https://doi.org/10.31925/farmacia.2024.6.22 **ORIGINAL ARTICLE**

INHIBITORY EFFECTS OF DIFFERENT ALKALOIDS IN DENDROBIUM NOBILE EXTRACTS ON OXIDATIVE DAMAGE IN CATARACT LENS EPITHELIAL CELLS

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Abstract

Cataracts, a leading cause of acquired blindness, are associated with apoptosis of lens epithelial cells (LEC). This study investigates the mechanisms by which alkaloids extracted from *Dendrobium nobile*, a traditional Chinese medicinal plant, inhibit oxidative stress in LECs. Alkaloids were fractionated into four groups: fat-soluble alkaloids (FSA), weakly polar alkaloids (WPA), low-polarity alkaloids (LPA) and water-soluble alkaloids (WSA). Optimal extraction conditions were determined. Samples were collected from 10 cataract patients and 10 individuals with normal lenses, and LECs were isolated and cultured. An oxidative damage model was induced using 500 μmol/L H_2O_2, and alkaloid fractions were applied at 20 μg/mL. Apoptosis and proliferation were assessed *via* flow cytometry, while oxidative stress markers (GSH-Px, SOD, MDA) and protein expression (Bcl-2, Bax, p38 MAPK, p-JNK) were analyzed by ELISA and Western blot. Compared to normal LECs, cataract and oxidative stress models exhibited higher apoptosis, decreased GSH-Px/SOD and elevated MDA. Treatment with alkaloid fractions, particularly WSA, significantly reduced apoptosis, increased proliferation, restored oxidative balance and suppressed p38 MAPK/JNK pathway activation. These findings suggest *Dendrobium nobile* alkaloids effectively mitigate oxidative damage and apoptosis in cataract-affected LECs, with WSA demonstrating the most potent anti-oxidative effects.

Rezumat

Cataracta, o cauză majoră de orbire, este asociată cu apoptoza celulelor epiteliale ale cristalinului (LEC). Studiul de față investighează mecanismul prin care alcaloizii extrași din *Dendrobium nobile*, o plantă utilizată în medicina tradițională chineză, inhibă stresul oxidativ asupra LEC. Alcaloizii au fost separați în patru fracțiuni: liposolubili, slab polari, cu polaritate redusă și solubili în apă. Au fost stabilite condițiile optime de extracție. Au fost incluse probe de la 10 pacienți cu cataractă și 10 pacienți sănătoși, iar celulele epiteliale ale cristalinului au fost cultivate. Modelul de stres oxidativ a fost indus cu H_2O_2 (500 μmol/L), iar fracțiunile de alcaloizi au fost aplicate în doză de 20 μg/mL. Apoptoza și proliferarea au fost evaluate prin citometrie în flux, iar markerii de stres oxidativ (GSH-Px, SOD, MDA), expresia proteinelor Bcl-2, Bax și a căii p38 MAPK/JNK au fost analizate prin ELISA și Western blot. Comparativ cu grupul normal, grupul cataractă și modelul de stres oxidativ au arătat o creștere a apoptozei, reducerea GSH-Px/SOD și creșterea MDA. Alcaloizii, în special cei solubili în apă, au redus semnificativ apoptoza, au crescut proliferarea și au inhibat activarea căii p38 MAPK/JNK, demonstrând un efect anti-stres oxidativ promițător.

Keywords: *Dendrobium nobile*, alkaloids, cataract, lens, OD, p38 MAPK/JNK pathway

Introduction

Cataract is a common blinding disease caused mainly by lens dysfunction, with patients clinically experiencing reduced visual acuity. Cataract is the major eye disease that causes blindness, and trauma, surgery, diabetes, age and other factors predispose to cataract [1-3]. Lens epithelial cells (LEC) are the most active part of lens metabolism, which has functions such as lens growth, differentiation and repair [4]. In the process of cataract, the function and amount of LEC decrease, which is to some extent the result of apoptosis. Oxidative damage (OD) is involved in the process of apoptosis. Oxygen free radicals can stimulate apoptosis receptors and cause apoptosis through the activation of semi-conductors activating enzymes and damage to the mitochondrial respiratory chain [5]. Therefore, ROS is also one of the important mechanisms for the onset and development of cataract.

Currently, the use of Chinese herbal medicine in the treatment of cataract is mainly focused on improving the antioxidant capacity of the lens. Antioxidants or

antioxidant enzyme activators are used to eliminate or neutralize oxidative products in the lens, which can suppress or reverse lens opacity and ultimately slow or suppress the process of cataract [6, 7]. Dendrobium is an epiphytic plant of the *Dendrobium* family *Orchidaceae*. *Dendrobium nobile* has the functions of strengthening yin, nourishing essence, strengthening muscles and bones, tonifying kidney and prolonging life. *Dendrobium* decoction can alter the activity of lens-related enzymes, thereby inhibiting D-lactose cataract [8]. Ethyl acetate extract of *Dendrobium* can suppress the production of aldose reductase and lipid peroxide [9]. Guo *et al.* [10] pointed out that polysaccharides from dendrobium have antioxidant effects such as reducing free radicals, enhancing the antioxidant system, inhibiting nuclear factor-κb and downregulating inflammatory response. The chemical constituents isolated from *Dendrobium nobile* could be classified into alkaloids, phenanthrenes, benzenes, sesquiterpenoids and others, with a total of 185 compounds. Alkaloids are a class of important and potent chemical constituents first isolated from *Dendrobium*. They include anti-tumour, glycaemic, anti-inflammatory and anti-cataract pharmacological effects. Hu *et al.* [11] proposed that alkaloids in *Dendrobium* could inhibit lipopolysaccharide (LPS)-induced acute lung injury. Li *et al.* [12] proposed that *Dendrobium* alkaloids could suppress the release of pro-inflammatory cytokines and ameliorate 6-OHDA-induced neuronal degeneration, thus playing a neuro-protective role. *Dendrobium nobile* can regulate the levels of non-protein hydrophobic groups and reduced coenzyme II in cataractous lenses, restoring them to the level of normal lenses and acting in the treatment of cataract. Wu *et al.* [13] found that *Dendrobium* extracts can suppress aldose reductase activity and AR gene expression, and thus act to improve diabetic cataract. In this article, the mechanism of *Dendrobium nobile* alkaloid extracts on H_2O_2-induced proliferation, apoptosis and OD of LEC in cataract was further investigated to provide help for the anti-cataract results of *Dendrobium nobile* alkaloid extracts.

Materials and Methods

Reagents and instruments

Reagent materials: Fetal bovine serum (FBS) (Gibco, USA); Dulbecco's Modified Eagle Medium culture medium (Gibco, USA); Phosphate buffer (Thermo Fisher Scientific, China); Trypsin (Gibco Co, USA); *Dendrobium nobile* (Guangxi Shenli Pharmaceutical Co., LTD., China); Chloroform (Anhui Fulltime Specialized Solvents & Reagents Co., Ltd., China); N-butanol (Anhui Fulltime Specialized Solvents & Reagents Co., Ltd., China); Methylthiazolyldiphenyl-tetrazolium bromide (MTT) cell proliferation and toxicity assay kit (Sigma-Aldirch, Inc., USA); Dimethyl sulfoxide (DMSO) reagent (Thermo Fisher Scientific, China); Annexin V-FITC/PI kit (Thermo Fisher Scientific, China); GSH-Px, MDA, SOD test kit (Shanghai Youkewei Biotech Co., LTD., China); Radio Immunoprecipitation Assay (RIPA) lysate (Beijing Solarbio Technology Co., LTD., China); Bicinchoninic acid (BCA) protein Quantitative Assay kit (Thermo Fisher Scientific, China); Bcl-2, Bax, p-p38 MAPK, c-Jun N-terminal kinase (JNK), p-JNK and glyceraldehde-3-phosphate dehydrogenase (GAPDH) primary antibodies (Abcam Corporation, UK); Horseradish peroxidase labelled IgG secondary antibody (Abcam, UK); Chemiluminescence protein visualization kit (Beijing Solarbio Technology Co., LTD., China).

Instrument: SW-CJ Ultra clean table (Hunan Lichen Instrument Technology Co., LTD., China); D180 carbon dioxide incubator (RWD Life Science, China); KH22R centrifuge (Beijing Kaida Science and Technology Co., LTD., China); YRE2020Z automatic rotary evaporator (Shanghai Yuhua Instrument & Equipment Co., LTD., China); Varioskan LUX automatic microplate Reader (Thermo Fisher Scientific, China); DYCP-38C electro-phoresis apparatus (Beijing Liuyi Biotechnology Co., LTD., China); HD-NC600L fully automatic gel imager (Shandong Horde·Electric, China).

Preparation of extracts from Dendrobium nobile

A total of 50 g of dried *Dendrobium nobile* was ground to a coarse powder and then extracted by reflux with 98% ethanol at 85°C for 3 times, 4 h each time. Following combining the extracts, the falling film concentration was carried out to a small volume. Later, the ethanol was evaporated at 65°C to obtain a concentrated aqueous solution. Rotary evaporation was continued to concentrate at 90°C to obtain a black extract, which was added to the prepared 5% hydrochloric acid solution for acidification. After the extract was dissolved, the acid solution was filtered, and NaCl was added to adjust to saturation. According to the ratio of 3:1, the mixture of acid solution and chloroform was applied to extract 10 times, and the chloroform was recovered by rotary evaporation. The recovered chloroform was rinsed to neutral with purified water, and the ratio was 3:1 (chloroform: water). The chloroform was recovered after rotary evaporation at 50°C to obtain FSA.

After 70% of the concentrated water solution was extracted with chloroform, the ratio was 3:1 (acid water solution:chloroform), the chloroform layer was combined, and the chloroform was recovered by rotary evaporation to obtain WPA.

The pH value of the acid water layer after chloroform extraction was adjusted to 9 ~ 10 by concentrated ammonia water and the alkali water layer was extracted by chloroform with the extraction ratio of 3:1 (alkali water layer:chloroform). Following combining the chloroform layer, the chloroform was recovered by rotary evaporation, and the LPA was obtained after rinsing to neutral.

The n-butyl alcohol solution was used to extract the alkali liquid after chloroform extraction, the extraction ratio was 3:1 (alkali water layer:n-butyl alcohol), and

the n-butyl alcohol was recovered by rotary evaporation following combining the n-butyl alcohol layer. Pure water extraction of n-butanol, 3:1 (n-butanol:water), combined with n-butanol layer extraction of aqueous solution; The n-butyl alcohol layer was extracted by saturated water with n-butyl alcohol, 3:1 (n-butyl alcohol layer:n-butyl alcohol saturated water). The n-butyl alcohol was recovered by rotary evaporation to obtain WSA.

Experimental subjects

From October 2022 to October 2023, 10 patients with cataract without other eye diseases who were treated in the Aier Eye Hospital of Mudanjiang, Mudanjiang, Heilongjiang Province, China, were enrolled. All the patients received phacoemulsification, and fresh anterior capsule tissue of the lens was collected. The normal anterior lens capsule tissues from 10 patients with high myopia who underwent clear lens extraction during the same period were used as controls. There were 4 males and 6 females with an average age of (63.1 ± 8.6) years. There were 5 males and 5 females in the control group, with a mean age of (62.8 ± 7.5) years. The trial protocol was conducted in accordance with the Declaration of Helsinki and the trial was approved by the ethics committee of the Aier Eye Hospital of Mudanjiang, Mudanjiang, Heilongjiang Province, China. Written informed consent was obtained from the patients and their families.

In vitro culture of primary LECs

The anterior capsule tissue was removed on a sterile ultra-clean bench, and the anterior capsule was cut into two halves using micro-scissors. One half of the tissue was laid flat on the bottom of the culture dish, and the other half of the tissue was flattened in reverse to the bottom of the dish. The tissue was cultured in 5% CO_2 at 37°C for 6 h, then a 3 mL DMEM medium containing 20% FBS was applied, and the medium was changed every 3 days. When the degree of cell fusion reached more than 90%, the original culture medium was discarded, the cells were washed with phosphate buffer, and digested with 0.2% trypsin. The digestion was terminated by adding a culture medium, and then the cells were centrifuged.

Culture of the LEC cells

Human LECs in the log phase were collected and grouped as follows: (1) Normal: Normal human primary LEC were inoculated in DMEM medium having 20% FBS for routine culture; (2) Cataract: Primary cataract LEC in the same medium; (3) OD: Primary cataract LECs in DMEM medium having 500 μmol/L H_2O_2 and 20% FBS; (4) OD+FSA: primary cataract LEC in the medium with 500 μmol/L H_2O_2, 20% FBS and 20 μg/L FSA; (5) OD+WSA: primary cataract LEC in the medium having 500 μmol/L H_2O_2, 20% FBS and 20 μg/L WSA; (6) OD+WPA: primary cataract LEC in the medium having 500 μmol/L H_2O_2, 20% FBS and 20 μg/L WPA; (7) OD+LPA: primary cataract LEC in the medium with 500 μmol/L H_2O_2, 20% FBS and 20 μg/L LPA.

After drug treatment, the LEC was routinely cultured for 24 hours, repeating 3 times.

MTT assay for detecting cell proliferation

After treatment, 200 μL MTT reagent at a final concentration of 0.5 g/L was added to each well and the cells were cultured for 4 h. The original solution was discarded and 150 μL DMSO reagent was added and the mixture was shaken for 10 min. An automated microplate reader was used to read the absorbance (A value) of each well at 490 nm. The inhibition rate of cell proliferation was calculated using equation (1).

$$\text{Proliferation inhibition rate (\%)} = [(A_{\text{value of control group}} - A_{\text{value of experimental group}})/A_{\text{value of control group}}] \times 100, \quad (1)$$

Cell apoptosis detected by flow cytometry

After treatment, the cells were rinsed with phosphate buffer and centrifuged at 1000 rpm for 5 minutes. They were resuspended by the addition of 200 μL binding buffer. An amount of 5 μL annexin V-FITC reagent was added and incubated in the dark at 25°C for 10 min, 1000 rpm/min for 5 min, and the cells were resuspended with 200 μL binding buffer. Then, 5 μL PI reagent was added and the apoptosis rate was determined. Flow cytometry was performed with the upper left quadrant representing injured cells, the lower left quadrant representing normal cells, the upper right quadrant representing late apoptotic cells and the lower right quadrant representing early apoptotic cells. The relative apoptosis rate was calculated using equation (2).

$$\text{Relative apoptosis (\%)} = [(\text{early apoptotic} + \text{late apoptotic cells})/\text{total cells}] \times 100, \quad (2)$$

Determination of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD) and malondialdehyde (MDA) levels

For the harvested cells, the top liquid was taken after the addition of cell lysate. The content of GSH-Px in the upper liquid was detected by the 2-nitrobenzoic acid method, and the absorbance was determined at 412 nm using an automatic microplate reader. The content of SOD in the upper liquid was determined by the xanthine oxidase method, and the absorbance was measured at 550 nm. The content of MDA in the upper liquid was determined by the thiobarbituric acid method and the absorbance was measured at 532 nm. The detection method was performed strictly according to the kit instructions.

Detection of protein expression by western blot

After treatment, cells were lysed with radio-immuno-precipitation assay lysate and total protein was extracted and quantified using the bicinchoninic acid protein quantitative detection kit. According to the sample volume of 50 μg *per* well, samples were loaded and subjected to sodium dodecyl sulphate-polyacrylamide gel electrophoresis gel electrophoresis to separate proteins. Proteins were transferred to a polyvinylidene fluoride membrane and blocked with 50 g/L skim milk powder

for 1 h at 25°C. Diluted primary antibodies against B-cell lymphoma-2 (Bcl-2, 1:2000), Bcl-2 Associated X Protein (Bax, 1:2000), p38 mitogen-activated protein kinase (p38 MAPK, 1:1000), phospho-p38 mitogen-activated protein kinase (p-p38 MAPK, 1:2000), c-Jun N-terminal kinase (JNK, 1:2000), phospho-c-Jun N-terminal kinase (p-JNK, 1:1000) and Glyceraldehyde-3-Phosphate Dehydrogenase (GAPDH, 1:5000) were then applied. Cells were incubated overnight at 4°C. Diluted horseradish peroxidase-labelled IgG secondary antibody (1:10000) was then applied and incubated for 30 min at 25°C. Protein bands were visualized by electrochemiluminescence, and the relative grey value of the target protein was quantitatively analysed using GAPDH as a control.

Statistical analysis

SPSS 22.0 (International Business Machines Corporation, USA.) was used. All data were expressed as mean ± sd ($\bar{x} \pm s$) and a one-way ANOVA variance test was used. When $p < 0.05$, the difference was statistically significant.

Results and Discussion

Single factor analysis of solvent extraction method

Most alkaloids are insoluble or difficult to dissolve in water but are readily soluble in organic solvents such as ethanol and chloroform. In this article, ethanol solution was chosen as the extraction solvent for alkaloids from *Dendrobium nobile*. The factors affecting the extraction yields of LPA, WPA, WSA and FSA were analysed. Firstly, the effect of the ethanol extract concentration on the extraction yield of different alkaloids was analysed (Figure 1A). As the ethanol concentration gradually increased from 10% to 100%, the extraction yields of LPA, WPA, WSA and FSA showed a trend of first increasing and then decreasing. When the ethanol concentration was 80%, the extraction yields of LPA, WPA, WSA and FSA were the highest, being 0.11%, 0.34%, 0.05% and 0.42%, respectively. Figure 1B illustrates the effect of extraction times on the extraction yield of different alkaloids. As the extraction times increased from 1 to 10 times, the extraction rates of LPA, WPA, WSA and FSA showed a gradual increasing trend. When the number of extractions was 10 times, the extraction rates of LPA, WPA, WSA and FSA were the highest, being 0.18%, 0.62%, 0.16% and 0.66% respectively.

Figure 1C illustrates the effect of extraction time on the extraction yield of different alkaloids. As the extraction time increased from 1 h to 6 h, the extraction rates of LPA, WPA, WSA and FSA also showed a gradual increasing trend. When the extraction time was 6 h, the extraction rates of LPA, WPA, WSA and FSA were the highest, being 0.21%, 0.65%, 0.15% and 0.71% respectively.

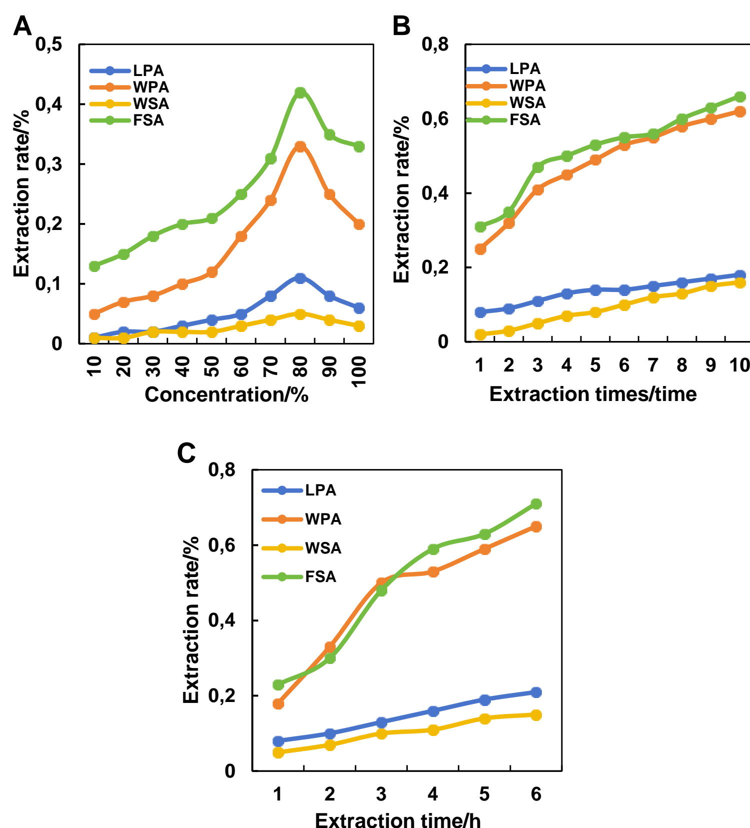

Figure 1.

Alkaloid extraction rate test. A: extraction solvent concentration; B: extraction times; C: extraction time

However, too many extraction times will result in a waste of reagents and too long an extraction time will also affect the stability of the extract. For this study, 80% ethanol concentration, 5 times and 3 hours was chosen as the final extraction of alkaloids from the *Dendrobium nobile*.

Dendrobium nobile alkaloids on LEC proliferation in cataract

Figure 2 illustrates the effects of *Dendrobium nobile* alkaloids on the proliferation of cataract LEC after oxidative stress injury. Compared to the normal group, the proliferative activity of LEC in the cataract group showed a visible decrease. Compared to the cataract group, the OD group showed significantly decreased proliferative activity. Compared to the OD group, OD+LPA, OD+WPA, OD+WSA and OD+FSA groups showed visibly increased proliferation activity (all $p < 0.05$).

Dendrobium nobile alkaloids effects on LEC apoptosis in cataract

Figure 3 illustrates the results of *Dendrobium nobile* alkaloids on the apoptosis rate of LEC after oxidative stress injury in cataract. Compared with the normal group, the apoptosis rate of the cataract group showed a significant increase. Compared with the cataract group, the apoptosis rate of the OD group showed a significant increase. Compared to the OD group, the apoptosis rate of OD+LPA, OD+WPA, OD+WSA and OD+FSA groups was significantly decreased (all $p < 0.05$).

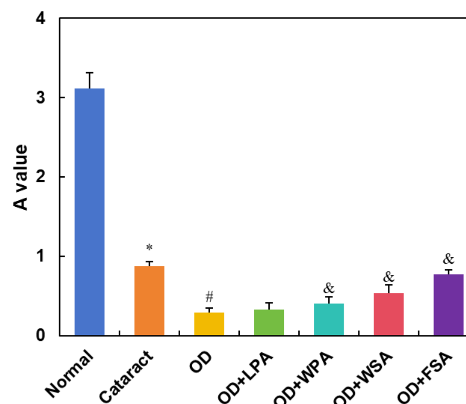

Figure 2.
Viscosity curves and the statistical mean of differences expressed by the regression lines

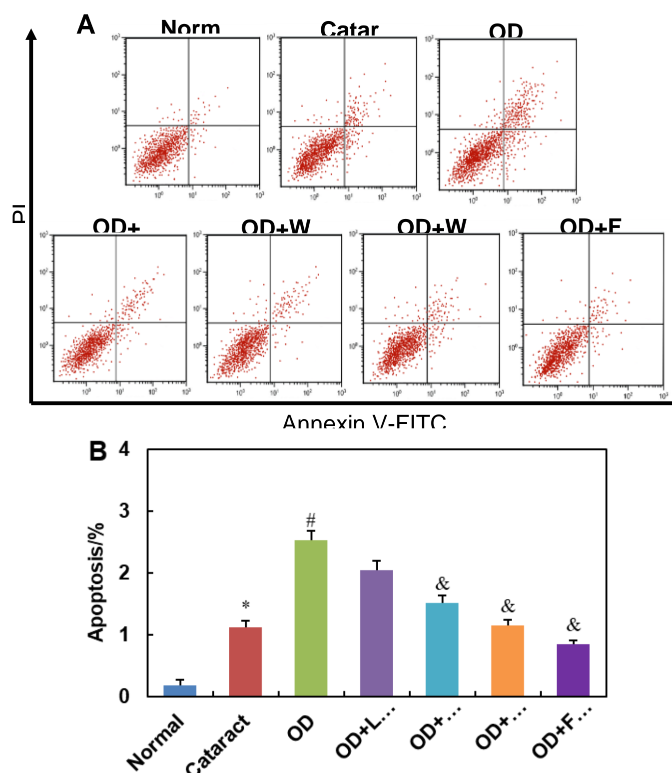

Figure 3.
Contrast of LEC apoptosis rates (A: flow cytometry; B: contrast of apoptosis rates)
* $p < 0.05$ compared with Normal group; # $p < 0.05$ compared with Cataract group; & $p < 0.05$ compared with OD group

Figure 4 shows that the expression of Bcl-2 protein was significantly decreased and the expression of Bax protein was significantly increased in the cataract group compared to the normal group. Compared to the cataract group, the expression of Bcl-2 protein was significantly decreased and the expression of Bax protein was significantly increased in the OD group. Compared to the OD group, the expression of Bcl-2 protein was significantly increased and the expression of Bax protein was significantly decreased in the

OD+LPA, OD+WPA, OD+WSA and OD+FSA groups (all $p < 0.05$).

Effects of Dendrobium nobile alkaloids on oxidative stress of LEC in cataract

Figure 5 shows the effects of different *Dendrobium nobile* alkaloids on oxidative stress damage to LEC in cataract. Compared to the normal group, the levels of GSH-Px and SOD in the upper fluid were significantly decreased, and MDA was significantly increased in the cataract group. Compared to the cataract group, the levels of GSH-Px and SOD were significantly decreased and MDA was significantly increased in the OD group. Compared to the OD group, the levels of GSH-Px and SOD were significantly increased, whereas MDA was significantly decreased in the OD+LPA, OD+WPA, OD+WSA and OD+FSA groups (all $p < 0.05$).

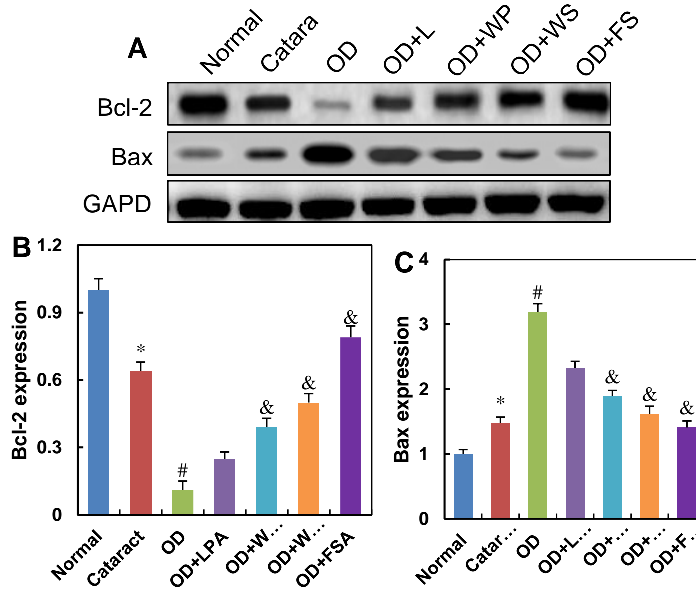

Figure 4.

Contrast of expression of LEC apoptosis-related proteins

(A: WB; B: Bcl-2 protein expression; C: Bax protein expression)

* $p < 0.05$ compared with Normal group; # $p < 0.05$ compared with Cataract group; & $p < 0.05$ compared with OD group

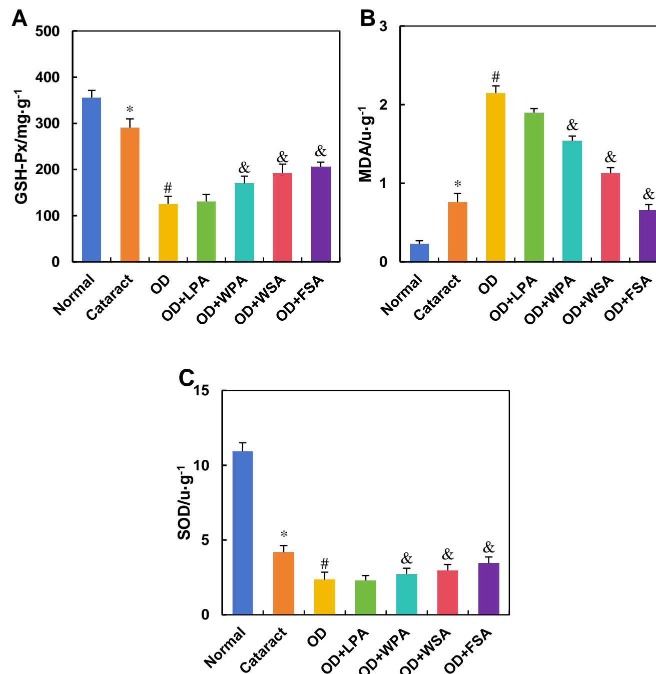

Figure 5.

Contrast of the contents of oxidative stress factors secreted by LEC (A: GSH-Px; B: MDA; C: SOD)

* $p < 0.05$ compared with Normal group; # $p < 0.05$ compared with Cataract group; & $p < 0.05$ compared with OD group

Dendrobium nobile alkaloids on p38 MAPK/JNK pathway in LEC of cataract

Figure 6 shows that the phosphorylation levels of p-p38 MAPK and p-JNK proteins were significantly increased in the cataract group compared to the normal group. Compared with the cataract group, the phosphorylation levels were significantly increased in the OD group. Compared to the OD group, the phosphorylation in the OD+LPA, OD+WPA, OD+WSA and OD+FSA groups was significantly decreased (all $p < 0.05$).

Cataract is a common cause of blindness worldwide and its pathogenesis is complex. OD is an important cataract induction mechanism [14]. OD can cause degeneration of lens proteins, leading to partial or total opacification of the lens and the development of cataracts [15]. The LEC is the most active metabolic site in the lens and is its anti-OD centre. The LEC contains a large number of antioxidant enzymes that play an important role in maintaining the repair, normal structure and function of the lens [16].

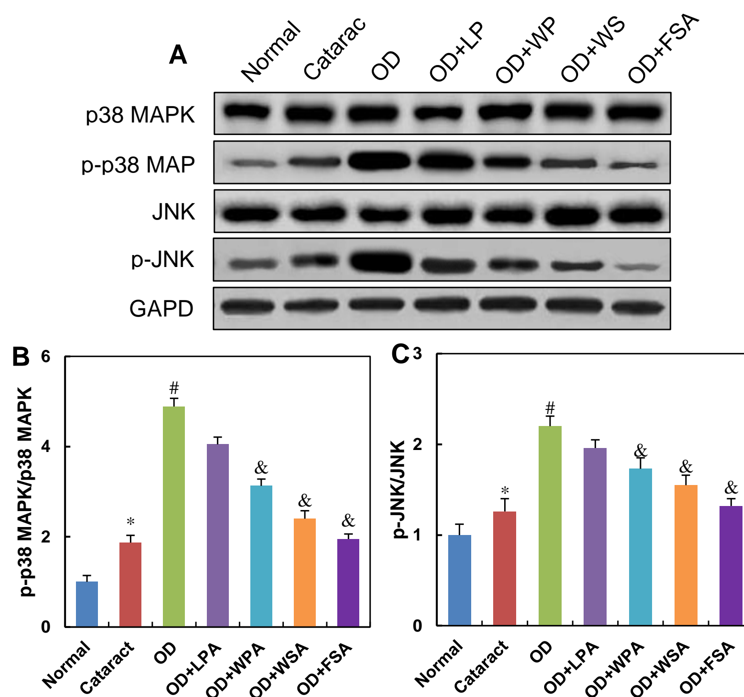

Figure 6.

Contrast of p38 MAPK/ JNK-related protein expression in LEC

(A: WB; B: p-p38 MAPK/p38 MAPK protein; C: p-JNK/JNK protein)

* $p < 0.05$ compared with Normal group; # $p < 0.05$ compared with Cataract group; & $p < 0.05$ compared with OD group

When LEC is damaged, the growth rate of the lens slows down or stops, and the stability of the lens itself is affected in severe cases. Therefore, LEC proliferation or apoptosis can indirectly reflect the anti-OD outcome of the drug and evaluate its anti-cataract outcome. *Dendrobium nobile* has immunomodulatory, anti-tumour, antioxidant, anti-inflammatory and anti-cataract effects [17-19]. Dendrobium has also been used in the treatment of eye diseases. Han *et al*. found that Funicin and Vinetorin, the extracts of Dendrobium endophytic fungus *Biscogniauxia Petrensis* MFL UCC14-0151, could antagonize the growth of *Streptococcus agalactiae* and *Staphylococcus aureus*, and their MIC values were 10.35 μM and 10.21 μM and 5.17 μM and 20.42 μM, respectively, which might be natural antibacterial agents [20]. In this article, alkaloid extracts with different polarity were isolated from *Dendrobium nobile* and applied in the treatment of cataract LEC. They were found to promote cell proliferation.GSH-Px, an antioxidant enzyme, was found to be abundant in the lens. MDA is a compound formed after the decomposition of lipid peroxides and is a common indicator of lipid peroxide levels in cells and tissues [21]. SOD can catalyse the dismutation of superoxide anion free radicals to produce oxygen and hydrogen peroxide, which are present in many types of cytoplasm [22]. SOD and MDA may reflect the balance of the oxidation/anti-oxidation system in cells and tissues. Changes in the molecular structure of lens proteins caused by factors such as OD will increase the level of insoluble proteins in the lens, and a large number of such proteins can affect the light scattering of the lens and lead to its loss of transparency [23-25]. This article first found that MDA activity increased, GSH-Px content, and SOD activity decreased in cataract LEC. It indicated that there is obvious OD in the cataractous lens. Secondly, MDA activity decreased and GSH-Px content and SOD activity increased in cataract LEC after treatment with

alkaloid extracts of different polarity from *Dendrobium nobile*, among which FSA had the best effect. This meant that the different polarity alkaloid extracts from *Dendrobium nobile* could inhibit cataract by improving the antioxidant capacity of the cataractous lens. This is similar to the results of Hsu *et al*. [26], who found that Dendrobium extracts can protect retinal pigment epithelial cells from oxidative stress damage and play a therapeutic role in eye diseases by regulating MAPK and Nrf2/HO-1 signalling pathways.

The pathogenesis of cataract includes OD, apoptosis and crystallin degeneration. The structure and function of LECs are involved in maintaining lens transparency [27]. LEC apoptosis leads to a reduction in cell density and affects the growth and quality of lens fibres. LEC defects will also lead to a loss of fibre osmotic pressure, affecting the transparency and integrity of lens fibres in this area [28, 29]. The Bcl-2 family is widely distributed in mammals and can be divided into anti-apoptotic and pro-apoptotic proteins according to the regulation of apoptosis [30]. The anti-apoptotic protein Bcl-2 can be used as an antioxidant to regulate the redox state of cells and block the destruction of cellular components by oxidation. It can also suppress the release of cytochrome C, which has a pro-apoptotic effect [31, 32]. Bax is a member of the Bcl-2 protein family, which can promote cell apoptosis as a dimer with family proteins [33]. This article showed that the apoptosis rate of LEC in cataract was increased, the expression of the pro-apoptotic protein Bax was up-regulated, and the expression of the anti-apoptotic protein Bcl-2 was downregulated. LEC in cataract have obvious apoptosis, which may induce cataract through cell membrane disruption and chromosome condensation. Second, the apoptosis rate of cataractous LEC was decreased, the expression of pro-apoptotic protein Bax was down-regulated, and the expression of anti-apoptotic protein Bcl-2 was up-regulated after treatment with different polarity alkaloid extracts from *Dendrobium nobile*. Different polarity alkaloid extracts from *Dendrobium nobile* could suppress LEC apoptosis and thereby inhibit cataract progression.

p38 MAPK is a relatively conserved serine/threonine mitogen-activated protein kinase. Upon stimulation, cells can activate this signalling pathway and promote the binding of substrates to adenine nucleoside tri-phosphate and ultimately participate in biological processes such as inflammation, cell cycle regulation and cell death [34-36]. Bai *et al*. [37] pointed out that H_2O_2 can induce phosphorylation of p38 MAPK. This is consistent with the present article, which found increased p38 MAPK phosphorylation and the JNK phosphorylation in cataract LEC after H_2O_2 induction. The p38 MAPK pathway is closely associated with oxidative stress. This article showed that phosphorylation levels of p38 MAPK and JNK were increased, while the phosphorylation levels of p38 MAPK and JNK were decreased in cataract LEC after treatment with different polar alkaloid extracts from *Dendrobium nobile*. It meant that different polar alkaloid extracts from *Dendrobium nobile* could suppress the activation of p38 MAPK/JNK signalling pathway, thereby inhibiting the apoptosis and oxidative stress response of LEC and inhibiting the process of cataract.

Conclusions

FSA extract of *Dendrobium nobile* has strong anti-oxidant result, which can promote the proliferation and suppress the apoptosis of LEC in cataract, and suppress the activation of p38 MAPK/JNK pathway to act in antioxidative stress injury. This article can provide a basis for further investigation of the anti-cataract agents of *Dendrobium nobile* and its antioxidant mechanism, and provide a reference for the research and development of anti-cataract drugs. However, the medicinal value of *Dendrobium nobile* FSA extracts for anti-cataract activity needs to be further investigated.

Conflict of interest

The authors declare no conflict of interest.

References

1. Ang MJ, Afshari NA, Cataract and systemic disease: A review. *Clin Exp Ophthalmol.*, 2021; 49(2): 118-127.
2. Dascalu AM, Tudosie MS, Smarandache GC, Serban D, Impact of the COVID-19 pandemic upon the ophthalmological clinical practice. *Rom J Leg Med.*, 2020; 28: 96-100.
3. Minkus CL, Pistilli M, Dreger KA, Fitzgerald TD, Payal AR, Begum H, Kaçmaz RO, Jabs DA, Nussenblatt RB, Rosenbaum JT, Levy-Clarke GA, Sen HN, Suhler EB, Thorne JE, Bhatt NP, Foster CS, Buchanich JM, Kempen JH, Risk of Cataract in Intermediate Uveitis. *Am J Ophthalmol.*, 2021; 229: 200-209.
4. Novo SG, Faranda AP, Shihan MH, Wang Y, Garg A, Duncan MK, The Immediate Early Response of Lens Epithelial Cells to Lens Injury. *Cells*, 2022; 11(21): 3456.
5. Suceveanu AI, Mazilu L, Katsiki N, Parepa I, Voinea F, Pantea-Stoian A, Rizzo M, Botea F, Herlea V, Serban D, Suceveanu AP, NLRP3 Inflammasome Biomarker-Could Be the New Tool for Improved Cardiometabolic Syndrome Outcome. *Metabolites*, 2020; 10(11): 448.
6. Atalay E, Oğurel T, Derici MK, The role of oxidative damage in cataract etiopathogenesis. *Ther Adv Ophthalmol.*, 2023; 15: 25158414231168813.
7. Liu Y, Li J, Ye Z, Ma T, Li Z, Protective Effects of Piceatannol against Selenite-Induced Cataract and Oxidative Damage in Rats. *Curr Eye Res.*, 2022; 47(9): 1272-1278.
8. Ling J, Chan CL, Ho CY, Gao X, Tsang SM, Leung PC, Wong CK, The Extracts of *Dendrobium* Alleviate Dry Eye Disease in Rat Model by Regulating Aquaporin Expression and MAPKs/NF-κB Signalling. *Int J Mol Sci.*, 2022; 23(19): 11195.
9. Guo J, Yang N, Zhang J, Huang Y, Xiang Q, Wen J, Chen Y, Hu T, Qiuyan L, Rao C, Neurotoxicity study

of ethyl acetate extract of *Zanthoxylum armatum* DC. on SH-SY5Y based on ROS mediated mitochondrial apoptosis pathway. *J Ethnopharmacol.*, 2024; 319(Pt 3): 117321.
10. Guo L, Qi J, Du D, Liu Y, Jiang X, Current advances of *Dendrobium officinale* polysaccharides in dermatology: A literature review. *Pharm Biol.*, 2020; 58(1): 664-673.
11. Hu Y, Yang H, Ding X, Liu J, Wang X, Hu L, Liu M, Zhang C, Anti-inflammatory octahydroindolizine alkaloid enantiomers from *Dendrobium crepidatum*. *Bioorg Chem.*, 2020; 100: 103809.
12. Li DD, Wang GQ, Wu Q, Shi JS, Zhang F, *Dendrobium nobile* Lindl alkaloid attenuates 6-OHDA-induced dopamine neurotoxicity. *Biotechnol Appl Biochem.*, 2021; 68(6): 1501-1507.
13. Wu J, Li X, Wan W, Yang Q, Ma W, Chen D, Hu J, Chen CO, Gigantol from *Dendrobium chrysotoxum* Lindl. binds and inhibits aldose reductase gene to exert its anti-cataract activity: An in vitro mechanistic study. *J Ethnopharmacol.*, 2017; 198: 255-261.
14. Zych M, Borymska W, Urbisz K, Kostrzewski M, Kaczmarczyk-Zebrowska I, Two bioactive compounds, rosmarinic acid and sinapic acid, do not affect the depleted glutathione level in the lenses of type 2 diabetic female rats. *Farmacia*, 2022; 70(4), 607-616.
15. Stefanescu R, Fülöp E, Demian L, Vari C, Osz BE, Grosan A, Laczko-Zöld E, Chibelean CB, Efficacy of natural polyphenolic compounds from bilberry and blueberry on the metabolic alterations. *Farmacia*, 2022; 70(4): 658-664.
16. Sugiyama Y, Nakazawa Y, Sakagami T, Kawata S, Nagai N, Yamamoto N, Funakoshi-Tago M, Tamura H, Capsaicin attenuates TGFβ2-induced epithelial-mesenchymal transition in lens epithelial cells *in vivo* and *in vitro*. *Exp Eye Res.*, 2021; 213: 108840.
17. Chen P, Wu Q, Feng J, Yan L, Sun Y, Liu S, Xiang Y, Zhang M, Pan T, Chen X, Duan T, Zhai L, Zhai B, Wang W, Zhang R, Chen B, Han X, Li Y, Chen L, Liu Y, Huang X, Jin T, Zhang W, Luo H, Chen X, Li Y, Li Q, Li G, Zhang Q, Zhuo L, Yang Z, Tang H, Xie T, Ouyang X, Sui X, Erianin, a novel dibenzyl compound in *Dendrobium* extract, inhibits lung cancer cell growth and migration via calcium/calmodulin-dependent ferroptosis. *Signal Transduct Target Ther.*, 2020; 5(1): 51.
18. Liu H, Liang J, Zhong Y, Xiao G, Efferth T, Georgiev MI, Vargas-De-La-Cruz C, Bajpai VK, Caprioli G, Liu J, Lin J, Wu H, Peng L, Li Y, Ma L, Xiao J, Wang Q, *Dendrobium officinale* polysaccharide alleviates intestinal inflammation by promoting small extracellular vesicle packaging of miR-433-3p. *J Agric Food Chem.*, 2021; 69(45): 13510-13523.
19. Ling J, Chan CL, Ho CY, Gao X, Tsang SM, Leung PC, Hu JM, Wong CK, The Extracts of *Dendrobium* Alleviate Dry Eye Disease in Rat Model by Regulating Aquaporin Expression and MAPKs/NF-κB Signalling. *Int J Mol Sci.*, 2022; 23(19): 11195.
20. Han L, Zheng W, He Z, Qian S, Ma X, Kang J, Endophytic fungus *Biscogniauxia petrensis* produces antibacterial substances. *PeerJ.*, 2023; 11: e15461.
21. Szyguła-Jurkiewicz B, Szczurek-Wasilewicz W, Osadnik T, Frycz-Kurek AM, Macioł-Skurk K, Małyszek-Tumidajewicz J, Skrzypek M, Romuk E, Gąsior M, Banach M, Jóźwiak JJ, Oxidative stress markers in hypertrophic cardiomyopathy. *Medicina*, 2021; 58(1): 31.
22. Henning Y, Blind US, Larafa S, Matschke J, Fandrey J, Hypoxia aggravates ferroptosis in RPE cells by promoting the Fenton reaction. *Cell Death Dis.*, 2022; 13(7): 662.
23. Liu S, Jin Z, Xia R, Zheng Z, Zha Y, Wang Q, Wan X, Yang H, Cai J, Protection of Human Lens Epithelial Cells from Oxidative Stress Damage and Cell Apoptosis by KGF-2 through the Akt/Nrf2/HO-1 Pathway. *Oxid Med Cell Longev.*, 2022; 2022: 6933812.
24. Pfaff A, Chernatynskaya A, Vineyard H, Ercal N, Thiol antioxidants protect human lens epithelial (HLE B-3) cells against tert-butyl hydroperoxide-induced oxidative damage and cytotoxicity. *Biochem Biophys Rep.*, 2022; 29: 101213.
25. Thompson B, Davidson EA, Chen Y, Orlicky DJ, Thompson DC, Vasiliou V, Oxidative stress induces inflammation of lens cells and triggers immune surveillance of ocular tissues. *Chem Biol Interact.*, 2022; 355: 109804.
26. Hsu WH, Chung CP, Wang YY, Kuo YH, Yeh CH, Lee IJ, Lin YL, *Dendrobium nobile* protects retinal cells from UV-induced oxidative stress damage via Nrf2/HO-1 and MAPK pathways. *J Ethnopharmacol.*, 2022; 288: 114886.
27. Novo SG, Faranda AP, Shihan MH, Wang Y, Garg A, Duncan MK, The Immediate Early Response of Lens Epithelial Cells to Lens Injury. *Cells*, 2022; 11(21): 3456.
28. Vigneux G, Pirkkanen J, Laframboise T, Prescott H, Tharmalingam S, Thome C, Radiation-induced alterations in proliferation, migration, and adhesion in lens epithelial cells and implications for cataract development. *Bioengineering*, 2022; 9(1): 29.
29. Wernecke L, Keckeis S, Reichhart N, Strauß O, Salchow DJ, Epithelial-Mesenchymal Transdifferentiation in Pediatric Lens Epithelial Cells. *Invest Ophthalmol Vis Sci.*, 2018; 59(15): 5785-5794.
30. Xu Y, Zheng Y, Shen P, Zhou L, Role of long non-coding RNA KCNQ1 overlapping transcript 1/microRNA-124-3p/BCL-2-like 11 axis in hydrogen peroxide (H_2O_2)-stimulated human lens epithelial cells. *Bioengineered*, 2022; 13(3): 5035-5045.
31. Knight T, Luedtke D, Edwards H, Taub JW, Ge Y, A delicate balance – The BCL-2 family and its role in apoptosis, oncogenesis, and cancer therapeutics. *Biochem Pharmacol.*, 2019; 162: 250-261.
32. Suraweera CD, Banjara S, Hinds MG, Kvansakul M, Metazoans and Intrinsic Apoptosis: An Evolutionary Analysis of the Bcl-2 Family. *Int J Mol Sci.*, 2022; 23(7): 3691.
33. Aghaei M, KhanAhmad H, Aghaei S, Ali Nilforoushzadeh M, Mohaghegh MA, Hejazi SH, The role of Bax in the apoptosis of *Leishmania*-infected macrophages. *Microb Pathog.*, 2020; 139: 103892.
34. Pan J, Liu H, Wu Q, Zhou M, Scopoletin protects retinal ganglion cells from high glucose-induced injury in a cellular model of diabetic retinopathy via ROS-dependent p38 and JNK signaling cascade. *Cent Eur J Immunol.*, 2022; 47(1): 20-29.
35. Zhou T, Yang M, Zhang G, Kang L, Yang L, Guan H, Long non-coding RNA nuclear paraspeckle assembly transcript 1 protects human lens epithelial cells against

H_2O_2 stimuli through the nuclear factor kappa b/p65 and p38/mitogen-activated protein kinase axis. *Ann Transl Med.*, 2020; 8(24): 1653.

36. Du S, Shao J, Xie D, Zhang F, Decorin inhibits glucose-induced lens epithelial cell apoptosis via suppressing p22phox-p38 MAPK signaling pathway. *PLoS One.*, 2020;15(4):e0224251.
37. Bai J, Zheng Y, Wang G, Liu P, Protective Effect of D-Limonene against Oxidative Stress-Induced Cell Damage in Human Lens Epithelial Cells *via* the p38 Pathway. *Oxid Med Cell Longev.*, 2016; 2016: 5962832.