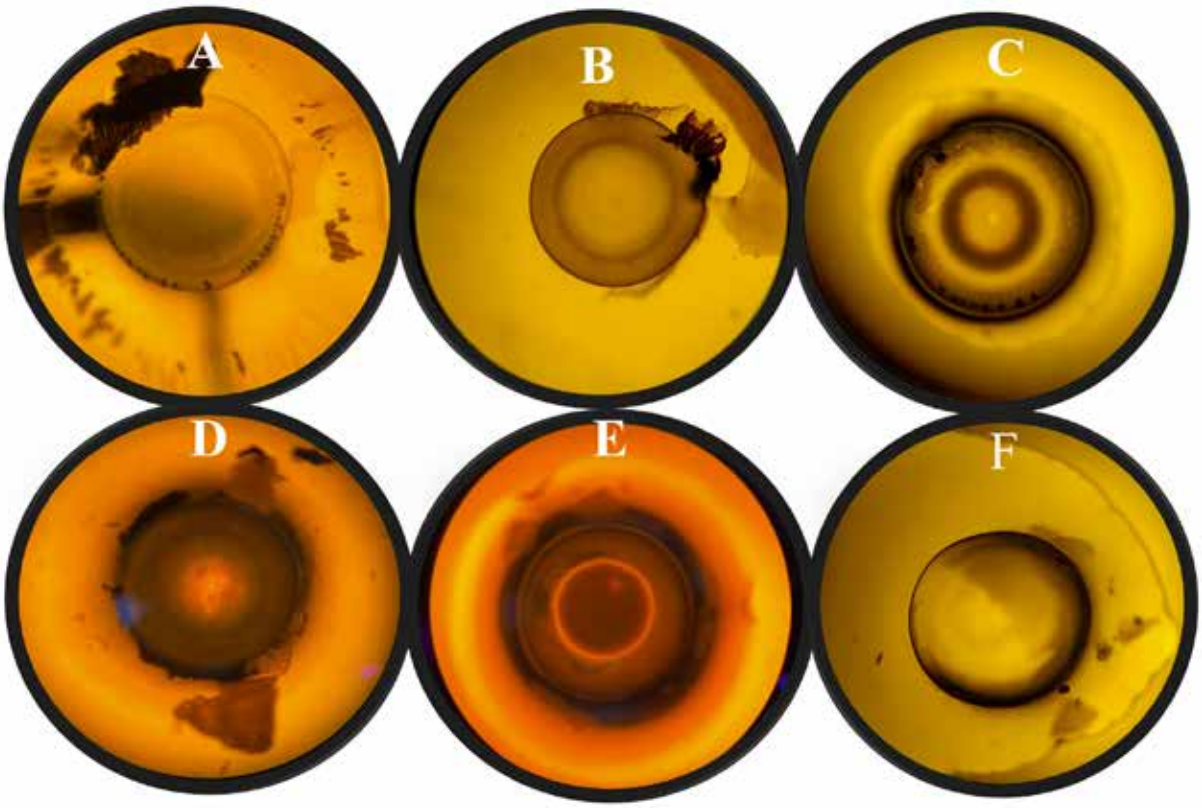

Figure 1. Microscopic postmortem in vitro imaging of cataract grades of the groups A: Grade 1 clear lens (Group 1) B: Grade 2 cataract (Group 6) C: Grade 3 cataract (Group 5) D: Grade 4 cataract (Group 4) E: Grade 5 cataract (Group 3) F: Grade 1 cataract (Group 6).

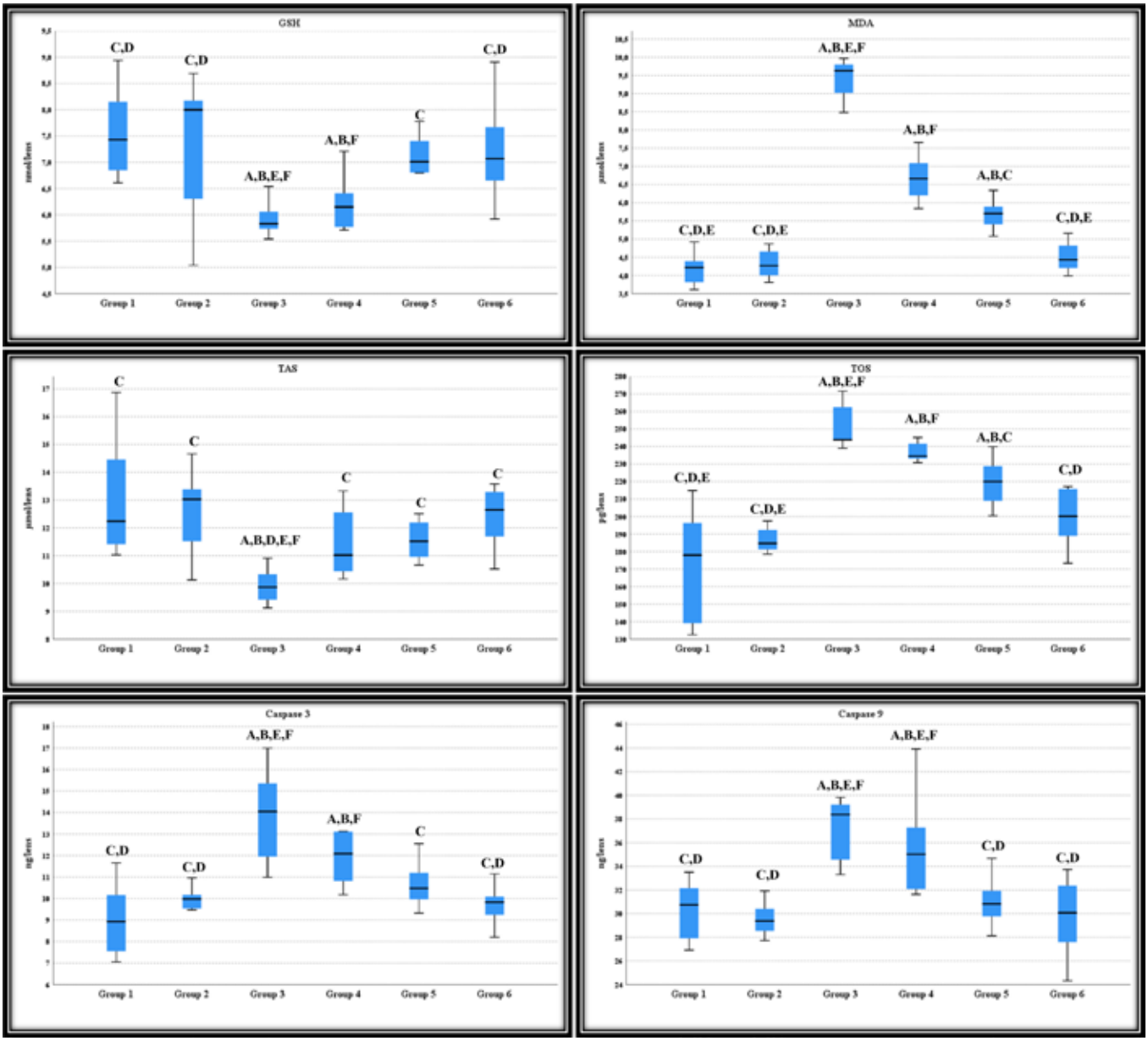

Figure 2. Levels of GSH, MDA, TAS, TOS, caspase 3, and caspase 9 in the chick embryo lenses of all groups on day 17. There is a statistically significant difference when compared with A) Group 1, B) Group 2, C) Group 3, D) Group 4, E) Group 5, and F) Group 6 ($p<0.05$).

5 (10.67 ng/lens) and 6 (9.69 ng/lens) compared to group 3 ($p<0.05$).

It was determined that the mean caspase 9 level increased due to HC administration and it was statistically significantly higher in group 3 compared to group 1 ($p<0.05$). While the mean caspase 9 level was determined as 29.33 ng/lens in group 1, this value was calculated as 39.71 ng/lens in group 3. However, caspase 9 levels in groups 5 (31 ng/lens) and 6 (29.72 ng/lens) decreased significantly with Chrysin administration compared to group 3 ($p<0.05$; Figure 2).

Immunohistochemical staining (TUNEL): The findings of the TUNEL (TUNEL positive cell count/Total cell) method in all groups were shown in Figure 3. The apoptotic rate was determined as 25.57 in groups 1 and 33.14 in group 2. No statistically significant difference was observed between these two groups ($p>0.05$). As a result of histopathological evaluations; It was determined that the rate of TUNEL positive cells increased in Group 3 (46,71) due to HC application, and this increase was statistically significant when compared to Groups 1 and 2 ($p<0.05$). It was determined that the rate of

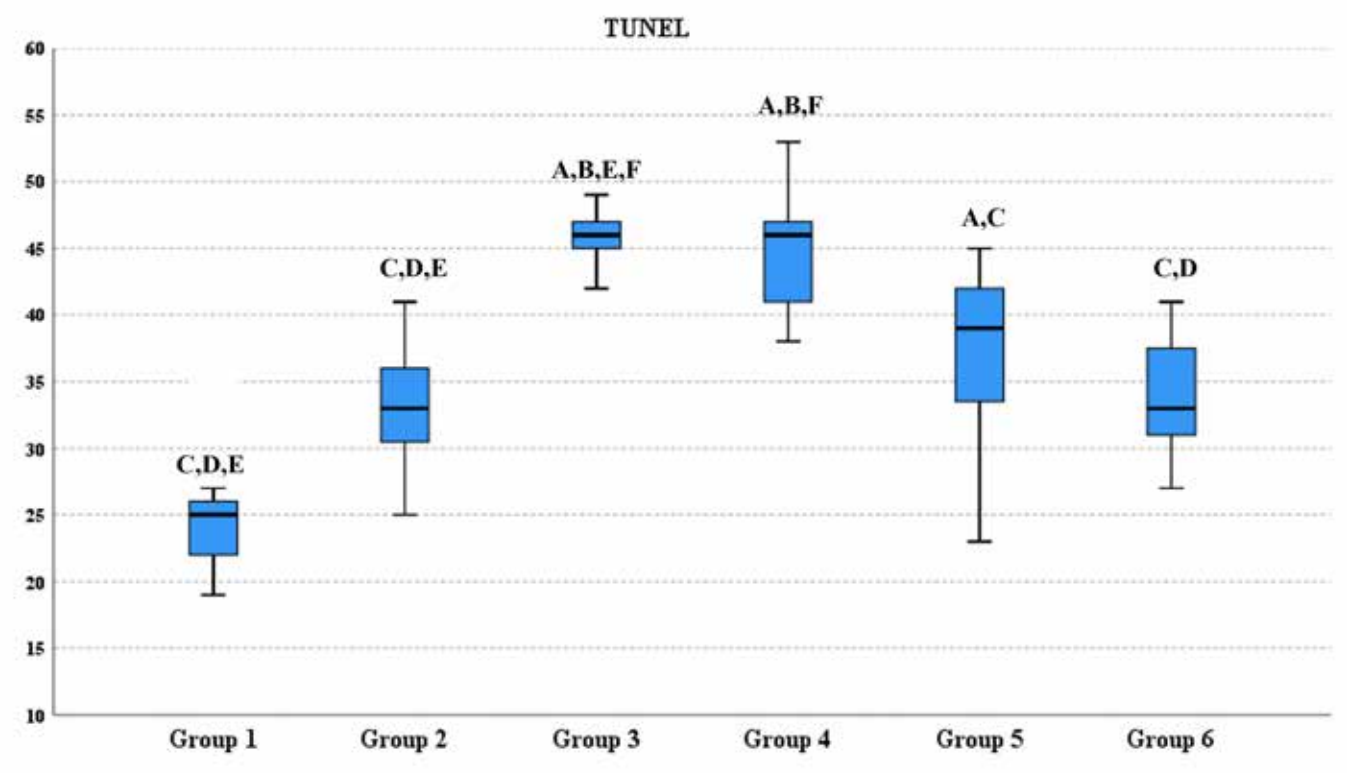

Figure 3. TUNEL results of all groups (number of TUNEL-positive cells/total cells). There is a statistically significant difference when compared with A) Group 1, B) Group 2, C) Group 3, D) Group 4, E) Group 5, and F) Group 6 ($p<0.05$).

TUNEL positive cells in groups 4 (44,71), 5 (36,86) and 6 (34) decreased compared to group 3 due to Chrysin application at different doses, and the difference between groups 3 and 6 was statistically significant ($p<0.05$). Microscopic images of the findings obtained in the TUNEL staining method were shown in Figure 4.

DISCUSSION

Cataract is one of the leading causes of blindness worldwide. The only treatment for cataract is surgical removal of the opaque lens and replacement with a clear lens. In order to delay or prevent cataract formation, it is important to know the cataract development mechanism and to find cataract preventive therapeutics [8]. The increase in reactive oxygen species (ROS) production and the decrease in endogenous antioxidant levels are thought to cause cataract formation [24,25]. Oxidative stress is one of the most important factors in cataract formation. The body has an antioxidant system consisting of enzymatic and non-enzymatic antioxidants to normalize the elevated free radical and ROS levels. Lens opacity develops when oxidative stress levels exceed the lens' natural antioxidant potential. Also, the level of GSH in the lens decreases. This increases the formation of opacity [16]. It has been reported that ROS is an effective factor in the development of cataracts in various experimental animal models and tissue culture studies [19,22–24].

The chick embryo cataract model is one of the frequently used methods to examine the etiopathogenesis of cataract and to investigate the mechanisms preventing cataract [16]. In this model, it was reported that different doses of HC that applied at various embryonic stages of chick induced cataract formation in the embryo lenses [16,17,23,26,27]. In this study, a steroid-induced cataract model was created. Injection of the planned dose of HC was done on day 15 of embryo development. Cataract grading in lenses was performed on the 17th day, 48 h after HC application. Consistent with the literature, the dose of HC was determined as 0.50 µmol, and as a result of our study, cataract formation was induced in all chick embryos of group 3 with HC application and the mean cataract degree was determined as 3.5 [16,26].

In the literature, anti-cataractogenic effects of various antioxidants such as piperine, astaxanthin, betaine and vitamin e have been investigated against HC-induced cataract in chick embryos [16–19]. Chrysin, a flavonoid belonging to

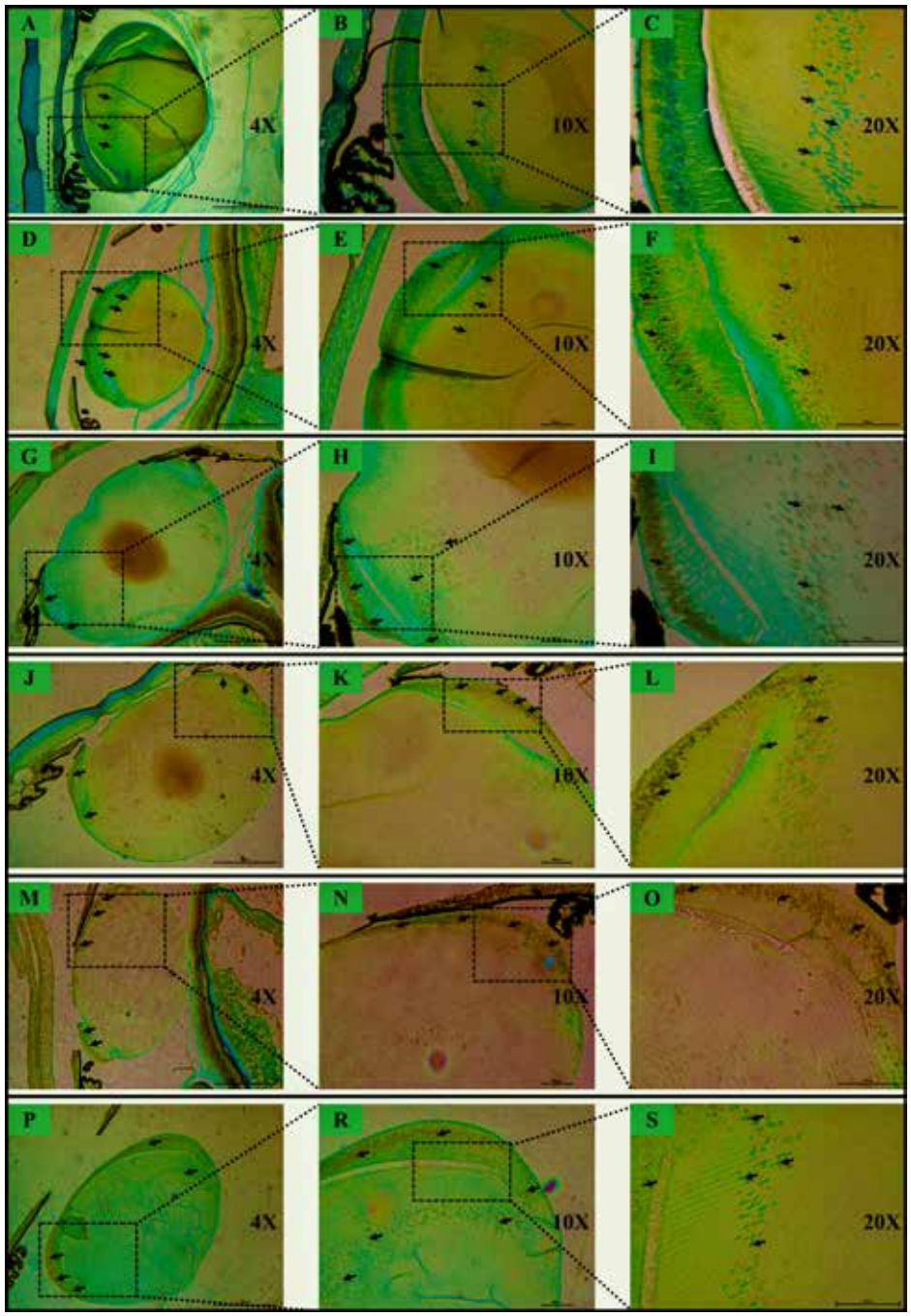

Figure 4. TUNEL staining of the lenses in all groups. Arrows indicate apoptosis-positive cells stained in the lenses. **A, B, C**; Group 1, **D, E, F**; Group 2, G, H, I) Group 3, **J, K, L**; Group 4, **M, N, O;** Group 5, **P, R, S**; Group 6 (X4, X10, X20 enlargement ratios, respectively).

the flavone subgroup, has antioxidant properties as well as many beneficial physiological effects [21].

Several recent studies have shown that chrysin has multiple biological activities with anti-inflammatory, anticancer and antioxidation effects [28–30]. In the literature, therapeutic effects of chrysin on patients with age-related macular degeneration and other diseases that develop choroidal neovascularization have been reported. In a study using in vitro and in vivo retinal neovascularization models, chrysin showed therapeutic effect by reducing the induction of HIF-la, VEGF, VEGFR2, Ang-1, Ang-2 and Tie-2 proteins and inhibiting the formation of acellular capillaries. These results indicate that chrysin may encumber retinal

neovascularization with vascular permeability and blood-retinal barrier breakdown detected in diabetic retina [31,32]. However, there are limited studies investigating the effect of Chrysin on cataract development. Sundararajan et al. incubated rat lenses with selenite and chrysin+selenite in vitro. In their study, the effect of a single dose of chrysin (200 µM chrysin) against the cataract formation induced by selenite applied at different time intervals was evaluated. In our study, an in ovo cataract model was created with HC application in chick embryos while they were still in the incubation period, and the therapeutic effect of chrysin (25, 50 and 100 mg/kg) at different doses was investigated. The two studies differ from each other in that the experimental methods used are in vitro and in ovo, and inducing cataract formation by different agents. In addition, while Sundararajan et al. evaluated the effect of a single dose of chrysin at the morphological, biochemical and molecular level in their study, we evaluated the therapeutic effect of chrysin morphologically, biochemically and histologically in our study [30].

In the experimental groups treated with Chrysin and HC, the cataract grades decreased in related to the amount of dose administered, and the mean cataract grades were calculated as 3.1, 2.3 and 1.5, respectively, from low dose to high dose. Our results were similar to the findings of the study conducted by Sundararajan et al in terms of reducing the grades of steroid-induced (HC) cataracts with Chrysin treatment [30].

GSH is a low molecular weight antioxidant synthesized in cells [33]. While TAS determines the overall antioxidative state, TOS is used to determine the oxidation state [34]. MDA, on the other hand, is defined as a measure of oxidative stress, which is produced at a high rate during lipid peroxidation [35]. All these parameters are important in terms of examining the balance between antioxidant-oxidant in lenses. Studies have reported decreased GSH and increased MDA levels in rat lenses in which cataract formation was induced [30,36,37]. In our study, an increase in TOS and MDA levels, and a decrease in GSH and TAS levels, especially in group 3, occurred as a result of HC application. These findings are similar to the results of other studies in the literature using HC to induce cataract formation [18,38]. In the groups treated with Chrysin; It was determined that decreased GSH and TAS levels increased with HC application, while increased MDA and TOS levels decreased and these changes were statistically significant. These data vary depending on the dose of Chrysin. Our biochemical results shows that HC application increases oxidative stress and triggers cataract formation by disrupting the antioxidant-oxidant balance in chick embryo lenses, while Chrysin application suppresses lens opacity by exhibiting antioxidant effect.

Factors such as overexposure to UVB rays and increase in oxidative stress cause damage to lens epithelial cells. It is predicted that this damage to lens epithelial cells occurs due to apoptosis [39,40]. Studies in biochemistry and genetics have revealed that caspases play an important role in the initiation and maintenance of the apoptotic process [41]. In experimental studies in which cataract formation was induced, it was reported that caspase 3 and caspase 9 levels in lenses increased after inducing agent application [30,42–46]. In a study examining the protective effect of parthenolide against apoptosis caused by oxidative stress in human lens epithelial (HLE) cells; H_2O_2 (cataract-inducing agent) was applied to HLE cells and increased caspase 3 and caspase 9 activities were detected in the cells, and it was reported that parthenolide inhibited the activation of caspase 3 and caspase 9 in HLE cells treated with Parthenolide [42]. In another study, Andersson et al. reported that in human lens epithelial cells incubated with staurosporine, caspase 3 activity increased in relation to staurosporine (cataract-inducing agent) concentration [43]. In a study examining the relationship between cataract formation and exposure to UVB rays, HLE cells were exposed to UVB rays at different intensities and durations. It has been reported that caspase 3 levels in HLE cells increase 2-5 times in related to proportion to the exposure time to UVB irradiation [8]. In an in vitro study in which Wistar rat lenses were incubated with selenite and/or chrysin; It has been reported that caspase 3 and 9 levels, which play a role in apoptosis, are statistically significantly higher in the only selenite treated group (cataract group; %98.7 and %85.5) compared to the control group (%71.8 and %66.1). However, it was reported that these values decreased significantly and approached the control group in the treatment groups in which chrysin and selenite were administered simultaneously [30]. In the studies that mentioned above, a positive correlation was reported between the applied cataract-inducing agent and the increase in caspase levels, which is an indicator of apoptosis. All these findings show that the change in caspase levels in lens epithelial cells and the resulting apoptosis are effective in the mechanism of cataract formation. Similar to the studies in the literature, caspase 3 and caspase 9 values increased in lenses after HC application (cataract-inducing agent) in this study. It was determined that caspase 3 and caspase 9 values decreased in related to proportion to the chrysin dose in the HC+chrysin treated groups, approaching the control group. These findings suggest that HC application causes apoptosis by activating caspase pathways in lens epithelial cells, and chrysin, which we apply as a therapeutic antioxidant, has an

antiapoptotic effect by inhibiting caspase 3 and caspase 9 related pathways.

The apoptosis process has a complex mechanism, and depending on this process, many metabolic, physiological and biochemical changes take place in the cells. The cells begin to shrink and condense, the cytoskeleton disintegrates and the nuclear membrane partially melts. Nuclear DNA is fragmented. Buds called apoptotic bodies are formed, which engulf the cell contents and break off from apoptotic cells in the form of membrane-enclosed vesicles [47]. One of the histological methods used in the literature to examine these changes that occur during apoptosis is the TUNEL staining method. This method is used to test endonuclease cleavage products by enzymatically labeling DNA strand breaks [48]. As applied in our study, there are studies in the literature in which apoptosis inducing cataract formation was histologically determined by TUNEL test [44,49].

In studies that examining the development of cataract due to H_2O_2, UVB rays, galactose and selenite application in rat lens organ culture [50] and rat lens epithelial cells [44,49,51]; It was determined that the rate of TUNEL positive cells was increased in the groups that only inducing agent was applied, and it was reported that apoptosis could be triggered by oxidative stress and also induced lens epithelial cell apoptosis could be an initiating mechanism in cataract formation. Similar to the studies in the literature, in our study, it was determined that cataract formation was observed in HC applied groups and the rate of TUNEL positive cells increased statistically significantly compared to the control group as a result of HC application (Figure 3). According to the results of histopathological evaluation, in our study; While the rate of TUNEL positive cells was determined as 25.57 in the control group, the rate was calculated as 46.71 in the HC group only. In HC+chrysin groups, a decrease in TUNEL positive cell rate occurred in related to proportion to the dose of chrysin administered, and the rate of TUNEL positive cells from low dose to high dose was determined as 44.71, 36.86 and 34, respectively. As far as we have researched, there are limited studies in the literature evaluating the anti-cataract effects of chrysin [22,30]. In only one of these studies, the effects of chrysin on apoptosis during cataract development were evaluated through the expression levels of apoptotic cascade proteins. As a result of the study; It has been reported that chrysin inhibits caspase-related apoptosis by reducing or blocking the apoptotic cascade activation [30].

In some experimental studies on cataract induction and treatment; the techniques used for the determination of apoptosis had the disadvantages that they were not completely specific for apoptosis and could be confused with the presence of necrotic cells. However, in this study, the apoptosis process was determined by two methods, biochemically and histologically. First, caspase 3 and caspase 9 levels were examined using biochemical kits to detect apoptosis, while TUNEL Positive cell ratio was calculated using TUNEL staining method, which is the histopathological parameter. Biochemical and histological evaluation results were compatible with each other in terms of cataract induction and prevention in the experimental groups.

Conclusion: In summary, our findings showed that cataract formation was induced in chick embryo lenses by HC application. Potential underlying cataract formation mechanisms may be associated with the disruption of the antioxidant–oxidant balance caused by HC application in the lenses, as well as HC's capacity to increase apoptosis in lens epithelial cells. A significant decrease in cataract grades was observed in the chrysin-treated groups. We posit that this effect may be due to the antioxidant and antiapoptotic properties of chrysin. However, further research is needed to clarify the anti-cataract effects of chrysin.

ACKNOWLEDGMENTS

This research was supported by the Afyonkarahisar Health Sciences University Scientific Research Projects Commission, Grant/Award Number: 20.LİS.001. This study is the master's thesis of one of the authors, Gülan Albaş Kurt, under the supervision of Tolga Ertekin. Author contributions: Research idea: TE, GAK, EA; Design of the study: TE, EA; Acquisition of data for the study: GAK, AS; Analysis of data for the study: EA, AB; Interpretation of data for the study: EA, AB; Drafting the manuscript: GAK, AS; Revising it critically for important intellectual content: GAK, AS; Biochemical analysis: HBK; Histological analysis: EA; Final approval of the version to be published: TE, EA, GAK.

REFERENCES

1. McCarty CA. Cataract in the 21st century: Lessons from previous epidemiologic research. Clin Exp Optom 2002; 85:91-6. [PMID: 11952404].

2. Liu YC, Wilkins M, Kim T, Malyugin B, Mehta JS. Cataracts. Lancet (London, England) 2017; 390:600-12. [PMID: 28242111].

3. Flaxman SR, Bourne RRA, Resnikoff S, Ackland P, Braithwaite T, Cicinelli MV, Das A, Jonas JB, Keeffe J, Kempen JH, Leasher J, Limburg H, Naidoo K, Pesudovs K, Silvester A, Stevens GA, Tahhan N, Wong TY, Taylor HR. Global causes of blindness and distance vision impairment 1990-2020: a systematic review and meta-analysis. Lancet Glob Health 2017; 5:e1221-34. [PMID: 29032195].

4. Nam GE, Han K, Ha SG, Han BD, Kim DH, Kim YH, Cho KH, Park YG, Ko BJ. Relationship between socioeconomic and lifestyle factors and cataracts in Koreans: the Korea National Health and Nutrition Examination Survey 2008-2011. Eye (Lond) 2015; 29:913-20. [PMID: 25976646].

5. Varma SD. Scientific basis for medical therapy of cataracts by antioxidants. Am J Clin Nutr 1991; 53:SUPPL.[PMID: 1985407].

6. Gupta VB, Rajagopala M, Ravishankar B. Etiopathogenesis of cataract: An appraisal. Indian J Ophthalmol 2014; 62:103-10. [PMID: 24618482].

7. Mares-Perlman JA, Klein BEK, Klein R, Ritter LL. Relation between Lens Opacities and Vitamin and Mineral Supplement Use. Ophthalmology 1994; 101:315-25. Internet[PMID: 8115152].

8. Ji Y, Cai L, Zheng T, Ye H, Rong X, Rao J, Lu Y. The mechanism of UVB irradiation induced-apoptosis in cataract. Mol Cell Biochem 2015; 401:87-95. [PMID: 25445170].

9. Kelly SP, Thornton J, Edwards R, Sahu A, Harrison R. Smoking and cataract: review of causal association. J Cataract Refract Surg 2005; 31:2395-404. [PMID: 16473237].

10. Turno-Krecicka A, Grzybowski A, Misiuk-Hojło M, Patryn E, Czajor K, Nita M. Ocular changes induced by drugs commonly used in dermatology. Clin Dermatol 2016; 34:129-37. [PMID: 26903180].

11. Wan ES, Qiu W, Baccarelli A, Carey VJ, Bacherman H, Rennard SI, Agustí A, Anderson WH, Lomas DA, DeMeo DL. Systemic steroid exposure is associated with differential methylation in chronic obstructive pulmonary disease. Am J Respir Crit Care Med 2012; 186:1248-55. [PMID: 23065012].

12. Liu D, Ahmet A, Ward L, Krishnamoorthy P, Mandelcorn ED, Leigh R, Brown JP, Cohen A, Kim H. A practical guide to the monitoring and management of the complications of systemic corticosteroid therapy. Allergy Asthma Clin Immunol 2013; 9:30-[PMID: 23947590].

13. Bayley KD, Read RA. Sub-Tenon's anesthesia for canine cataract surgery. Vet Ophthalmol 2018; 21:601-11. [PMID: 29411508].

14. Dave A, Craig JE, Alamein M, Skrzypiec K, Beltz J, Pfaff A, Burdon KP, Ercal N, de Iongh RU, Sharma S. Genotype, Age, Genetic Background, and Sex Influence Epha2-Related Cataract Development in Mice. Invest Ophthalmol Vis Sci 2021; 62:3-[PMID: 34495288].

15. Okuno T, Kojima M, Yamaguchi-Sekino S, Ishiba Y, Suzuki Y, Sliney DH. Cataract Formation by Near-infrared Radiation in Rabbits. Photochem Photobiol 2021; 97:372-6. [PMID: 33064904].

16. Ishikawa S, Hashizume K, Nishigori H, Tezuka Y, Sanbe A, Kurosaka D. Effect of astaxanthin on cataract formation induced by glucocorticoids in the chick embryo. Curr Eye Res 2015; 40:535-40. [PMID: 25110808].

17. Vurmaz A, Duman R, Sabaner MC, Ertekin T, Bilir A. Antioxidant effects of piperine in in-vivo chick embryo cataract model induced by steroids. Cutan Ocul Toxicol 2019; 38:182-9. Internet[PMID: 30678496].

18. Vurmaz A, Ertekin A, Sabaner MC, Atay E, Bozkurt E, Bilir A. Effects of vitamin E in a glucocorticoid induced cataract model in chicken embryos. Biotech Histochem 2021; 96:431-8. Internet[PMID: 32957855].

19. Duman R, Ertekin T, Duman R, Vurmaz A, Çetinkaya E, Güzel H. Anticataractogenic effect of betaine in chick embryo hydrocortisone-induced cataract model. Indian J Med Res 2019; 150:407-11. [PMID: 31823923].

20. Zhang X, Peng L, Dai Y, Xie Q, Wu P, Chen M, Liu C. Anticataract effects of coconut water in vivo and in vitro. Biomed Pharmacother 2021; 143:112032[PMID: 34488080].

21. Naz S, Imran M, Rauf A, Orhan IE, Shariati MA. Iahtisham-Ul-Haq, et alChrysin: Pharmacological and therapeutic properties. Life Sci 2019; 235:116797[PMID: 31472146].

22. Wojnar W, Zych M, Borymski S, Kaczmarczyk-Sedlak I. Chrysin reduces oxidative stress but does not affect polyol pathway in the lenses of type 1 diabetic rats. Antioxidants 2020; 9:1-19. [PMID: 32079112].

23. Lee JW, Iwatsuru M, Nishigori H. Glucocorticoid-Induced Cataract of Developing Chick Embryo as a Screening Model for Anticataract Agents. J Ocul Pharmacol Ther 1995; 11:533-41. [PMID: 8574817].

24. Ho MC, Peng YJ, Chen SJ, Chiou SH. Senile cataracts and oxidative stress. J Clin Gerontol Geriatr. 2010; 1:17-21. .

25. Chang D, Zhang X, Rong S, Sha Q, Liu P, Han T, Pan H. Serum antioxidative enzymes levels and oxidative stress products in age-related cataract patients. Oxid Med Cell Longev 2013; 2013:587826[PMID: 23781296].

26. Duman R, Ertekin T, Duman R, Vurmaz A, Köken T, Bilir A. Comparison of the Efficacy of Different Hydrocortisone-Induced Cataract Models in Developing Chick Embryos. J Pharm Res Int. 2018; 21:1-7. .

27. Nishigori H, Lee JW, Yamauchi Y, Maruyama K, Iwatsuru M. Analysis of glucose levels during glucocorticoid-induced cataract formation in chick embryos. Invest Ophthalmol Vis Sci 1987; 28:168-74. [PMID: 3804647].

28. Zeinali M, Rezaee SA, Hosseinzadeh H. An overview on immunoregulatory and anti-inflammatory properties of chrysin and flavonoids substances. Biomed Pharmacother 2017; 92:998-1009. [PMID: 28609844].

29. Kasala ER, Bodduluru LN, Madana RM. V AK, Gogoi R, Barua CC. Chemopreventive and therapeutic potential of chrysin in cancer: mechanistic perspectives. Toxicol Lett 2015; 233:214-25. [PMID: 25596314].

30. Sundararajan M, Thomas PA, Archana Teresa P, Anbukkarasi M, Geraldine P. Regulatory effect of chrysin on expression of lenticular calcium transporters, calpains, and apoptotic-cascade components in selenite-induced cataract. Mol Vis 2016; 22:401-23. [PMID: 27168717].

31. Kang MK, Park SH, Kim YH, Lee EJ, Antika LD, Kim DY, Choi YJ, Kang YH. Dietary compound chrysin inhibits

retinal neovascularization with abnormal capillaries in db/db mice. Nutrients 2016; 8:872-[PMID: 27918469].

32. Song JH, Kim YH, Lee SC, Kim MH, Lee JH. Inhibitory Effect of Chrysin (5,7-Dihydroxyflavone) on Experimental Choroidal Neovascularization in Rats. Ophthalmic Res 2016; 56:49-55. [PMID: 27058958].
33. Wu G, Fang YZ, Yang S, Lupton JR, Turner ND. Glutathione Metabolism and Its Implications for Health. J Nutr 2004; 134:489-92. [PMID: 14988435].
34. Jin CQ, Dong HX, Cheng PP, Zhou JW, Zheng BY, Liu F. Anti-oxidant status and oxidative stress in patients with chronic ITP. Scand J Immunol 2013; 77:482-7. [PMID: 23551069].
35. Ayala A, Muñoz MF, Argüelles S. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid Med Cell Longev 2014; 2014:360438[PMID: 24999379].
36. Gupta SK, Trivedi D, Srivastava S, Joshi S, Halder N, Verma SD. Lycopene attenuates oxidative stress induced experimental cataract development: An in vitro and in vivo study. Nutrition 2003; 19:794-9. [PMID: 12921892].
37. Nakazawa Y, Oka M, Bando M, Takehana M. Hesperetin prevents selenite-induced cataract in rats. Mol Vis 2015; 21:804-10. [PMID: 26283862].
38. Duman R, Vurmaz A. Role of innate immunity and oxidative stress in steroid-induced cataracts in developing chick embryos. Cutan Ocul Toxicol 2018; 37:281-5. Internet[PMID: 29542353].
39. Martinez G, de Iongh RU. The lens epithelium in ocular health and disease. Int J Biochem Cell Biol 2010; 42:1945-63. [PMID: 20883819].
40. Yan Q, Liu J-P, Li DW-C. Apoptosis in lens development and pathology. Differentiation 2006; 74:195-211. [PMID: 16759286].
41. Adrain C, Martin SJ. The mitochondrial apoptosome: A killer unleashed by the cytochrome seas. Trends Biochem Sci 2001; 26:390-7. [PMID: 11406413].
42. Yao H, Tang X, Shao X, Feng L, Wu N, Yao K. Parthenolide protects human lens epithelial cells from oxidative stress-induced apoptosis via inhibition of activation of caspase-3 and caspase-9. Cell Res 2007; 17:565-71. [PMID: 17339884].
43. Andersson M, Honarvar A, Sjöstrand J, Peterson A, Karlsson JO. Decreased caspase-3 activity in human lens epithelium from posterior subcapsular cataracts. Exp Eye Res 2003; 76:175-82. [PMID: 12565805].
44. Tamada Y, Fukiage C, Nakamura Y, Azuma M, Kim YH, Shearer TR. Evidence for apoptosis in the selenite rat model of cataract. Biochem Biophys Res Commun 2000; 275:300-6. [PMID: 10964662].
45. Wang HM, Li GX, Zheng HS, Wu XZ. Protective effect of resveratrol on lens epithelial cell apoptosis in diabetic cataract rat. Asian Pac J Trop Med 2015; 8:153-6. Internet[PMID: 25902031].
46. Cao J, Wang T, Wang M. Investigation of the anti-cataractogenic mechanisms of curcumin through in vivo and in vitro studies. BMC Ophthalmol 2018; 18:48-[PMID: 29454324].
47. Cohen JJ. Programmed cell death and apoptosis in lymphocyte development and function. Chest 1993; 103:Suppl99S-101S. [PMID: 8428550].
48. Elmore S. Apoptosis: A Review of Programmed Cell Death. Toxicol Pathol 2007; 35:495-516. [PMID: 17562483].
49. Takamura Y, Kubo E, Tsuzuki S, Akagi Y. Apoptotic cell death in the lens epithelium of rat sugar cataract. Exp Eye Res 2003; 77:51-7. [PMID: 12823987].
50. Li WC, Kuszak JR, Dunn K, Wang RR, Ma W, Wang GM, Spector A, Leib M, Cotliar AM, Weiss M, John Espy J, Howard G, Farris RL, Auran J, Donn A, Hofeldt A, Mackay C, Merriam J, Mittl R, Smith TR. Lens epithelial cell apoptosis appears to be a common cellular basis for non-congenital cataract development in humans and animals. J Cell Biol 1995; 130:169-81. [PMID: 7790371].
51. Li WC, Spector A. Lens epithelial cell apoptosis is an early event in the development of UVB-induced cataract. Free Radic Biol Med 1996; 20:301-11. [PMID: 8720900].

Articles are provided courtesy of Emory University and the Zhongshan Ophthalmic Center, Sun Yat-sen University, P.R. China. The print version of this article was created on 5 November 2023. This reflects all typographical corrections and errata to the article through that date. Details of any changes may be found in the online version of the article.