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Lanosterol Fails to Restore Lens Transparency in Murine Cataract: *In Vivo* and *In Vitro* Evidence

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LW designed the experiment. XC analyzed the data. IA collected the data. ZL created figures and tables. AM drafted the manuscript. WS supervised the study. All the authors have approved the submission.

Key words
BALB/c Mice, Cataract, Lanosterol, Lens, Protein aggregation, Oxidative stress, Drug delivery

ABSTRACT

The conventional belief that cataracts are irreversible and require surgical intervention for vision restoration has been challenged. This study aimed to investigate whether oxysterols, lanosterol, exert effects by binding to crystallin proteins and contribute to the disaggregation of lenticular opacities. Twelve aged BALB/c mice, consisting of equal numbers of males and females, were divided into two groups (6 males and 6 females in each group). Lanosterol was injected subcutaneously in the orbital region around the eyes, and the therapeutic effect of lanosterol on lens structure collapse and opacification was investigated using a cataract model of aged BALB/c mice. The lenses of each eye were removed by dissection, and the lens epithelial cells were cultured in sterile TC-199 bicarbonate media supplemented with 30 mM fructose and 20 U/mL of penicillin-streptomycin. The cells were cultured in a humidified incubator with an atmosphere of 95% air and 5% CO_2 at 37°C. After 48 h, the cells were transferred to 24-well culture plates containing 2 ml of standard culture media. The treatment failed to reverse the lens opacities and/or prevent the progression of cataracts to the nuclear stage. Lanosterol does not show therapeutic effects under the tested conditions to increase the mRNA expression of crystalline protein for the dissolution of cataracts.

INTRODUCTION

Vision impairment represents a significant global health issue, and cataracts stand out as a leading contributor to blindness on a worldwide scale (Fang *et al*., 2022). Cataracts manifest as cloudiness or dysfunction in the eye's lens. The prevalence of cataracts dramatically increases with age, reaching 47% in those aged 55-64 years and a staggering 88% in individuals over 75 years old (Congdon *et al*., 2004; Klein *et al*., 2002). Surgical treatment is the standard approach for individuals with cataracts, constituting a substantial portion of healthcare expenses due to its prevalence among aging populations in developed nations. Conversely, developing countries face considerable morbidity related to cataracts, primarily due to limited access to surgical care. Therefore, there is an urgent need to develop effective and safe medications for the treatment of cataracts. Unfortunately, a potential drug specifically for human cataracts has yet to be applied

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Abbreviations
SPF, Specific Pathogen-Free; NS, Normal Saline; ARVO, Association for Research in Vision and Ophthalmology; MLECs, Mice Lens Epithelial Cells; ANOVA, Analysis of Variance; SSC, Side Scatter.

in clinical practice. Lanosterol, which is the initial sterol in the lipid biosynthetic pathway, undergoes a conversion process via enzymes, such as lanosterol synthase, squalene epoxidase, and 3-hydroxy-3-methylglutaryl-coenzyme A reductase, with acetyl-CoA. Regarding in vitro and in cells, studies in human genetics have shown that lanosterol can dissolve crystalline aggregates and restore lens transparency in rabbits as well as dogs with age-related cataracts (Zhao *et al*., 2015). Recently, findings indicate that the effectiveness of lanosterol in dissolving protein aggregates or restoring clarity to lens nuclei in cataractous lenses from age-related cataracts in humans is uncertain (Daszynski *et al*., 2019; Shanmugam *et al*., 2015). However, it is unclear whether lanosterol achieves lens clarity by enhancing its ability to physically dissolve protein aggregates or by denaturing the amyloid, such as fibril proteins, in cataractous lenses. Kang *et al*. (2018) reported that the disruption of human γD-crystallin aggregation by lanosterol occurs through its binding to the hydrophobic dimerization interface. This interaction may be linked to oxidative stress processes. Lanosterol provides the potential to protect against early stages of crystallin denaturation and lens epithelial cell apoptosis caused by UV-B exposure-induced oxidative damage (Hua *et al*., 2019). Huang *et al*. (2019) demonstrated that a combination of lanosterol and hesperetin provides the potential to delay cataracts induced by selenite. Intriguingly, the incorporation of lanosterol into ophthalmic formulations provides potential in the field of ophthalmology. However, designing ophthalmic formulations that contain lanosterol is challenging due to its limited solubility.

In this study, we administer subcutaneous injections of lanosterol in the orbital region surrounding the cataractous eyes of BALB/c mice to investigate the therapeutic effects of lanosterol on lens structure collapse and opacification.

MATERIALS AND METHODS

Animal and drug administration

Aged-specific pathogen-free (SPF) BALB/c mice (≥14 months old) were obtained from the School of Basic Medical Sciences, Shanxi Medical University. To determine the effective dosage of lanosterol, we conducted an experiment using aged BALB/c mice. Twelve aged BALB/c mice, consisting of equal numbers of males and females, were divided into two groups (6 males and 6 females in each group: treatment group (10, 20, and 40 μg lanosterol per injection) and one saline control group. Based on the doses, mice received subcutaneous injections of lanosterol at doses of 10 μg, 20 μg, or 40 μg once a week in the orbital region around the eye, respectively. The mice received normal saline (NS) injections once a week as a control. All mice received four injections in total. We monitored the cataract ratios in the animals during the study. Results showed that all three doses of lanosterol exhibited similar therapeutic effects at 10 μg, 20 μg, and 40 μg. However, the 40 μg dose of lanosterol did not show any side effects, and its therapeutic effect was not higher than that of 20 μg (P = 0.190 for alleviating cataract symptoms; P = 0.761 at 10 weeks; P = 0.883). We focused on studying the pharmacokinetics as well as the therapeutic effects of 20 μg lanosterol for subsequent experiments. All experimental procedures that involve live animals were carried out in compliance with the national institutes of health guide for the care and use of laboratory animals and the association for research in vision and ophthalmology (ARVO) statement for the use of animals in ophthalmic and vision research. The experiments were approved by the experimental ethics committee of Shanxi Medical University.

In vitro *lens culture studies*

The eyes of aged BALB/c mice were promptly enucleated upon carbon dioxide asphyxiation-induced death. The intact lens from each eye was then carefully dissected from a posterior approach. The lens epithelial cells were cultured in sterile TC-199 bicarbonate media supplemented with 30 mM fructose and 20 U/mL of penicillin-streptomycin. The cell culture was maintained in a humidified incubator at 37°C with an atmosphere of 95% air and 5% CO_2 (Zhang *et al*., 2012). Subsequently, the cells were transferred to 24-well culture plates, with each well containing 2 ml of standard culture media, and incubated for 48 h. Two groups were established, with 6 lenses assigned to each group. The first group served as the untreated control, while the lenses in the second group were subjected to experimental treatment with lanosterol. Following the initial 48-hour culture period, the culture media for each lens were replaced with fresh TC-199 bicarbonate media containing 15 mM lanosterol liposomes, and the lens epithelial cells were cultured for an additional 48 h. Further, at the end of the second 48-hour culture period, the lens epithelial cells were gently washed with a PBS solution and transferred to culture plates that contained the PBS solution.

Lens clarity evaluation

To facilitate imaging, an antibody incubation container was sterilized using high-pressure steam and placed within a vertical clean bench. For clarity assessment, lenses were excised and photographed under a stereomicroscope. Alcohol spray was used for sterilizing instruments and not applied to the lenses. Next, 24-well cell culture clusters were delicately removed from the incubator and carefully

positioned on the antibody incubation container to ensure no air bubbles formed, and only a layer of 75% alcohol separated the clusters from the container. A Nikon D90 camera set to the highest magnification was used to capture the image. This method enabled the observation of the transparency of the entire lens area, with a black image signifying a transparent lens and a gray image indicating an opaque lens.

Expression analysis of genes using RT-qPCR

The cultured mice lens epithelial cells (MLECs) were collected at different time points, and total RNA was extracted using TRIzol reagent (SBS Gene Technology Co., LTD, Beijing, China) following the manufacturer's instructions. The RNA was then precipitated with the ethyl alcohol and dissolved in the diethyl pyrocarbonate-treated water. Next, 1.0 µg of RNA was reverse transcribed in a 20-µl reaction volume using the RevertAid First Strand cDNA Synthesis Kit (ThermoScientific, USA). The reverse transcription process involved incubation at 25°C for 10 min, followed by 42°C for 60 min and 72°C for 10 min, with subsequent cooling on ice. The resulting cDNA was amplified using a 7500 Real-Time PCR system (Applied Biosystems, Foster City, CA, USA) with the following cycling conditions: an initial step of 95°C for 2 min, followed by 40 cycles of 95°C for 10 seconds and 53°C for 40 seconds (Zhao *et al*., 2019). Amplification was carried out using BlazeTaq™ SYBR® Green qPCR Mix 2.0 (GeneCopoeia, USA) with 2 µl of the reverse-transcribed product in the 20-µl reaction volume. Mouse GAPDH was used as an internal control. The primers used are shown in Table I.

Statistical analysis

Each experiment was replicated a minimum of three times, and the data were presented as mean ± standard deviation. Statistical assessments were conducted using GraphPad Prism 9.5.1 software (GraphPad Software Inc., La Jolla, CA, United States). The differences between the two groups were analyzed through an unpaired t-test, while comparisons involving more than two datasets were performed using one-way analysis of variance (ANOVA). A *P*-value less than 0.05 was deemed statistically significant.

Table I. Primers used for quantitative real-time PCR.

Gene	Primer sequences 5' -3'	Primer length
m-CRYAA	F: 5'-ACG AGA GGC AGG ATG ACC AT3` R: 5'-CCA AAC CGG ACT GGA CCT T3`	20 19
m-CRYAB	F: 5'-TGA CAC CGG ACT CTC AGA GAT G3` R: 5'-TGT TCG TCC TGG CGT TCT TC3`	22 20
m-CRYBA2	F: 5'-CAG TGG CCA CCA CAG CAA3` R: 5'-CCC ATG GAA GGC AGT GAT G3`	18 19
m-CRYBB1	F: 5'-CTG CCT TCC GTG GAG AGA TG3` R: 5'-CCC CTT CGA ACA GGC AGA T3`	20 19
m-CRYBB2	F: 5'-GCT CTC TGA GGC CCA TCA AA3` R: 5'-GCA CGG AAG ACA CCT TTT CC3`	20 20
m-CRYGD	F: 5'-GCA GTG GAT GGG TTT CAG TGA3` R: 5'-TGG AAT CGG TCC TGG AGA GA3`	21 20
m-CRYGC	F: 5'-CTA CCA GGG CCA CCA GTA CTT C3` R: 5'-TCC ATC ATG ACA CCT TTG TGA TCT3`	22 24
m-MIP	F: 5'-GAG ATC TTC TTG ACG CTC CAG TTC3` R: 5'-CAT CCC CGC ACC AGT GTA AT3`	24 20
m-PAX6	F: 5'-GCC CTC ACC AAC ACG TAC AGT3` R: 5'-ATC ATA ACT CCG CCC ATT CACT3`	21 22
m-PROX1	F: 5'-ACC TTA TTC AGG AAG CGC AAT G3` R: 5'-TGC GAG GTA ATG CAT CTG TTG3`	22 21
m-GAPDH	F: 5'-GCC ACC CAG AAG ACT GTG GAT-3` R: 5'-GGA AGG CCA TGC CAG TGA-3`	21 18

F, forward primer; R, reverse primer

RESULTS

Cellular uptake of lanosterol

Efficient internalization of lanosterol by MLECs should be considered to exert its antioxidant effects. Considering the uptake experiment, the flow cytometer detection chart revealed a concentration as well as a time-dependent increase in the side scatter (SSC) of MLECs, indicating successful internalization of lanosterol into the cells (Fig. 1A, B). To assess cytotoxicity following internalization, a CCK8 assay was performed. The results demonstrated minimal cell damage with an increase in the concentrations of lanosterol (2, 4, 8, 16, 32 μg/mL) and incubation times (24 and 48 h) (Fig. 1C, D). These findings indicate the biocompatibility of lanosterol and its suitability for subsequent studies.

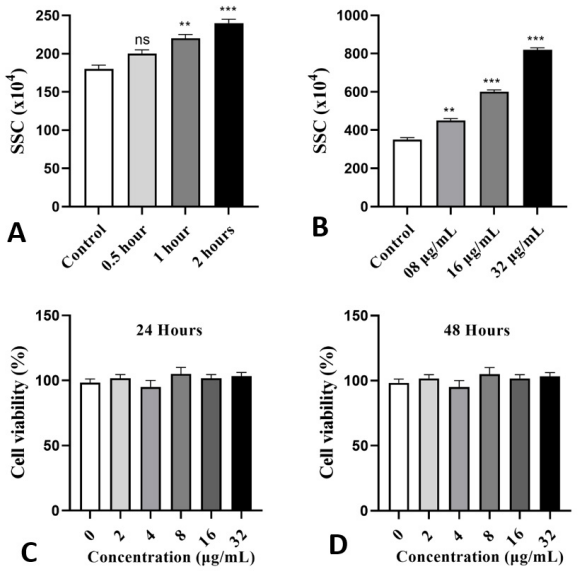

Fig. 1. Cellular uptake *in vitro*. (A) SSC values of HLECB3 cells treated with lanosterol with different incubation times via flow cytometry analysis. (B) SSC values of MLECs treated with different concentrations of lanosterol via flow cytometry analysis. (C) Cell viability of MLECs treated with different concentrations of lanosterol for 24 h. (D) Cell viability of MLECs treated with different concentrations of Lanosterol for 48 h. ** and *** indicate $P < 0.01$ and $P < 0.001$; ns indicated statistically not significant.

Lanosterol injection in the transparency of the lenses

To check the therapeutic effect of lanosterol, we divided the aged BALB/c mice into two groups. The first group was injected with 20 μg of lanosterol subcutaneously in the orbital region, while the second group was injected with NS once a week. After 10 weeks, we observed the ratio of cataracts through a slit lamp microscope. The result showed that the lanosterol injection group had no improvement in the symptoms of cataracts (Fig. 2) as compared to the control group injected with NS. The lenses were carefully extracted from the eyeballs through a posterior incision with the help of a research stereo microscope (Nikon SMZ800) and subsequently observed with the help of a dark-field microscope. The transparency of the lenses in the lanosterol treatment group showed no significant difference (Fig. 2B) from that in the NS group (Fig. 2A). These findings suggest that lanosterol provides no therapeutic effects on cataracts by comparing it with the young normal mouse lens (Fig. 2C).

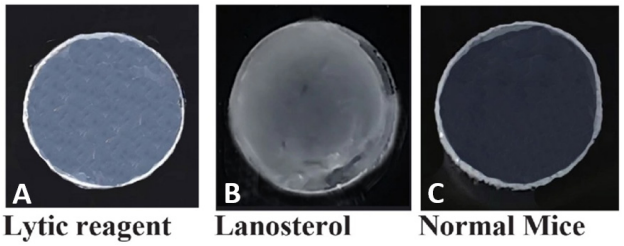

Fig. 2. Lanosterol significantly increased lens transparency in cataract BALB/c mice. (A) Representative slit-lamp microscopic images of cataracts treated with lanosterol. The lens of mice treated with lanosterol was not clear. The transparency of the lens in the lanosterol treatment group showed no significant difference from that of the lens treated with the NS control group. (B) The representative lens image of the control group mice was treated with normal saline. (C) Representative lens image of normal young mice. The lens is transparent.

Lanosterol injection alleviates the symptoms of cataract

To check the therapeutic effect of lanosterol, we divided the aged BALB/c mice into two groups. Both groups have 6 cataracts of BALB/c mice (3 males and 3 females), and the ratio of cataracts in both groups is the same. The first group was injected with 20 μg of lanosterol subcutaneously in the orbital region, while the second group was injected with NS once a week. After 10 weeks, we observed the ratio of cataracts in the lanosterol treatment group and the control group through a slit lamp microscope. The severity of cataracts before and after the treatment in both groups is presented in Table II.

Lanosterol increases the mRNA expression of crystalline genes

One of the factors contributing to cataract formation is the reduced expression of crystalline genes. The crystalline genes include *CRYAA* (αA-crystallin), *CRYAB* (αB-cystallin), *CRYBA2* (βA2-cystallin), *CRYBB1* (β-crystallin B1), *CRYBB2* (β-crystallin B2), *CRYGC* (GC-cystallin)

Table II. Effect of lanosterol on lens opacity in murine cataract.

Mouse number	Control group				Lanosterol group			
	Beginning		After 10-weeks		Beginning		After 10-weeks	
	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye	Left eye	Right eye
1	±	±	+₌	±	±	±	+₌	±
2	±	±	+₌	+₌	±	±	+₌	+₌
3	±	+₌	±	±	±	+₌	±	±
4	+˙	±	+₌	±	+˙	±	+₌	±
5	±	±	±	+₌	±	±	±	+₌
6	±	+˙˙	+₌	+	±	+˙˙	+₌	+
% of cataracts accounts for the total lens area	23.44 ±0.08		24.58 ±0.83		23.44 ±0.08		24.58 ±0.83	

+₌ indicates that the opacity of the lens makes up 15% of the total lens. ± indicates that the opacity of the lens makes up 20% of the total lens + indicates that the opacity of the lens makes up 25% of the total lens. +˙ indicates that the opacity of the lens makes up 30% of the total lens. +˙˙ indicates that the opacity of the lens makes up 35% of the total lens.

and CRYGD (GD-crystallin). We also aimed to find the mRNA expression of the *PAX6* and *PROX1* genes. After the successful transfection of lanosterol, we used RT-qPCR to detect the mRNA expression of these genes (Fig. 3).

DISCUSSION

The mammalian lens contains numerous densely packed fiber cells that develop from specialized lens epithelial cells throughout the lifespan of an individual. These fiber cells are divided into three groups of crystallin proteins: α-crystallin, which resembles small heat shock proteins, and β- as well as γ-crystallins, which play important roles in maintaining the lens transparency and high refractive index. The formation of lens opacities, which cause light scattering, is directly linked to the clumping together of β- as well as γ-crystallins. To prevent this clumping, Horwitz (2000) reported that small heat-shock proteins, like αA- as well as αB-crystallins, act as chaperones, safeguarding the lens against protein aggregation. According to the protein unfolding hypothesis for age-related cataracts, β- as well as γ-crystallins undergo gradual modifications to lower their energy levels for unfolding, which facilitates their binding to α-crystallin. It is believed that lanosterol and 25-hydroxycholesterol can enhance the ability of α-crystallins to bind with unfolded (aggregated) β- as well as γ-crystallins. The proposed outcome is the solubilization of these insoluble, light-scattering, aggregated proteins within the lens, leading to an increase in soluble proteins and a decrease in insoluble proteins, dissolving the cataract.

We investigated the potential use of lanosterol as a subcutaneous injection in the orbital region. Lanosterol showed high biocompatibility and low toxicity. In the present study, we observed no detrimental effects on lens epithelial cells exposed to lanosterol and no signs of irritation, opacity, or muddiness after the subcutaneous injection of lanosterol in the orbital region of mice. Results show that lanosterols are safe and support their potential application in subcutaneous injections that target the orbital region.

The first group was injected with 20 µg of lanosterol subcutaneously in the orbital region, while the second group was injected with NS once a week. After 10 weeks, we observed the ratio of cataracts through a slit lamp microscope. The result showed that the lanosterol injection group showed no improvement in the symptoms of cataracts (Fig. 2) compared to the control group injected with NS. The lenses were removed from a posterior incision in the eyeballs under a research stereo microscope (Nikon SMZ800) and observed under a dark-field microscope. Transparency of the lenses in the lanosterol treatment group showed no significant difference (Fig. 3B) from that in the NS group (Fig. 3A). After in vitro transfection of lanosterol to MLECs, the RT-qPCR result indicates that the mRNA expression level of crystalline genes in the lanosterol group exhibited no significant difference compared to the CPT reagent group. These findings suggest that lanosterol provides no therapeutic effects on cataracts.

Risk factors contribute to age-related cataracts, such as oxidative stress, which is widely recognized as a significant factor. The development of cataracts is affected by the modifications, denaturation, and aggregation of lens

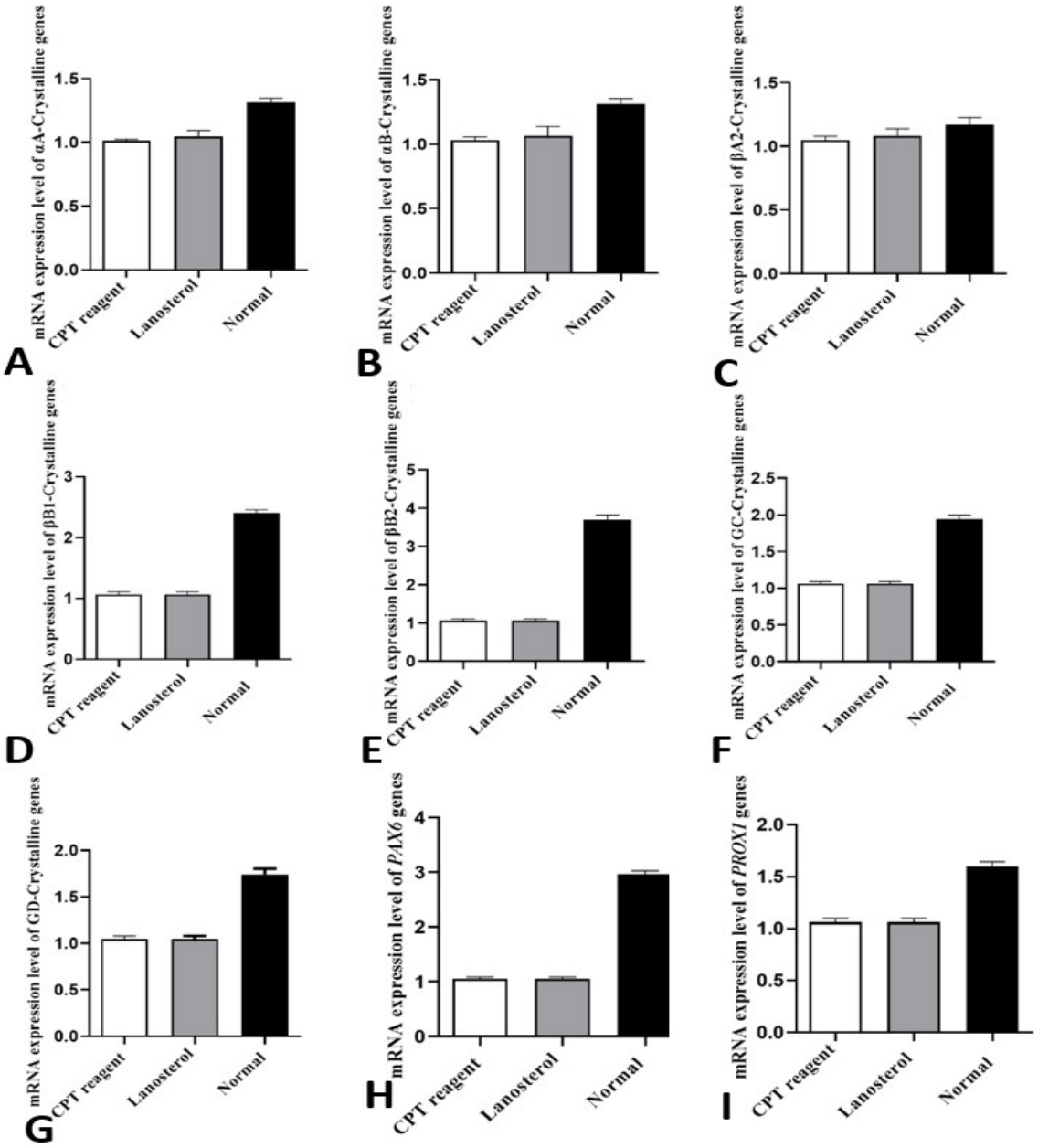

Fig. 3. Lanosterol increases the mRNA expression of crystalline genes. (A) The mRNA expression level of the *CRYAA* (αA-crystallin) gene. In the lanosterol group, the expression is not higher compared to the CPT reagent group. (B) The mRNA expression level of the *CRYAB* (αB-cystallin) gene. In the lanosterol group, the expression is not higher compared to the CPT reagent group. (C) The mRNA expression level of *CRYBA2* (βA2-cystallin) indicates that the lanosterol group expression is not higher compared to the CPT reagent group. (D) The mRNA expression level of the *CRYBB1* (β-crystallin B1) gene. Lanosterol group expression is not higher compared to the CPT reagent group. (E) The mRNA expression level of the *CRYBB2* (β-crystallin B2) gene. In the lanosterol group, the expression is not higher compared to the CPT reagent group. (F) The mRNA expression level of the *CRYGD* (GC-cystallin) gene. In the lanosterol group, the expression is not higher compared to the CPT reagent group. (G) The mRNA expression level of the *CRYGD* (GD-crystallin) gene. In the lanosterol group, the expression is not higher compared to the CPT reagent group. (H) The mRNA expression level of the *PAX6* gene. In the lanosterol group, the expression is not higher compared to the CPT reagent group. (I) The mRNA expression level of the *PROX1* gene. In the lanosterol group, the expression is not higher compared to the CPT reagent group.

proteins, including enzymes and crystallins, as a result of oxidative stress (Berthoud and Beyer, 2009). Oxidative stress not only affects enzymatic activities but also influences cell proliferation. The exact mechanism through which lanosterol synthase protects lens transparency remains unclear despite extensive research on its role in the nervous system. It is suggested that inhibiting lanosterol synthase could lead to secondary apoptosis in neurons due to an increase in reactive oxygen species (ROS) (Cenedella, 2009). After the inhibition of lanosterol, the activity of catalase (CAT) and superoxide dismutase (SOD) decreases in astrocytes, leading to impaired lipid metabolism in the cerebral cortex (Copetti-Santos *et al*., 2015).

Additional research is required to establish an approach for preparing a subcutaneous injection formulation with elevated concentrations of lanosterol. It is important to develop an eye drop formulation capable of effectively delivering lanosterol to the lens. Previous findings indicated that solid drug NPs in ophthalmic formulations can be absorbed into the corneal epithelium through energy-dependent endocytosis, demonstrating significant transcorneal penetration rates (Ishii *et al*., 2019). The present finding focuses on the therapeutic effects of lanosterol administered subcutaneously in the orbital region in BALB/c mice.

Limitations

This study used a cataract model in aged BALB/c mice, which may not fully represent the complex nature of cataracts in human patients. The findings may not directly translate to human cataract treatment. The therapeutic effect of lanosterol was investigated over a limited treatment duration. A longer-term study may be necessary to assess the potential of lanosterol to produce delayed or cumulative effects on cataract progression. The specific concentration and subcutaneous administration of lanosterol used may not have been optimal for achieving the desired therapeutic effects. Different doses or routes of administration could yield different outcomes. The present study aimed to determine whether lanosterol binds to crystallin protein and contributes to the dissolution of opacities. However, no mechanistic insights were provided (Daszynski *et al*., 2019). The use of cultured lens epithelial cells in vitro provides a simplified model of cataract development and may not fully capture the complexity of the in vivo lens environment. The study focused specifically on lanosterol, but there are other oxysterols that could potentially have different effects on cataract development. The findings may not be applicable to other oxysterols or their combinations. Limitations include small sample size, restricted dosing range, and use of a single delivery route. Future work should explore higher dosing, alternative formulations, and extended treatment durations.

Future perspectives

The present findings suggest that lanosterol alone may not be effective in reversing cataract progression. Future research could explore the potential of the combination of lanosterol with other compounds or therapeutic approaches to enhance its anti-cataractogenic activity and promote cataract dissolution (Deguchi *et al*., 2022). However, other oxysterols or related compounds may provide promise for cataract treatment. Investigating different oxysterols and their mechanisms of action could reveal novel therapeutic options. Further research is needed to elucidate the underlying mechanisms by which lanosterol interacts with crystallin protein and influences cataract dissolution. Understanding these mechanisms in detail could uncover new targets for intervention and guide the development of more effective treatments. Using animal models that more accurately mimic human cataract development and progression could provide valuable insights. Developing animal models that better recapitulate the complexity of human cataracts would enhance the translational relevance of preclinical studies. Despite the negative findings, the potential of lanosterol or other oxysterols for cataract treatment should not be dismissed entirely. Well-designed clinical trials are necessary to determine the safety and efficacy of these compounds in human patients, considering factors like dosage, administration route, and treatment duration. The present study investigated the potential of lanosterol, but there may be other innovative therapeutic approaches for cataract treatment that warrant exploration. Advancements in drug delivery systems, gene therapy, or regenerative medicine techniques could offer new avenues for restoring vision in individuals with cataracts. Investigating interventions at earlier stages of cataract development could be crucial. Identifying biomarkers or early indicators of cataract formation may enable targeted interventions to prevent or delay cataract progression, potentially reducing the need for surgical intervention. Understanding the genetic and environmental factors that contribute to cataract development could pave the way for personalized treatment approaches. Tailoring interventions based on individual characteristics may improve treatment outcomes and optimize vision restoration for patients with cataracts (Liu *et al*., 2025; Mehmood *et al*., 2021, 2023; Wang *et al*., 2024; Yin *et al*., 2021).

CONCLUSION

Lanosterol, administered at 10–40 µg doses in BALB/c

mice, did not reverse cataract formation. Lanosterol failed to reverse the nuclear opacity of cataractous nuclei.

DECLARATIONS

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Ethical approval

The experiments were performed at the School of Basic Medical Sciences, Shanxi Medical University, and were approved by the Experimental Ethics Committee of Shanxi Medical University, Taiyuan 030001, China. All applicable international, national, and/or institutional guidelines for the care and use of animals were followed. The methods are reported in accordance with ARRIVE guidelines (https://arriveguidelines.org) for the reporting of animal experiments.

Data availability

All processed data used in this study can be obtained from the corresponding author upon reasonable request.

Informed consent statement

This article does not contain any studies with human participants performed by any of the authors.

Generative AI and AI-assisted technology statement

The authors declare that no generative AI and AI assisted technology was used in this manuscript.

Statement of conflict of interest

The authors have declared no conflict of interest.

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