International Journal of Pharmacology

ISSN 1811-7775

science alert

ansinet
Asian Network for Scientific Information

OPEN ACCESS International Journal of Pharmacology

ISSN 1811-7775
DOI: 10.3923/ijp.2025.189.199

Research Article
Protective Mechanism of Licochalcone A-Mediated p53/AMPK/mTOR Pathway Against Retinal Ganglion Cell Injury in Glaucoma

Chen Wu, Yuesheng Qiu, Yueqing Zhu and Tiantian Yao

Department of Ophthalmology, The First People's Hospital of Jiashan, Jiashan, 314199, Zhejiang, China

Abstract

Background and Objective: Licochalcone A (Lico A) has anti-inflammatory, oxidative and bacterial activities. However, the effect of Lico A on the oxidative damage of RGC in glaucoma has not been reported. This study investigated the mechanism of Lico A-mediated regulation of Hydrogen Peroxide (H_2O_2)-induced oxidative damage of RGC in glaucoma via p53/AMPK/mTOR pathway. **Materials and Methods:** The Lico A was extracted from the licoherbs residues. Oxidative damage of glaucoma retinal ganglion cells model (Mod group) was constructed using H_2O_2-induced RGC-5 cells and effects of 5, 10 and 20 μmol/L Lico A on cell proliferation, apoptosis, oxidative stress and expression of p53/AMPK/mTOR pathway-related proteins were analyzed. **Results:** The Lico A was successfully extracted and with the increase of Lico A concentration, the clearance of DPPH, ABTS, O_2^- and H_2O_2 also increased. As against Mod, the proliferation activity of RGC-5 was increased, the AR was decreased, the avidity of SOD and GSH-Px had an increase, the degree of MDA and the expression of p53, AMPK and LC3II/LC3I was decreased and the expression of mTOR was visibly raised in the 5, 10 and 20 μmol/L Lico A treatment groups. **Conclusion:** The Lico A has a strong antioxidant effect. In addition, Lico A can inhibit H_2O_2-induced apoptosis and autophagy in glaucomatous RGC through p53/AMPK/mTOR signaling pathway and improve OS injury.

Key words: Lico A, antioxidant, glaucoma, RGC, p53/AMPK/mTOR signaling pathway

Citation: Wu, C., Y. Qiu, Y. Zhu and T. Yao, 2024. Protective mechanism of licochalcone A-mediated p53/AMPK/mTOR pathway against retinal ganglion cell injury in glaucoma. Int. J. Pharmacol., 20: XX-XX.

Corresponding Author: Chen Wu, Department of Ophthalmology, The First People's Hospital of Jiashan, Jiashan, 314199, Zhejiang, China

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Glaucoma is a progressive optic nerve disease[1]. Optic atrophy, visual field defects and loss of RGCs are the main pathological features of glaucoma[2]. Many factors induce RGC damage, including OS, glial cell activation and deficiency of neurotrophic factors[3,4]. Prevention and mitigation of RGC damage is a new therapeutic target for glaucoma.

Licorice is the dried root and rhizome of licorice, liquorice biloba or liquorice glabrata. The nature of liquorice is sweet and flat and it can return to the heart, lungs, spleen and stomach. It has the functions of tonifying qi, relieving pain and detoxifying. Licorice contains triterpene saponins, flavonoids and other compounds[5]. The Lico A is a licorice flavonoid with a variety of physiological activities, which participates in and regulates various physiological activities in the body[6]. The Lico A can inhibit the growth of a variety of pathogenic bacteria. The Lico A can visibly reduce the secretion of enterotoxins SEA and SEB[7]. The Lico A can inhibit the biofilm formation of *Staphylococcus aureus*[8]. The Lico A can induce apoptosis of tumor cells through various pathways and inhibit cell proliferation, migration, invasion and tumor angiogenesis[9,10]. Moreover, Lico A can regulate the activation of inflammation-related pathways TLR4/MyD88/NF-κB and PPARγ and finally exert effects[11]. In addition, Lico A had anti-OS effect. The Lico A can play a neuroprotective role[12]. However, the mechanism of action of Lico A in the oxidative damage of RGC in glaucoma has not been reported.

In this study, Lico A was extracted from licorice residue and its antioxidant activity was evaluated by detecting DPPH, ABTS, O_2^- and H_2O_2 clearance *in vitro*. Secondly, the oxidative damage model of glaucomatous RGC was established by using Hydrogen Peroxide (H_2O_2)-induced RGC-5. The aim was to provide an experimental basis for understanding the mechanism of Lico A in the treatment of glaucoma and to find new therapeutic targets for glaucoma.

MATERIALS AND METHODS

Study area: The study was performed in The First People's Hospital of Jiashan from December, 2022 to October, 2023.

Isolation and identification of Lico A: The 10 g licorice residue was extracted by heating and refluxing with 95% ethanol (Sigma-Aldrich, USA) 3 times, 2 hrs each time and the liquid was concentrated under reduced pressure. After purification by HPD100 macroporous resin (Tianjin Haoju Resin Technology Co., Ltd., China), the liquid was eluted with distilled water, 75% ethanol and 100% ethanol in turn. The eluent was collected and purified by a polyamide chromatographic column and then eluted with methanol-water (Sigma-Aldrich, USA) gradient. The 20 g of total flavonoids of licorice were separated and purified by silica gel chromatographic column, then eluted by dichloromethane methanol gradient and the 9:1 ratio of dichloromethane methanol eluent was separated by polyamide chromatographic column and then eluted by methanol-water gradient and 60% of the methanol eluent was Lico A. The reference substance of Lico A (3.693 mg) was precisely weighed and dissolved in 2 mL methanol solution and then stored at 4°C for later use. The extraction of Lico A was determined by K2025 high performance liquid chromatography (Haineng Future Technology Group Co., Ltd., China). The powder of Lico A (15 mg) was dissolved in 10 mL methanol solution and shaken well. The chromatographic conditions were set on a 5 μm Agilent TC-C_{18} column of 150×4.5 mm, the mobile phase was acetonitrile:water (40:60, V/V), 254 nm, 16°C, 1.0 mL/min, 10 μL.

In vitro antioxidant activity of Lico A

DPPH clearance assay: According to the instructions of the DPPH scavenging ability test kit (Shanghai Huicheng Biotechnology Co., Ltd., China), 0.1 mM DPPH-ethanol solution was prepared, 650 μL Lico A solution was mixed with 195 μL DPPH-ethanol solution and the reaction was carried out at 37°C in the dark for half an hour and the absorbance (OD) was detected at 517 nm through Varioskan LUX multifunctional enzyme-linked immunosorbent assay (Thermo Fisher Scientific, China). Each sample was detected three times and DPPH clearance rate (CR) was calculated.

ABTS clearance determination: According to the instructions of the ABTS free radical (FR) scavenging ability test kit (Shanghai Shangbao Biotechnology Co., Ltd., China), 2.45 mM potassium persulfate solution was prepared with 7.0 mM ABTS-aqueous solution as the diluent, incubation overnight at 25°C in the dark. Then, 650 μL Lico A solution was mixed with 195 μL potassium persulfate diluent and the reaction was carried out at 37°C in the dark for half an hour. The OD was detected at 734 nm.

O_2^- clearance determination: According to the instructions of the superoxide anion (O_2^-) radical scavenging ability test kit (Shanghai Huicheng Biotechnology Co., Ltd., China), 0.1 M pH = 7.4 sodium phosphate buffer (NaPB) was used as diluent and 150 μM nitrotetrazolium chloride solution, 468 μM

reduced coenzyme II solution and 60 μM potassium persulfate solution were prepared. The 1 mL Lico A, nitrotetrazolium blue chloride solution and each reduced coenzyme II solution were mixed and then 1 mL potassium bisulfate solution was added, at 37°C for 5 min and the OD was detected at 560 nm.

Determination of H_2O_2 clearance: According to the instructions of the H_2O_2 scavenging ability test kit (Shanghai Solarbio Biotechnology Co., Ltd., China), a 43 mM hydrogen peroxide solution was configured with 0.1 M pH = 7.4 NaPB as the diluent. The 340 μL Lico A and 600 μL hydrogen peroxide solution were mixed, measurement of OD at 230 nm.

Culture and grouping of RGC: The mouse retinal ganglion cell line RGC-5 (Ningbo Mingzhou Biotechnology Co., Ltd., China) was inoculated in Dulbecco's minimum essential medium (DMEM) high sugar culture medium (Gibco, USA) containing 10% fetal bovine serum (Gibco, USA) and 1% penicillin-streptomycin (Gibco, USA), at 37°C, 5% CO_2 and saturated humidity CellXPert C170 cell culture incubator (Eppendorf, Germany). When the confluence degree of the cells reached about 85%, the cells were digested with 0.2% trypsin and passaged. A model of oxidative damage of glaucoma retinal ganglion cells (Mod group) was constructed using H_2O_2-induced RGC-5 cells. Normal RGC-5 was used as Ctrl. The cells were treated with 5, 10 and 20 μmol/L Lico A: Low-Lico A group (LG), Middle-Lico A group (MG) and High-Lico A group (HG).

The RGC-5 in Ctrl was cultured in a normal medium. In Mod, LG, MG and HG, RGC-5 was cultured in a medium containing 200 μmol/L H_2O_2 (Sigma-Aldrich, USA). The LG, MG and HG cells were treated with 5, 10 and 20 μmol/L Lico A solution, respectively.

Detection of RGC indexes

Cell proliferation by CCK-8 detection: The RGC-5 in log phase in a 96-well plate at 1×10^5/well, following cell adhesion, they were grouped and cultured for 48 hrs. According to the instructions of the Cell Counting Kit-8 (CCK-8) detection kit (Sigma Aldrich, USA), 20 μL CCK-8 reagent was applied and incubated for 3 hrs. After removal, it was shaken and mixed for 10 min. The OD was detected at 450 nm and the cell survival rate (SR) was computed, repeated 3 times and the mean value was obtained.

Apoptosis detection by flow cytometry: The RGC-5 in log phase in a 24-well plate at 2×10^5/well, following the cells adhered to the wall, they were grouped, culture for 24 hrs and the original medium was discarded. According to the instructions of the Annexin V-FITC/PI apoptosis detection kit (Sigma Aldrich, USA), the cells were collected by centrifugation, washed with phosphate buffer (PB) and subjected to resuspension in 1×binding buffer. The 5 μL of Annexin V-FITC working solution (WS) was applied, in the dark for 10 min. The 10 μL of PI WS was applied and incubation in the dark for 5 min. Apoptosis was subjected to detection by CytoFLEX flow cytometer (Beckman Kurt, USA), thrice and the mean value was obtained.

Immunofluorescence assay for ROS expression: The RGC-5 in log phase in a 96-well plate at 1×10^5/well, following cell adhesion, they were divided into groups, culture for 48 hrs. They were washed 3 times with PB, the addition of 4% paraformaldehyde and fixed at 25°C for 20 min. They were rinsed, covered with 200 μL of ROS (Beijing Geneline Huake Biotechnology Co., Ltd., China) staining WS and incubated for 60 min at 25°C. They were rinsed, then 200 μL DAPI staining WS was applied to cover the cells for nuclear staining, at 25°C for half an hour. The cells were rinsed, the excess liquid was removed by suction, the fluorescence mounting solution was added and the cells were observed and photographed under a C^{2+} confocal fluorescence microscope (Nikon, Japan). Five fields were randomly selected to count the number of ROS-positive cells, thrice to obtain the mean value.

Enzyme linked immunosorbent assay for level of OS index: The RGC-5 in log phase in a 6-well plate at 1.5×10^6/well, following cell adhesion, they were grouped and culture for 24 hrs. After centrifugation, the cells were collected and serum-free medium (5 mL) was added, culture for 12 hrs. The upper liquid was collected to determine the activity of SOD and the contents of malondialdehyde (MDA) and GSH-Px based on the guide of the ELISA kit (Shanghai Beyotime Biotechnology Co., Ltd., China).

Western blotting: The RGC-5 in log phase in a 24-well plate at 2×10^5/well, following the cells adhered to the wall, they were grouped, culture for 24 hrs and the original medium was discarded. The cells were lysed with RIPA lysate and the protein concentration was detected by the BCA method. A total of 30 μg of protein was taken as a sample and the protein was separated by polyacrylamide gel electrophoresis with DYCP-31DN gel electrophoresis instrument (Beijing Liuyi Biotechnology Co., Ltd., China). Proteins to the PVDF membrane were blocked with 5% skim milk powder for 1 hr. Then, first antibodies (Abcam, UK) against p53, AMPK, mTOR,

LC3-I, LC3-II and β-actin diluted 1:1000 were added and incubated overnight at 4°C. The membranes were rinsed three times with PB for 10 min each time, the addition of horseradish peroxidase-labeled IgG secondary antibody (Abcam, UK) diluted 1:2000, incubated for 1 hr at 25°C. The membranes were rinsed and development with chemiluminescence solution. Under WD-9413C Gel Imager (Beijing Liuyi Biotechnology Co., Ltd., China), ImageJ software was used for quantitative analysis of protein bands and the relative expression (RE) of each target was computed using β-actin as a reference.

Statistical analysis: All data were presented as Mean±SD. One-way ANOVA test and LSD-t method were adopted. The $p<0.05$ was considered statistically meaningful. The SPSS 22.0 software was employed.

RESULTS

Determination of Lico A: In this study, Lico A was first isolated from licorice residue and the extract was identified by HPLC. It was found that Lico A separated and purified from licorice residue had the same high characteristic peak as Lico A standard (Fig. 1).

***In vitro* antioxidant activity of Lico A:** This study used vitamin C (Vc) as the control to detect DPPH (Fig. 2a), ABTS (Fig. 2b), O_2^- (Fig. 2c) and H_2O_2 (Fig. 2d) CR *in vitro* at different concentrations of Lico A to evaluate its antioxidant activity *in vitro*. With the increase of Lico A concentration, its scavenging of DPPH, ABTS, O_2^- and H_2O_2 also increased and higher than the Vc control (Fig. 2).

Lico A promoting the proliferative activity of RGC-5 after H_2O_2 injury: In this study, the oxidative damage model of RGC induced by H_2O_2 was established. As against the Ctrl, the proliferation activity of RGC-5 in the Mod was visibly decreased ($p<0.05$). Secondly, when the cells were treated with Lico A, it was found that as against the Mod, the LG, the MG and the HG had visibly increased RGC-5 proliferation activity ($p<0.05$) and suggested concentration-dependent characteristics (Fig. 3).

Lico A inhibition of apoptosis of RGC-5 after H_2O_2 injury: As shown in Fig. 4, as against the Ctrl, the apoptosis rate of RGC-5 in the Mod was visibly raised ($p<0.05$). As against the Mod, the apoptosis rate of RGC-5 in the LG, MG and HG was visibly decreased ($p<0.05$) and suggested a concentration dependent characteristic (Fig. 4a is a flow chart of apoptosis rate detection and Fig. 4b is statistics of apoptosis rate).

Lico A improving the OS injury of RGC-5 after H_2O_2 injury: According to Fig. 5a, ROS staining is red; Fig. 5b is ROS positive stained cells number. As against the Ctrl, the number of ROS-positive cells in Mod was visibly raised ($p<0.05$). As against the Mod, the number was markedly reduced in the LG, MG and HG ($p<0.05$) and suggested a concentration dependent feature (Fig. 5).

As against Ctrl, SOD (Fig. 6a) was significantly reduced and MDA (Fig. 6b) was significantly increased and GSH-Px (Fig. 6c) was also significantly reduced in Mod group ($p<0.05$); As against the Mod, the avidity of the LG, MG and HG had a marked raise and the degree of MDA was markedly decreased (all $p<0.05$), showing concentration-dependent characteristics (Fig. 6).

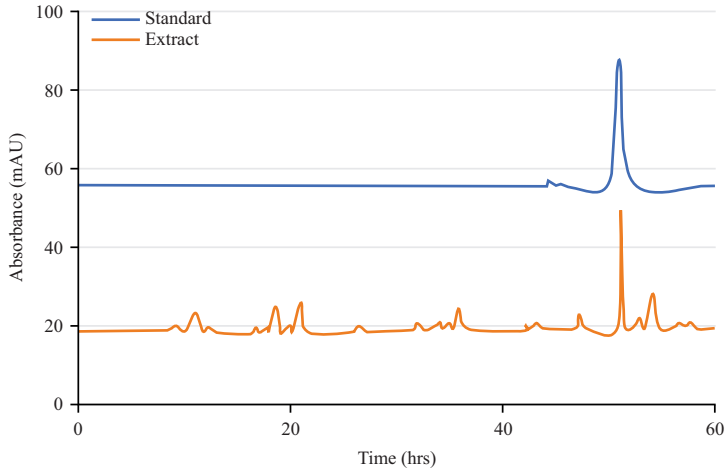

Fig. 1: HPLC profile of Lico A

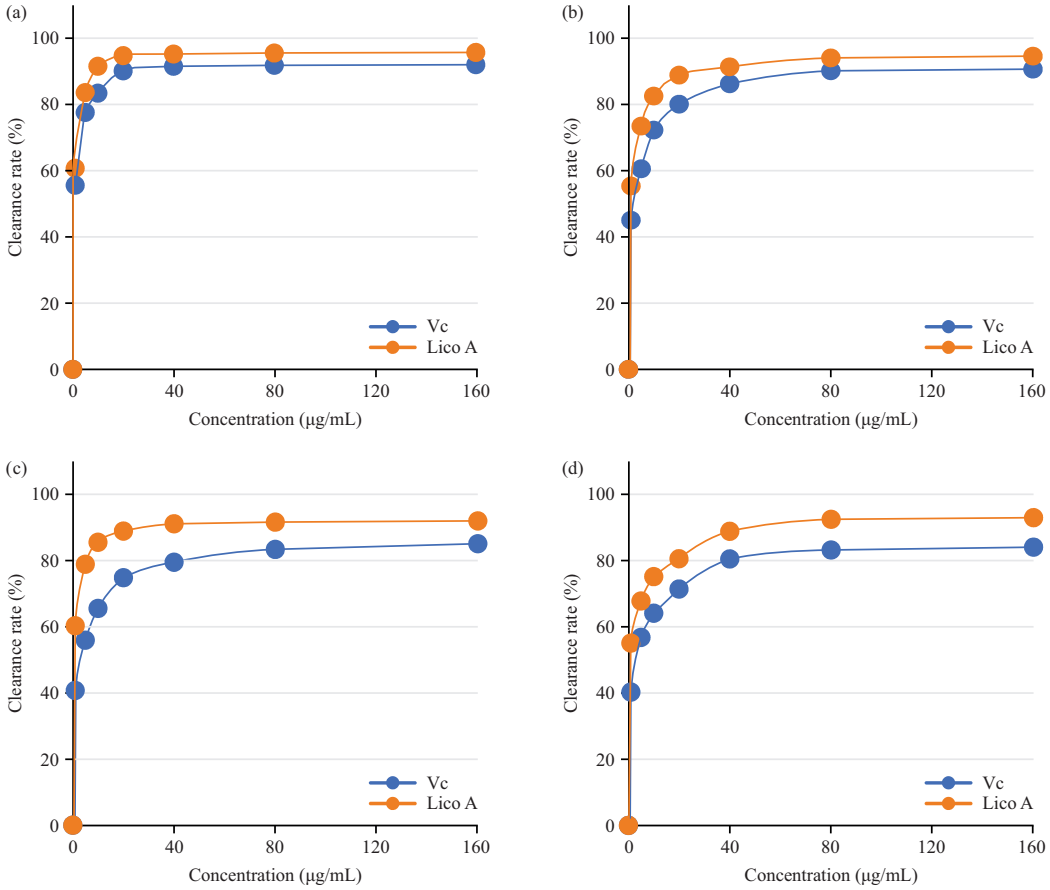

Fig. 2: Curve of Lico A concentration-FR CR, (a) DPPH CR, (b) ABTS CR, (c) O_2^- CR and (d) H_2O_2 CR

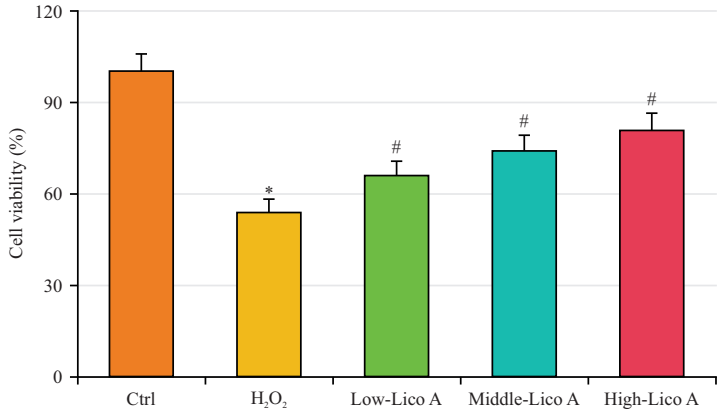

Fig. 3: RGC-5 proliferative activity

*As against the Ctrl, #As against the Mod and all p<0.05

Lico A suppression of the activation of the p53/AMPK/mTOR pathway in RGC-5 after H_2O_2 injury: According to Fig. 7a, the expression levels of different proteins in different groups are different. As against the Ctrl, p53 (Fig. 7b), AMPK (Fig. 7c) and LC3II/LC3I (Fig. 7e) in RGC-5 of the Mod had a marked raise and mTOR (Fig. 7d) had a marked decrease; As against the Mod, those in RGC-5 in the LG, MG and HG were markedly decreased and mTOR was markedly raised (all p<0.05), suggesting a concentration-dependent characteristic (Fig. 7).

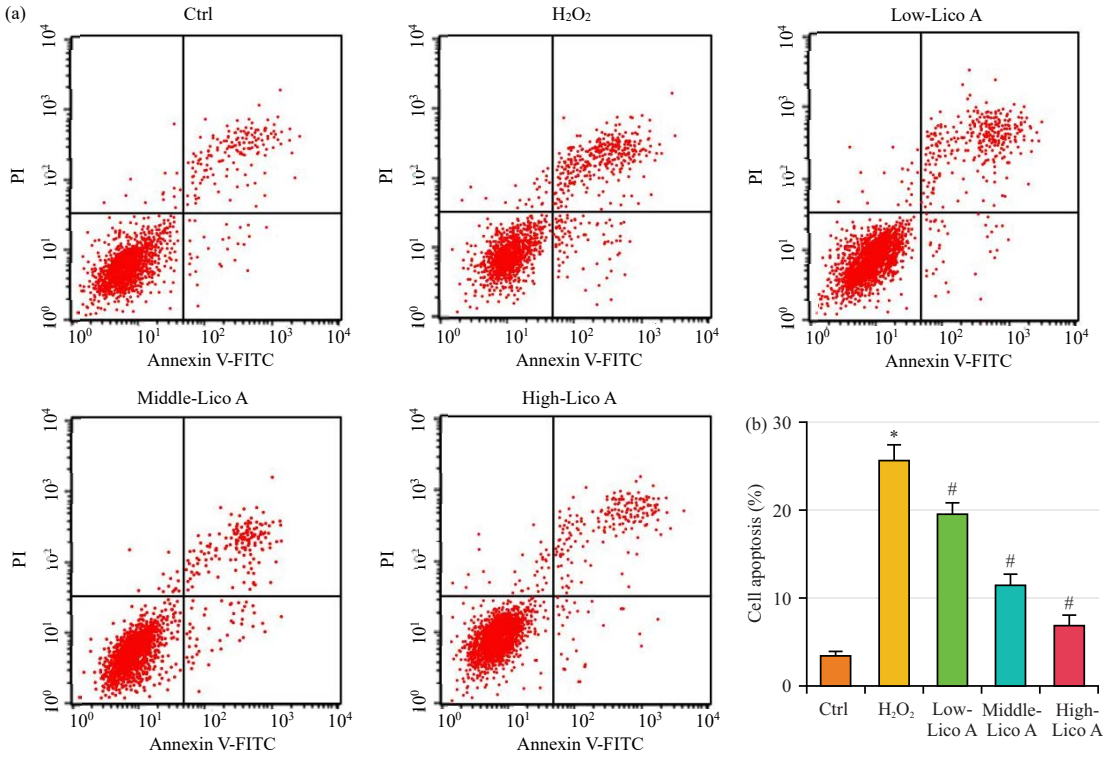

Fig. 4(a-b): Detection and comparison of apoptosis rate of RGC-5, (a) Flow cytometry and (b) Contrast of AR
*As against the Ctrl, p<0.05 and #As against the Mod, p<0.05

DISCUSSION

This study explores the mechanism by which Licochalcone A (Lico A) regulates oxidative damage of Retinal Ganglion Cells (RGCs) in glaucoma through the p53/AMPK/mTOR pathway. Experimental extraction from licorice residue successfully isolated Lico A, confirmed by HPLC detection showing high consistency with the characteristic peak of Lico A standard. The Lico A exhibits various biological activities including antibacterial, anti-inflammatory and antioxidant effects. Increasing concentrations of Lico A enhance its ability to scavenge DPPH, ABTS, O_2^- and H_2O_2 radicals, indicating potent antioxidant capabilities.

In this study, Lico A was extracted and separated from licorice residue and the HPLC detection showed that it was highly consistent with the characteristic peak of Lico A standard. It indicated that Lico A was successfully isolated from licorice residue. The Lico A exhibits various biological activities, including antibacterial, anti-inflammatory and antioxidant effects[13-15]. With the increase in Lico A concentration, its ability to scavenge DPPH, ABTS, O_2^- and H_2O_2 also increases, indicating that Lico A has strong antioxidant capabilities. Numerous studies have confirmed the significant antioxidant activity of Lico A[16-18] and the results of this study were consistent with those findings. The Lico A not only effectively neutralizes and eliminates various types of free radicals such as DPPH, ABTS, O_2^- and H_2O_2, but also captures and neutralizes free radicals, reducing damage to cells and tissues[19]. Additionally, Lico A can enhance the activity of intracellular antioxidant enzymes, thereby eliminating generated free radicals. Other studies have pointed out that Lico A can reduce cells' sensitivity to oxidative stress by regulating redox status and cellular signaling pathways, thus protecting cells from damage[20].

Loss of Retinal Ganglion Cells (RGCs) is a major pathological feature of glaucoma[21,22]. Effective inhibition of RGC apoptosis and enhancement of survival are crucial for delaying or preventing optic nerve damage progression. Treatment with different doses of Lico A significantly increases proliferation and reduces apoptosis rates in RGC-5 cells in a concentration-dependent manner, suggesting Lico A promotes RGC proliferation and inhibits apoptosis. Recent studies have indicated that Lico A inhibits proliferation and promotes apoptosis of glioma cells, with potential implications for cancer therapy[23], which aligned with findings in this study. The Lico A has also been shown to promote

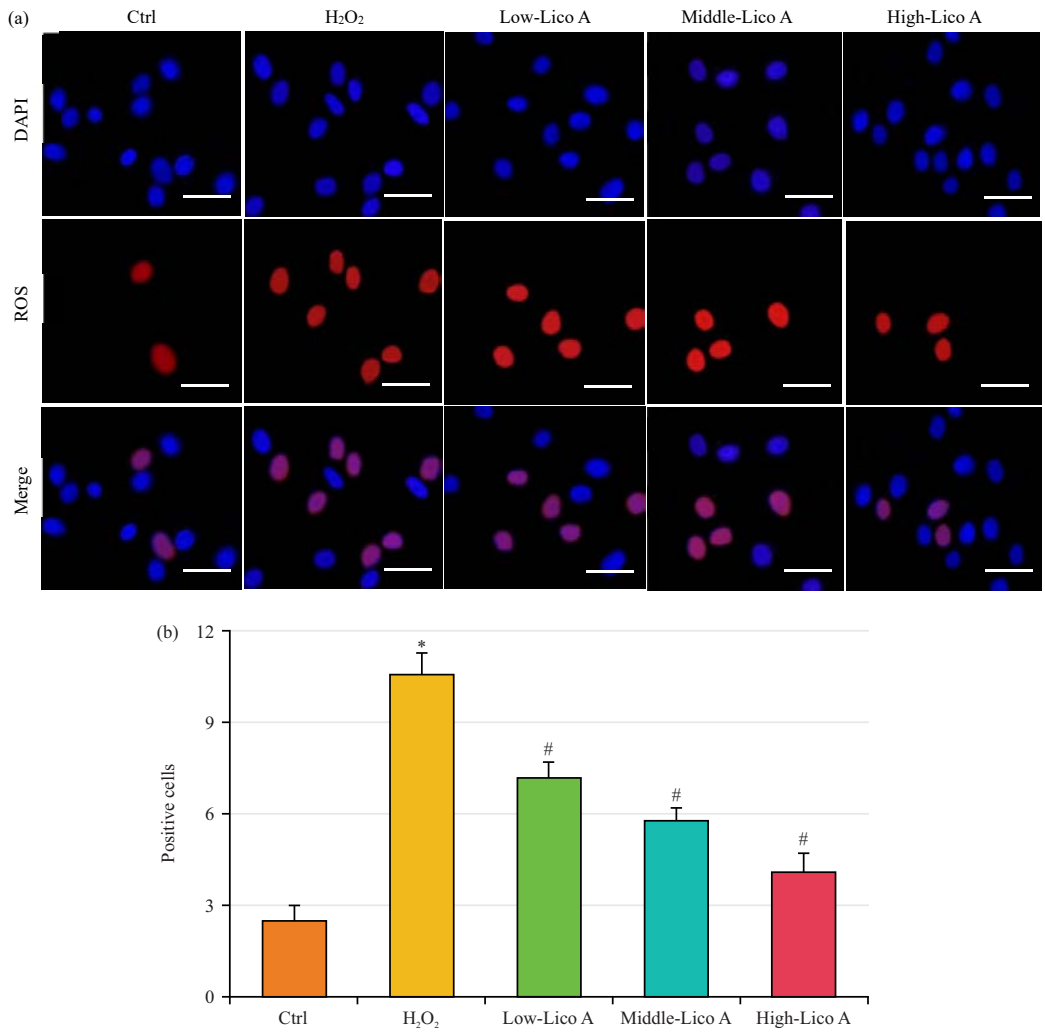

Fig. 5(a-b): ROS immunofluorescence staining of RGC-5, (a) Cellular immunofluorescence staining, ×400 and (b) Contrast of the number of ROS positive cells

*As against the Ctrl, $p<0.05$ and #As against the Mod, $p<0.05$

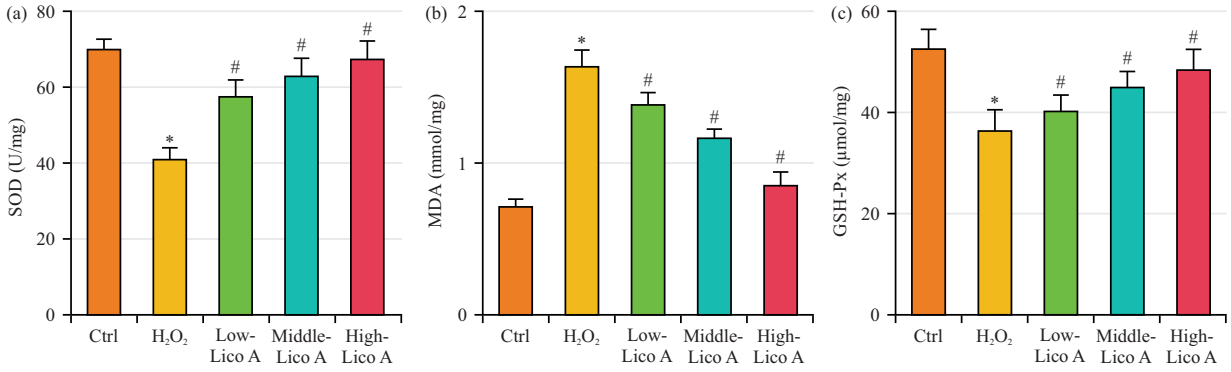

Fig. 6(a-c): Detection of OS indicators in RGC-5 supernatant, (a) Contrast of SOD activity, (b) Contrast of MDA content and (c) contrast of GSH-Px content

*As against the Ctrl, $p<0.05$ and #As against the Mod, $p<0.05$

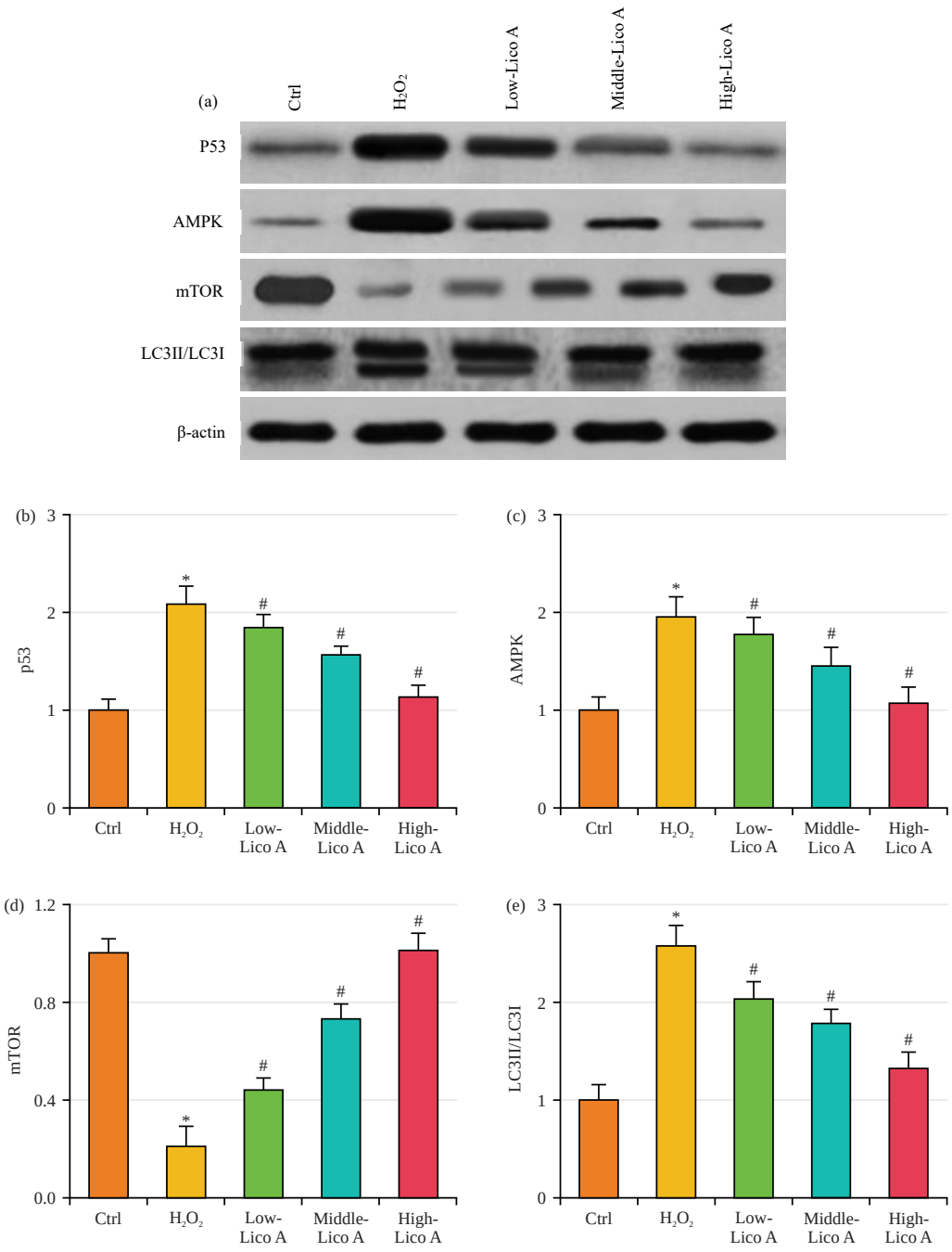

Fig. 7(a-e): RGC-5, p53/AMPK/mTOR pathway protein detection, (a) WB result, (b) RE of p53, (c) RE of AMPK, (d) RE of mTOR and (e) RE of LC3II/LCI

*As against the Ctrl, $p<0.05$ and #As against the Mod, $p<0.05$

cancer cell death and apoptosis through mechanisms such as reducing DNA synthesis and activating the FasL signaling pathway[24,25].

The ROS is one of the compounds causing oxidative damage and raised ROS levels can induce the death of RGC[26]. Effective removal of ROS in RGC *in vivo* and *in vitro* can visibly improve the symptoms of acute pathological glaucoma injury models[27]. In the study, it found that after treatment with different doses of Lico A, the number of ROS-positive stained cells in H_2O_2-induced RGC-5 cells significantly decreased, while SOD and GSH-Px activities increased and MDA content decreased, all in a concentration-dependent manner. This

indicates that Lico A plays an important role in regulating oxidative stress by enhancing the activity of SOD and GSH-Px antioxidant enzymes, reducing ROS generation and lowering the accumulation of oxidative products (MDA). Wu *et al*.[28] found that Lico A could target thioredoxin reductase 1 to enhance ROS generation and lead to apoptosis of colon cancer cells. Kühnl *et al*.[29] found that Lico A could induce Nrf2 nuclear translocation in human fibroblasts, increase GSH-Px expression in cells and reduce ROS concentration. The Lico A can regulate ROS production, which can suppress the ROS positive expression rate in RGC induced by H_2O_2. Huang *et al*.[30] found that Lico A could reduce inflammation in inflammatory bronchial epithelial cells, reduce MDA level and increase GSH-Px level. Bhatia *et al*.[31] found that Lico A could play an anti-inflammatory and anti-oxidative effect on microglia induced by lipopolysaccharide by suppressing the arachidonic acid/cyclooxygenase pathway. The Lico A could regulate the production of antioxidant enzymes and oxidative products in H_2O_2-induced RGC, thereby playing a role in regulating OS. The current research on Lico A in glaucoma treatment is limited, but the results mentioned in the article suggest that Lico A has potential application value in treating glaucoma, especially its potential to protect RGCs from oxidative damage.

Autophagy of RGC is closely correlated with the occurrence and development of glaucoma[32]. In the process of RGC damage and apoptosis, the level of autophagy is markedly raised[33]. Studies have found that in RGC-5 cells treated with different doses of Lico A, the expression levels of p53, AMPK and LC3II/LC3I proteins significantly increase, while the level of mTOR protein significantly decreases, showing a dose-dependent characteristic. Shen *et al*.[34] found that Lico A could suppress the proliferation activity of osteosarcoma cells and promote their apoptosis by inducing cell autophagy. Xue *et al*.[35] found that Lico A could activate the LC3-II pathway, suppress the activation of PI3K/Akt/mTOR pathway and promote autophagy and apoptosis of breast cancer cells. The results in the article indicate that Lico A can regulate the expression levels of genes and proteins related to the p53/AMPK/mTOR signaling pathway, thereby influencing RGC cell apoptosis. This provides a theoretical basis for studying the protective mechanisms of Lico A in treating damage to retinal ganglion cells in glaucoma.

CONCLUSION

The Lico A has a strong antioxidant activity *in vitro* and it can promote the proliferation of H_2O_2-induced RGC, suppress apoptosis and OS and suppress cell autophagy through p53/AMPK/mTOR signaling pathway. The Lico A plays a protective role in the oxidative damage of RGC in glaucoma. However, in the future, glaucoma animal models should be prepared to further explore the therapeutic effect of Lico A on glaucoma.

SIGNIFICANCE STATEMENT

The purpose of this study was to explore the mechanism of Licochalcone A inhibiting the progression of glaucoma and to find a new therapeutic target for glaucoma. It was found that Licochalcone A has a strong free radical scavenging effect *in vitro* and can inhibit hydrogen peroxide-induced apoptosis of retinal ganglion cells in glaucoma by regulating p53/AMPK/mTOR signaling pathway and affect the autophagy state and oxidative stress damage level of cells. This finding provides new drug candidates for the treatment of glaucoma. In the future, with the in-depth study of the mechanism of action and the optimization of extraction and purification technology, Licochalcone A has a broad application prospect in the field of ophthalmic drugs and is expected to become an important drug ingredient in treating glaucoma and other eye diseases.

REFERENCES

1. Schuster, A.K., C. Erb, E.M. Hoffmann, T. Dietlein and N. Pfeiffer. 2020. The diagnosis and treatment of glaucoma. Deutsches Ärzteblatt Int., 117: 225-234.
2. Adornetto, A., L. Rombolà, L.A. Morrone, C. Nucci, M.T. Corasaniti, G. Bagetta and R. Russo, 2020. Natural products: Evidence for neuroprotection to be exploited in glaucoma. Nutrients, Vol. 12. 10.3390/nu12103158.
3. Catalani, E., K. Brunetti, S.D. Quondam and D. Cervia, 2023. Targeting mitochondrial dysfunction and oxidative stress to prevent the neurodegeneration of retinal ganglion cells. Antioxidants, Vol. 12. 10.3390/antiox12112011.
4. Miao, Y., G.L. Zhao, S. Cheng, Z. Wang and X.L. Yang, 2023. Activation of retinal glial cells contributes to the degeneration of ganglion cells in experimental glaucoma. Prog. Retinal Eye Res., Vol. 93. 10.1016/j.preteyeres.2023.101169.
5. Ding, Y., E. Brand, W. Wang and Z. Zhao, 2022. Licorice: Resources, applications in ancient and modern times. J. Ethnopharmacol., Vol. 298. 10.1016/j.jep.2022.115594.
6. Olloquequi, J., M. Ettcheto, A. Cano, A. Fortuna and J. Bicker *et al*., 2023. Licochalcone A: A potential multitarget drug for Alzheimer's disease treatment. Int. J. Mol. Sci., Vol. 24. 10.3390/ijms241814177.
7. Qiu, J., H. Feng, H. Xiang, D. Wang and L. Xia *et al*., 2010. Influence of subinhibitory concentrations of licochalcone A on the secretion of enterotoxins A and B by *Staphylococcus aureus*. FEMS Microbiol. Lett., 307: 135-141.

8. Shen, F., X. Tang, Y. Wang, Z. Yang and X. Shi *et al*., 2015. Phenotype and expression profile analysis of *Staphylococcus aureus* biofilms and planktonic cells in response to licochalcone A. Appl. Microbiol. Biotechnol., 99: 359-373.
9. Laphanuwat, P., S. Kongpetch, L. Senggunprai, A. Prawan and V. Kukongviriyapan, 2022. Licochalcone A induces cholangiocarcinoma cell death via suppression of Nrf2 and NF-κB signaling pathways. Asian Pac. J. Cancer Prev., 23: 115-123.
10. Hou, X., S. Yang and Y. Zheng, 2019. Licochalcone A attenuates abdominal aortic aneurysm induced by angiotensin II via regulating the miR-181b/SIRT1/HO-1 signaling. J. Cell. Physiol., 234: 7560-7568.
11. Tian, M., N. Li, R. Liu, K. Li, J. Du, D. Zou and Y. Ma, 2022. The protective effect of licochalcone A against inflammation injury of primary dairy cow claw dermal cells induced by lipopolysaccharide. Sci. Rep., Vol. 12. 10.1038/s41598-022-05653-6.
12. Liu, X., Y. Ma, X. Wei and T. Fan, 2018. Neuroprotective effect of licochalcone A against oxygen-glucose deprivation/reperfusion in rat primary cortical neurons by attenuating oxidative stress injury and inflammatory response via the SIRT1/Nrf2 pathway. J. Cell. Biochem., 119: 3210-3219.
13. Wahab, S., S. Annadurai, S.S. Abullais, G. Das and W. Ahmad *et al*., 2021. *Glycyrrhiza glabra* (Licorice): A comprehensive review on its phytochemistry, biological activities, clinical evidence and toxicology. Plants, Vol. 10. 10.3390/plants10122751.
14. Authier, H., V. Bardot, L. Berthomier, B. Bertrand, C. Blondeau, S. Holowacz and A. Coste, 2022. Synergistic effects of licorice root and walnut leaf extracts on gastrointestinal candidiasis, inflammation and gut microbiota composition in mice. Microbiol. Spectr., Vol. 10. 10.1128/spectrum.02355-21.
15. Tibenda, J.J., Y. Du, S. Huang, G. Chen and N. Ning *et al*., 2023. Pharmacological mechanisms and adjuvant properties of licorice glycyrrhiza in treating gastric cancer. Molecules, Vol. 28. 10.3390/molecules28196966.
16. Liang, M., X. Li, X. Ouyang, H. Xie and D. Chen, 2019. Antioxidant mechanisms of echinatin and licochalcone A. Molecules, Vol. 24. 10.3390/molecules24010003.
17. Lv, H., Q. Xiao, J. Zhou, H. Feng, G. Liu and X. Ci, 2018. Licochalcone A upregulates Nrf2 antioxidant pathway and thereby alleviates acetaminophen-induced hepatotoxicity. Front. Pharmacol., Vol. 9. 10.3389/fphar.2018.00147.
18. Wu, Y., H. Wang, J. Zhu, H. Shen and H. Liu, 2021. Licochalcone A activation of glycolysis pathway has an anti-aging effect on human adipose stem cells. Aging, 13: 25180-25194.
19. Xu, K.D., Y. Miao, P. Li, P.P. Li, J. Liu, J. Li and F. Cao, 2022. Licochalcone A inhibits cell growth through the downregulation of the Hippo pathway via PES1 in cholangiocarcinoma cells. Environ. Toxicol., 37: 564-573.
20. Sahoo, B.M., B.K. Banik, P. Borah and A. Jain, 2022. Reactive oxygen species (ROS): Key components in cancer therapies. Anti-Cancer Agents Med. Chem., 22: 215-222.
21. Yadav, K.S., S. Sharma and V.Y. Londhe, 2020. Bio-tactics for neuroprotection of retinal ganglion cells in the treatment of glaucoma. Life Sci., Vol. 243. 10.1016/j.lfs.2020.117303.
22. Zhao, W.J., C.L. Fan, X.M. Hu, X.X. Ban and H. Wan *et al*., 2023. Regulated cell death of retinal ganglion cells in glaucoma: Molecular insights and therapeutic potentials. Cell. Mol. Neurobiol., 43: 3161-3178.
23. Mu, Y., J. Dong, H. Cui, J. Hu, J. Liang and L. Yan, 2022. Effect of Licochalcone-A combined with *Rab23* gene on proliferation of glioma U251 cells. Evidence-Based Complementary Altern. Med., Vol. 2022. 10.1155/2022/9299442.
24. Lu, W.J., G.J. Wu, R.J. Chen, C.C. Chang and L.M. Lien *et al*., 2018. Licochalcone A attenuates glioma cell growth *in vitro* and *in vivo* through cell cycle arrest. Food Funct., 9: 4500-4507.
25. Tseng, T.Y., C.H. Lee, H.L. Lee, C.Y. Su, C.Y. Kao, J.P. Tsai and Y.H. Hsieh, 2023. Licochalcone A suppresses renal cancer cell proliferation and metastasis by engagement of Sp1-mediated LC3 expression. Pharmaceutics, Vol. 15. 10.3390/pharmaceutics15020684.
26. Zhang, J.X., Y. Xiao, Y.Q. Li, Y.L. Zhu and Y.R. Li *et al*., 2023. Licochalcone A induces ferroptosis in hepatocellular carcinoma via reactive oxygen species activated by the SLC7A11/GPX4 pathway. Integr. Cancer Ther., Vol. 22. 10.1177/15347354231210867.
27. Rong, R., X. Zhou, G. Liang, H. Li and M. You *et al*., 2022. Targeting cell membranes, depleting ROS by dithiane and thioketal-containing polymers with pendant cholesterols delivering necrostatin-1 for glaucoma treatment. ACS Nano, 16: 21225-21239.
28. Wu, P., T. Yu, J. Wu and J. Chen, 2020. Licochalcone A induces ROS-mediated apoptosis through TrxR1 inactivation in colorectal cancer cells. BioMed Res. Int., Vol. 2020. 10.1155/2020/5875074.
29. Kühnl, J., D. Roggenkamp, S.A. Gehrke, F. Stäb, H. Wenck, L. Kolbe and G. Neufang, 2015. Licochalcone A activates Nrf2 *in vitro* and contributes to licorice extract induced lowered cutaneous oxidative stress *in vivo*. Exp. Dermatol., 24: 42-47.
30. Huang, W.C., C.Y. Liu, S.C. Shen, L.C. Chen, K.W. Yeh, S.H. Liu and C.J. Liou, 2019. Protective effects of licochalcone A improve airway hyper-responsiveness and oxidative stress in a mouse model of asthma. Cells, Vol. 8. 10.3390/cells8060617.

31. Bhatia, H.S., M. Apweiler, L. Sun, J. Baron, A. Tirkey and B.L. Fiebich, 2023. Licochalcone A inhibits prostaglandin E_2 by targeting the MAPK pathway in LPS activated primary microglia. Molecules, Vol. 28. 10.3390/molecules28041927.
32. Obanina, N.A., N.P. Bgatova, A.V. Eremina, A.N. Trunov and V.V. Chernykh, 2022. Autophagy in human retinal neurons in glaucoma. Bull. Exp. Biol. Med., 173: 468-474.
33. Mathew, B., M. Chennakesavalu, M. Sharma, L.A. Torres and C.R. Stelman *et al*., 2021. Autophagy and post-ischemic conditioning in retinal ischemia. Autophagy, 17: 1479-1499.
34. Shen, T.S., Y.K. Hsu, Y.F. Huang, H.Y. Chen, C.P. Hsieh and C.L. Chen, 2019. Licochalcone A suppresses the proliferation of osteosarcoma cells through autophagy and ATM-Chk2 activation. Molecules, Vol. 24. 10.3390/molecules24132435.
35. Xue, L., W.J. Zhang, Q.X. Fan and L.X. Wang, 2018. Licochalcone A inhibits PI3K/Akt/mTOR signaling pathway activation and promotes autophagy in breast cancer cells. Oncol. Lett., 15: 1869-1873.