Oridonin protects hydrogen peroxide-induced human lens epithelial cell damage by regulating the NLRP3/NF-κB pathway and Nrf2-mediated oxidative stress

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RESEARCH ARTICLE

Abstract

Cataract is the clouding of eye lens, and is the leading cause of visual impairment and blindness worldwide. There is an urgent need to develop new drugs to combat this disease. Oridonin (ORI), isolated from *Rabdosia rubescens*, is a natural substance that has been studied as an activator of nuclear factor erythroid 2-related factor 2 (Nrf2) and covalent inhibitor of NLR family pyrin domain containing 3 (NLRP3). Whether ORI has a therapeutic effect on human lens' epithelial cell injury needs further study. In this study, we used cataract lens epithelial cell lines HLE-B3 and SRA01/04. We found using the MTT assay that ORI treatment increased cell viability induced by hydrogen peroxide (H_2O_2). In addition, ORI suppressed the apoptosis of H_2O_2-induced HLE-B3 and SRA01/04 cells detected by flow cytometry and Western blot analysis. Our data further revealed that ORI regulated nuclear factor, erythroid 2 (NFE2) like bZIP transcription factor 2 (Nrf2)-mediated oxidative stress by enzyme-linked-immunosorbent serologic assay. We further found that ORI inhibited NLRP3/nuclear factor-κb (NF-κB) pathway in H_2O_2-induced HLE-B3 and SRA01/04 cells by Western blot analysis. Therefore, these results suggested that ORI could have the potential to serve as a promising drug to treat cataract.

Keywords: cataract; oridonin (ORI); oxidative stress; Nrf2; NLRP3; NF-κB

Introduction

Cataract, clouding of the eye lens, is a leading cause of visual impairment and blindness worldwide (Wang and Ren, 2022). It is estimated that more than 68% of people over the age of 79 have some form of lens opacity or cataract, and the incidence increases with age (Chang *et al.*, 2022). At a young age, human lens is usually protected from oxidative damage by a powerful oxygen-free radical scavenger system that uses glutathione (GSH) as its primary antioxidant to detoxify reactive oxygen species (ROS) (Hassan *et al.*, 2021). Perturbation in eye lens redox state is considered as the main cause of age-related cataracts (Sritrakoon *et al.*, 2021). Owing to its unclear pathogenesis, cataract surgery is currently the only effective treatment, but the risk of surgical complications is often associated with preoperative vision, age, and ocular complications. Thus, there is an urgent

requirement to develop new drugs to combat this disease.

Oridonin (ORI), isolated from *Rabdosia rubescens*, is a natural substance that has been studied as an activator of nuclear factor erythroid 2-related factor 2 (Nrf2) and covalent inhibitor of NLR family pyrin domain containing 3 (NLRP3; Kazantseva *et al.*, 2022). In addition, it has been shown to relieve acute lung injury and vascular inflammation by blocking the nuclear factor-κb (NF-κB) pathway (Zhao *et al.*, 2022). ORI ameliorated neurological damage by improving mitochondrial function and antioxidant capacity as well as restraining neuroinflammation (Yasuda *et al.*, 2022). ORI suppressed senescence-associated secretory phenotype (SASP) via inhibiting p38 and NF-κB pathways in senescent cells (Wang *et al.*, 2021). Whether ORI has therapeutic effect on human lens epithelial cell (HLEC) injury needs further study.

Nrf2, an important nuclear transcription factor, shows strong antioxidant activity and has been widely used as a promoter to suppress oxidative stress and the resulting inflammation (Li *et al.*, 2021a). With the occurrence of oxidative stress, Nrf2 enters the nucleus to initiate the transcription of antioxidant enzymes (GSH and superoxide dismutase [SOD]) and antioxidant genes (heme oxygenase [HO-1], NAD(P)H quinone oxidoreductase 1 [NQ-O1], GSH-synthesizing enzyme, and glutamate cysteine ligase modifier [GCLM]), and ultimately alleviates oxidative damage (Liu *et al.*, 2022). NF-κB acts as a sensor to initiate inflammatory response in response to certain stimuli, such as reactive oxygen species (ROS), free fatty acid (FFA), and proinflammatory factors (Hua *et al.*, 2021). Normally, NF-κB interacts with IkappaB (IκB) kinase in a silent state and has no effect on downstream genes (Li *et al.*, 2021a). However, when stimulation occurs, IκB is phosphorylated and NF-κB is released and activated to control expression of genes and inflammatory mediators.

In the present study, we investigated the effects of ORI on the progression of cataract *in vitro*. We revealed that ORI increased hydrogen peroxide (H_2O_2)-induced cell viability, reduced apoptosis, alleviated oxidative stress, and inhibited the NLRP3/NF-κB pathway. Therefore, ORI could have the potential to serve as a promising drug for the treatment of cataract.

Materials and Methods

Reagents

Oridonin with a purity >98% was purchased from the National Institute for Food and Drug Control (Beijing, China). H_2O_2 was obtained from Sigma Aldrich (St. Louis, MO, US). ORI was dissolved in dimethyl sulfoxide (DMSO).

Cell culture

Human lens epithelial cells (ATCC, Manassas, VA, US) and Human LECs (SRA01/04; Bluefbio, Shanghai, China) were cultured with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin at 37°C in a humidified hood with 5% CO_2. Cells were treated with H_2O_2 for 24 h, and then ORI was added for another 24 h.

Cell viability

Cells were plated into 96-well plates at a density of 1×10^3 cells/well. Cell viability was detected by 3-(4,5-dim ethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay. After treatment with ORI or H_2O_2, 10% MTT was added to cells following rinsing with phosphate-buffered saline solution (PBS). Cells were incubated for 4 h before the measurement of absorbance value in each well with a microplate reader at 490 nm. Absorbance in the experimental group was normalized with that of the control group.

Cell apoptosis

For the detection of apoptotic cell percentage, Annexin V/Propidium Iodide (PI) apoptosis detection was done following the manufacturer's protocol ((Sigma Aldrich). Briefly, cells were digested and mixed in reaction buffer containing Annexin V and PI for 5 min at room temperature in the dark. Cell proportion of intact cells (Annexin V^-/PI^-), early apoptotic cells (Annexin V^+/PI^-), and late apoptotic cells (Annexin V^+/PI^+) were analyzed by a flow cytometer (BD Biosciences).

Immunoblot assay

Proteins were extracted with radioimmunoprecipitation assay (RIPA) buffer (Beyotime, Beijing, China), and protein concentration was analyzed by bicinchoninic acid (BCA) assay kit. The proteins were separated by 9% sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS-PAGE), and transferred on polyvinylidene difluoride (PVDF) membranes, followed by blocking with 5% *bovine serum albumin* (BSA) in a mixture of tris-buffered saline and polysorbate 20 (TBST) buffer. Subsequently, membranes were conjugated with primary antibodies targeting Bcl-2 (1:1,000, ab32124; Abcam, Cambridge, UK), Bax (1:1,000, ab32503; Abcam), Nrf2 (1:1,000, ab62352; Abcam), HO-1 (1:1,000, ab305290, Abcam), NQO-1

(1:1,000, ab80588; Abcam), p-p65 (1:1,000, ab76302; Abcam), p65 (1:1,000, ab32536; Abcam), p-IκBα (1:1,000, ab133462; Abcam), IκBα (1:1,000, ab32518; Abcam), and b-actin (1:10,000, ab178787; Abcam) for 2 h at room temperature. Subsequently, the membranes were incubated with specific secondary antibodies at room temperature for 1 h. The blots were analyzed with enhanced chemiluminescent (ECL) Western blotting substrate kit.

Antioxidant activity detection

The levels of SOD, GSH, *myeloperoxidase* (MPO), and malondialdehyde (MDA) were measured with the detection kits obtained from from Nanjing Jiancheng Bio-Engineering Institute (Jiangsu, China) following the manufacturer's guidelines.

Statistics

GraphPad 6.0 was used for statistical analysis. All data were represented as mean ± standard error of mean (SEM). Three replicates were performed for each experiment. One-way ANOVA and post-hoc Turkey's test were used for statistical comparisons. For measurements, $P < 0.05$ was considered statistically significant.

Results

Oridonin increased cell viability in human lens epithelial cells induced by H_2O_2

To evaluate the cell viability exposed to ORI in HLE-B3 and SRA01/04 cells, MTT assay was performed. HLE-B3 and SRA01/04 cells were incubated with increasing dose of H_2O_2. The result showed that H_2O_2 treatment decreased cell viability of HLE-B3 and SRA01/04 cells (Figure 1A). The molecular formula of ORI is shown in Figure 1B. Cell viability was minimally affected by the low dose of ORI (0.5–25 μM) (Figure 1C). Then, cells exposed to H_2O_2 and ORI were subjected to MTT assay. H_2O_2 treatment led to reduced cell viability of HLE-B3 and SRA01/04 cells. ORI improved cell viability in a dose-dependent manner (Figure 1D). Collectively, ORI improves cell viability by H_2O_2 treatment.

Oridonin relieved cell apoptosis in HLE-B3 and SRA01/04 cells induced by H_2O_2

The effect of ORI on cell apoptosis was evaluated by flow cytometry (FCM) and immunoblot assay. H_2O_2 significantly induced the expression of Bax and reduced the expression of Bcl-2. Addition of ORI ameliorated the alterations of these proteins in HLE-B3 and SRA01/04 cells (Figure 2A). H_2O_2 treatment enhanced cell apoptosis, while ORI treatment inhibited cell apoptosis (Figure 2B). These data further demonstrated that ORI suppressed the H_2O_2-induced cell apoptosis.

Oridonin improved oxidative stress mediated by Nrf2 in H_2O_2-induced cells

H_2O_2 stimulation is believed to induce oxidative stress in cells, hence oxidative stress was determined in HLE-B3 and SRA01/04 cells. The results demonstrated that the levels of MDA and MPO were enhanced and that of SOD and GSH were reduced in

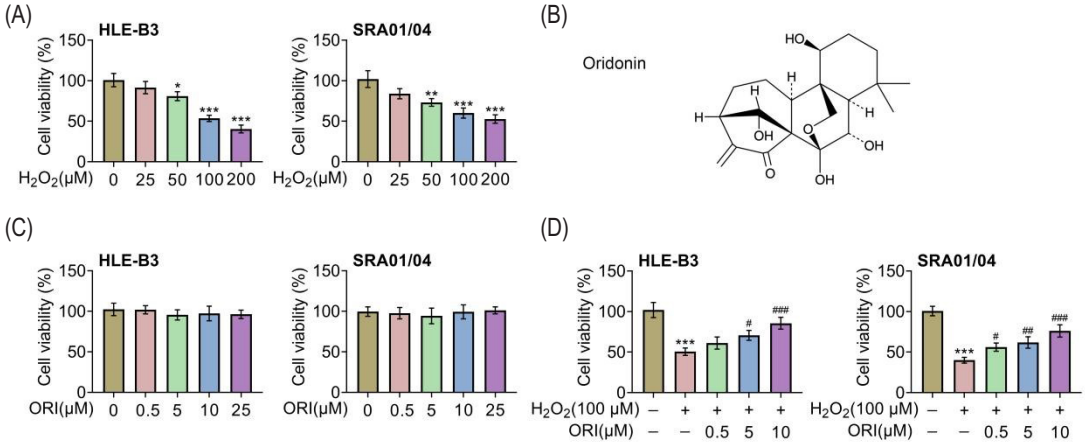

Figure 1. Oridonin increases cell viability in HLECs induced by H_2O_2. (A) The cell viability of HLE-B3 and SRA01/04 cells in response to increasing dose of H_2O_2. (B) The chemical structure of ORI. (C) The cell viability of HLE-B3 and SRA01/04 cells in response to the increasing dose of ORI. (D) The cell viability of HLE-B3 and SRA01/04 cells in response to H_2O_2 and elevated level of ORI. ***$P < 0.001$ vs. control, #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. H_2O_2.

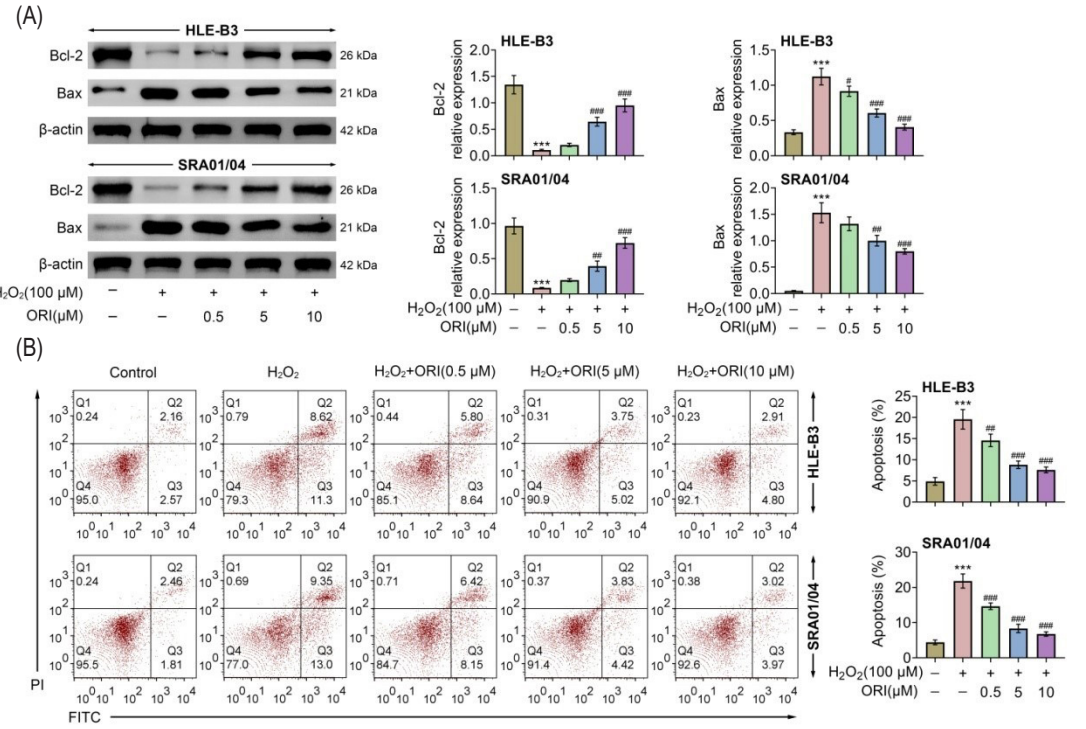

Figure 2. Oridonin relieves cell apoptosis in HLE-B3 and SRA01/04 cells induced by H_2O_2. (A) Expressions of Bcl-2 and Bax in response to H_2O_2 and elevated level of ORI. (B) HLE-B3 and SRA01/04 cells apoptosis in response to H_2O_2 and elevated level of ORI were detected by flow cytometry. ***$P < 0.001$ vs. control, #$P < 0.05$, ##$P < 0.01$, ###$P < 0.001$ vs. H_2O_2.

H_2O_2-stimulated cells (Figure 3A). ORI treatment significantly attenuated these changes, indicating its effects on anti-oxidative stress (Figure 3A). Moreover, the involvement of Nrf2 in oxidative stress was evaluated by immunoblot assay. H_2O_2 inhibited the levels of Nrf2, HO-1, and NQO-1. ORI treatment reversed the alterations of these proteins (Figure 3B). These data suggested that ORI improved Nrf2-mediated oxidative stress in H_2O_2-induced cells.

Oridonin inhibited NLRP3/NF-κB pathway in H_2O_2-induced HLE-B3 cells

To reveal the involved mechanisms underlying the role of ORI in apoptosis and oxidative stress, the NF-κB signaling pathway was detected. We observed the elevated levels of p-p65 and p-IκBα in H_2O_2-induced HLE-B3 and SRA01/04 cells (Figure 4). ORI treatment inhibited the elevation of these proteins. Our results indicated that ORI inhibited NLRP3/NF-κB pathway in H_2O_2-induced HLE-B3 cells.

Discussion

Cataract is a leading cause of blindness in the world, accounting for approximately 46% of global blindness (Gajraj and Mohan, 2022). Aging, genetic factors, local nutritional disorders, immune and metabolic abnormalities, trauma, poisoning, radiation, etc. can cause lens metabolism disorders, leading to the occurrence of cataract (Wei *et al.*, 2022). However, the precise pathogenesis of cataract still needs to be worked out. The symptoms of early cataract are not obvious, and the main treatment methods include surgical resection and drug therapy (Bhalerao *et al.*, 2021; Zhang *et al.*, 2017). Although the majority of cases can achieve good results, the effect of drug therapy is limited because of unclear pathogenesis (Twum *et al.*, 2021; Yin *et al.*, 2017). In this study, we found that ORI increased cell viability of H_2O_2-indued cells, reduced cell apoptosis, and alleviated oxidative stress. Thus, these results suggested that ORI could be used as a potential therapeutic agent for cataract.

We successfully established cataract lens epithelial cell model in HLE-B3 cells by using H_2O_2 (Hong *et al.*, 2021). Through a series of *in vitro* experiments, such as cell counting kit-8 (CCK-8) assay, flow cytometry, Western blot analysis, and enzyme-linked-immunosorbent serologic assay (ELISA), we revealed that ORI promoted the viability of H_2O_2-induced HLE-B3 cells, and suppressed apoptosis and oxidative stress. In fact, the biological activities of ORI have been widely revealed in different types of diseases (Duan *et al.*, 2021). ORI attenuated hind

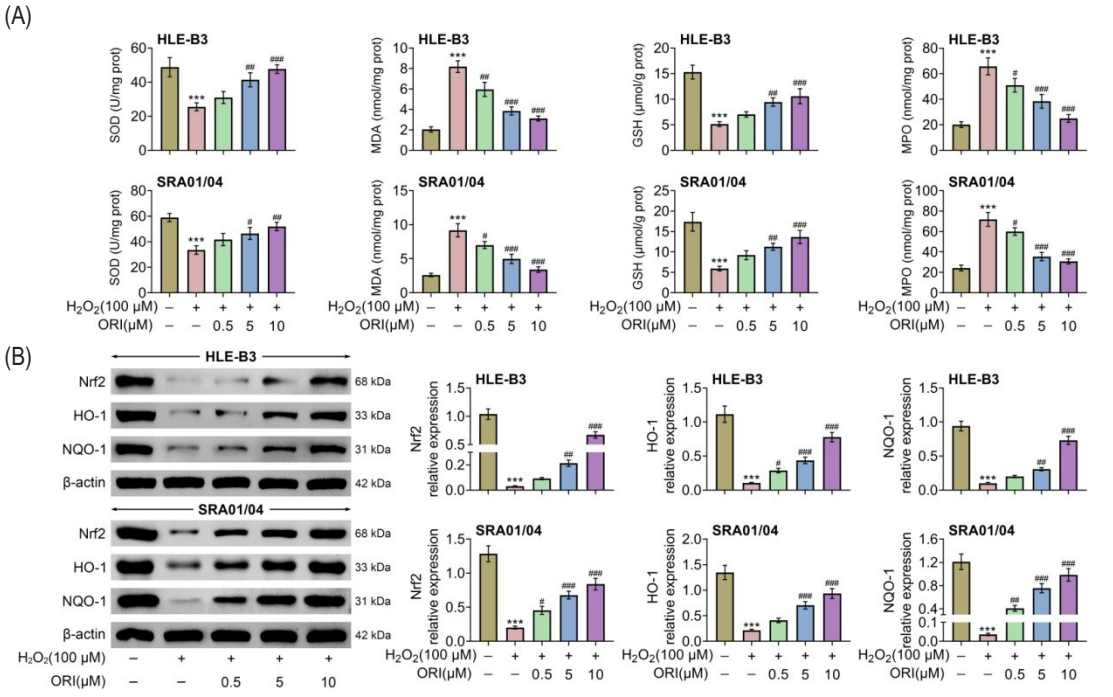

Figure 3. Oridonin improves oxidative stress mediated by Nrf2 in H_2O_2-induced cells. (A) The levels of SOD, MDA, GSH, and MPO were determined in each group. (B) The expression of Nrf2, HO-1, and NQO-1 in response to H_2O_2 and elevated level of ORI. $^{***}P < 0.001$ vs. control, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. H_2O_2.

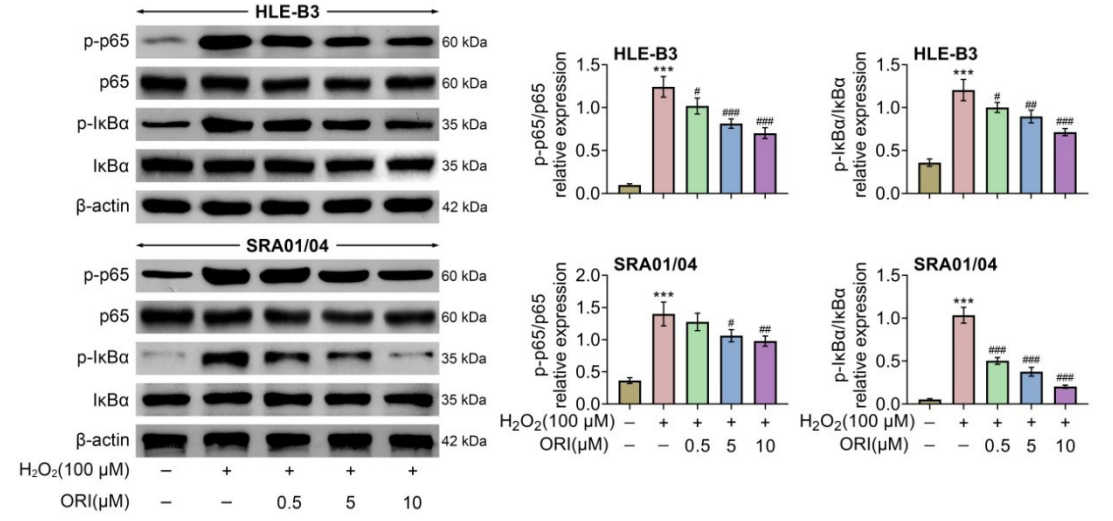

Figure 4. Oridonin inhibits NLRP3/NF-κB pathway in H_2O_2-induced HLE-B3 cells. Immunoblot assays depicted the expression of p-NF-κB and p-Iκba in H_2O_2- and ORI-induced HLE-B3 and SRA01/04 cells. $^{***}P < 0.001$ vs. control, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$ vs. H_2O_2.

limb ischemia-reperfusion injury via regulating Nrf2-mediated oxidative stress and NLRP3-mediated inflammation (Camilloni *et al.*, 2021; Du *et al.*, 2021). Similarly, here we revealed the effects of ORI on the oxidative stress of H_2O_2-induced HLE-B3 cells. We further revealed its effects on the NLRP3 pathway of HLE-B3 cells. Results of the previous studies established that ORI ameliorated traumatic brain injury-induced neurological damage by improving mitochondrial functions and antioxidant capacity through the Nrf2 pathway (Zhao *et al.*, 2022a, 2022b). The results also revealed that ORI suppressed neuroinflammation via this pathway. However, its effects on the inflammation of HLE-B3 cells required further examination.

In addition, the preventive and therapeutic effects of ORI were revealed on the nude mice hemi-spleen model of colon cancer liver metastasis (Zhou *et al.*, 2022). ORI induced oxidative stress-mediated cancer cells apoptosis by targeting thioredoxin reductase (Li *et al.*, 2021b; Zhu *et al.*, 2021). We revealed the effects of ORI on the apoptosis and oxidative stress of cells in a cataract *in vitro* cell model. Moreover, ORI also stimulated the anti-metastasis effects of oxaliplatin–cisplatin on colorectal cancer liver metastasis. These studies, together with our findings, confirmed that ORI could serve as a promising drug in different types of diseases.

In this study, we further revealed that ORI regulated Nrf2 and NF-κB pathways in H_2O_2-induced HLE-B3 cells. Nrf2 is known as an important nuclear transcription factor showing strong antioxidant activity which inhibits oxidative stress and inflammation. With the occurrence of oxidative stress, Nrf2 enters the nucleus to initiate the transcription of antioxidant enzymes to alleviate oxidative damage. NF-κB acts as a sensor to initiate inflammatory responses. Our results suggested that ORI mediated both Nrf2 and NF-κB pathways to protect against H_2O_2-induced human lens epithelial cell damage. Therefore, the Nrf2 and NF-κB pathways could serve as promising targets for cataract treatment. There are some limitations to the present study. First, the products of ORI degradation are not mentioned in this study. Second, presently no conditions and techniques are available for chromatographic analysis, hence the future research must be carried for such analysis.

Conclusion

In this study, we established a cataract cell model induced by H_2O_2. Our data revealed that ORI enhanced H_2O_2-induced HLE-B3 cell viability, and suppressed cell apoptosis and Nrf2-mediated oxidative stress. We further revealed that ORI could mediate both Nrf2 and NF-κB pathways, thereby protecting H_2O_2-induced human lens epithelial cell damage. Therefore, these findings suggested that ORI could serve as a drug for the treatment of cataract.

Data and Material Availability

All data generated or analyzed during this study are included in the published article.

Conflict of Interests

The authors stated that there was no conflict of interest to disclose.

Author Contributions

Jie Chen designed the study and supervised data collection. Wei Zeng analyzed and interpreted the data. Xili Xiao and Chengmin Lin prepared the manuscript for publication and reviewed its draft. All authors read and approved the final manuscript.

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