• Basic Research •

Effect of nano-selenium loaded with lycium barbarum polysaccharide on the proliferation of lens epithelial cells after UVB damage *in vitro*

Jing-Xiang Zhong, Shan-Shan Jin, Kang-Sheng Wu, Guo-Cheng Yu, Lei-Lei Tu, Lian Liu

Department of Ophthalmology, the First Affiliated Hospital of Jinan University, Guangzhou 510630, Guangdong Province, China

Correspondence to: Guo-Cheng Yu, Lei-Lei Tu, and Lian Liu. Department of Ophthalmology, the First Affiliated Hospital of Jinan University, 613 Huangpu Road, Guangzhou 510630, Guangdong Province, China. 260949346@qq.com; tuleileipat@foxmail.com; liulianbb@163.com

Received: 2021-03-25 Accepted: 2021-08-18

Abstract

● **AIM:** To investigate the effect of nano-selenium loaded with different concentrations of lycium barbarum polysaccharide (LBP-SeNPs) on the proliferation of human lens epithelial cells (HLECs) from UV irradiation.

● **METHODS:** LBP-SeNPs were prepared and their particle size was detected. HLECs (SRA01/04) were irradiated with UVB for different time (0, 10, 20, 30, 40, 50, 60min) to construct a damaged model, the survival rate of cells was determined by methylthiazol tetrazolium (MTT) assay. The 4',6-Diamidine-2'-phenylindole dihydrochloride (DAPI) staining was used to observe the status of cell nucleus and drug entering cytoplasm through cell membrane in SRA01/04 cells after adding LBP-SENPS loaded with coumarin fluorescence agent 24h under fluorescence microscope. SRA01/04 normal and UVB-damaged cells were treated with different amounts of LBP-SeNPs at different concentrations, cells proliferation were observed.

● **RESULTS:** The particle size of LBP-SeNPs was stable in the range of 150-200 nm. The survival rate changes with time after UVB irradiation were statistically significant. The 10min of UVB exposure as the time was chosen to construct the cell damage model. With DAPI staining, LBP-SeNPs were observed to enter the cytoplasm through the cell membrane under fluorescence inverted microscope. Cytotoxicity of SRA01/04 at different concentrations of LBP-SeNPs were measured. Cell survival rate was statistically different compared with the control group. The higher the loading concentration of LBP in nano-Se drugs was, the higher the cell proliferation rate was (P<0.05). The lower the concentration of LBP-SeNPs, the higher the cell proliferation rate, showing a negative growth trend (P<0.05). The group with the highest average cell proliferation rate was 0.5 μmol/L 2.0 mg/mL LBP-SeNPs (128.80%). When the 2.0 mg/mL LBP-SeNPs group was selected for cell photography, the cell density was higher at 0.5 μmol/L. With the increase of concentration, SRA01/04 cells appeared more cytoplasm dehydration, cell shrinkage and apoptotic bodies, and cell density decreased.

● **CONCLUSION:** LBP-SeNPs has moderate particle size and good stability. LBP-SeNPs can protect HLECs (SRA01/04) from UVB-induced damage, and the cell proliferation rate is further increased with increasing the amount of loaded LBP and decreasing nano-selenium concentration.

● **KEYWORDS:** lycium barbarum polysaccharide; nano-selenium; human lens epithelial cells; ultraviolet irradiation

DOI:10.18240/ijo.2022.01.02

Citation: Zhong JX, Jin SS, Wu KS, Yu GC, Tu LL, Liu L. Effect of nano-selenium loaded with lycium barbarum polysaccharide on the proliferation of lens epithelial cells after UVB damage *in vitro*. *Int J Ophthalmol* 2022;15(1):9-14

INTRODUCTION

Lycium barbarum is a well-known herbal medicine with a variety of biological effects, including antioxidant and cytoprotective effects[1-3]. Lycium barbarum polysaccharide (LBP) is the main component of lycium barbarum responsible for these biological activities[4]. It is well documented that LBP can prevent the generation and accumulation of reactive oxygen species (ROS) and has protective effects against damage in a variety of cells and tissues through mitochondria-related pathways[5]. Studies had shown that UVB irradiation can lead to cataract formation[6-7]. Oxidative stress caused by ROS which produced by UVB irradiation to lens epithelial cells (LECs) is thought to be the main factor in the development of cataracts[8]. Previous studies in our group had shown that LBP had a protective effect against oxidative

damage in human lens epithelial cells (HLECs) and retinal ganglion cells[2-3]. Nano-selenium (SeNPs), as a unique nano-scale elemental selenium, have high efficacy on diseased tissues due to their excellent pharmacokinetics and selectivity, low toxicity to normal tissues as well as high penetration to cells. In this experiment, we investigated the effect of nano-selenium loaded with LBP (LBP-SeNPs) on the proliferation of HLECs after UVB irradiation (Figure 1).

MATERIALS AND METHODS

Construction and Particle Size Detection of LBP-SeNPs The 1 mL sodium selenite was added to the solution containing different concentrations of LBP (0.5, 1.0, 1.5, 2.0 mg/mL) at room temperature and pressure. After that, 1 mL reduced vitamin C solution and 2 mL water were added. The solution was stirred for 8h and dialysis for 12h, LBP-SeNPs solution was collected. The particle size and stability of LBP-SeNPs were determined by nanoparticle size analyzer. The LBP-SeNPs used in this experiment were prepared and tested by the Institute of Chemical Materials of Jinan University.

Cell Culture The SV40 T-antigen-transformed HLECs[9], SRA01/04, was obtained from the Cancer Institute of the Chinese Academy of Medical Sciences (Beijing, China). Cells were cultured in DMEM supplemented with 10% FBS, 100 U/mL penicillin, and 100 mg/mL streptomycin and incubated at 37°C in a 5% CO_2 incubator[10].

UVB-Induced HLECs' Oxidative Damage Model SRA01/04 suspensions were placed in 96-well cell culture plates at a concentration of approximately 5×10^4 cells/mL and grouped according to the UV irradiation time (0, 10, 20, 30, 40, 50, 60min), each group was replicated in 6 wells with a volume of 100 μL. After 24h of cell culture, the culture medium was sucked out and 20 μL phosphate buffered saline (PBS) was added to prevent cell apoptosis due to excessive dryness during irradiation. Cells were irradiated in an ultraviolet light box at a wavelength of 302 nm, and one group was uncovered every 10min until the end of 60min. After that, the cells continued to be cultured for 24h. Methylthiazol tetrazolium (MTT) was used to determine the survival rate of the cells.

Detection of Toxic Effects of Drugs SRA01/04 cells were cultured for 24h and then administered in groups: 1) Experimental group: SeNPs loaded with different concentrations of LBP (0.5, 1.0, 1.5, 2.0 mg/mL), the stock solution was diluted in a gradient so that the concentrations were 16, 8, 4, 2, 1, and 0.5 μmol/L, respectively, and three replicate wells were set up for each concentration of each group of drugs, with 100 μL of drug added to each well; 2) Control group: An equal amount of complete culture solution was added to each well; 3) Blank group: Instead of planting cytosol, the plates were planted with complete culture solution, with 2 replicate wells, and the remaining steps were the same as the control group. After drug administration, the cells were continued to be incubated for 24h to observe cell morphology and MTT to detect cell activity.

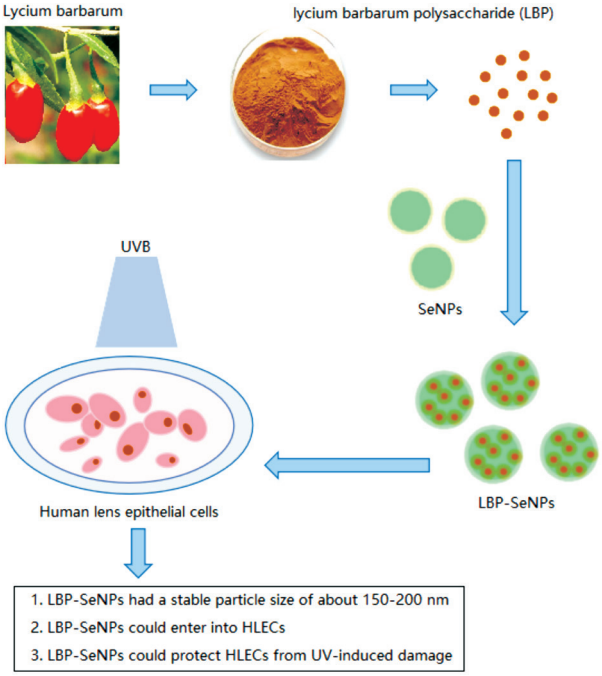

Figure 1 Schematic illustration of the preparation of LBP-SeNPs and application in HLECs model with UVB damaged.

Detection of Drug Protective Effects According to the above experimental steps, SRA01/04 cells were damaged by ultraviolet radiation for 10min. Different concentrations of LBP-SeNPs were administered immediately after that steps. Cell activity was detected by MTT after 24h incubation. The LBP-SeNPs group with the highest cell proliferation rate was photographed to observe the cell status.

Statistical Analysis Data were analyzed using the SPSS 18.0 (IBM, Armonk, NY, USA). Data were presented as the mean±SD. For comparison of the different groups, statistical comparisons were performed by one-way analysis of variance and Student's *t* test. Differences were considered significant at $P<0.05$.

RESULTS

Particle Size of LBP-SeNPs and Their Stability The particle sizes of the prepared LBP-SeNPs were examined using a nanoparticle size analyzer, and the average particle sizes corresponding to the LBP concentrations of 0.5, 1.0, 1.5, and 2.0 mg/mL are shown in Figure 2A, and the particle sizes of both loaded and unloaded LBP-SeNPs were in the range of 150-180 nm, and only the average particle size of 1.5 mg/mL LBP-SeNPs was less than 160 nm. As shown in Figure 2B, the particle size of LBP-SeNPs basically remained between 150-200 nm with time, indicating their stability.

Culture of SRA01/04 Cells SRA01/04 cells were observed under inverted microscope as shown in Figure 3. Most of the

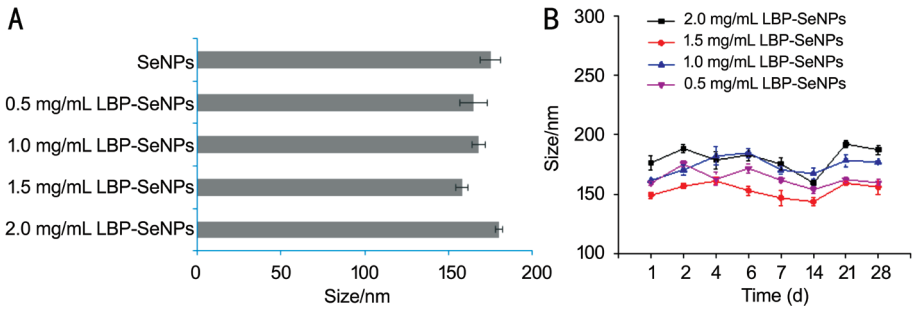

Figure 2 Particle size and stability of SeNPs and LBP-SeNPs A: Average particle size of SeNPs and LBP-SeNPs; B: Variation of particle size of LBP-SeNPs with preservation time.

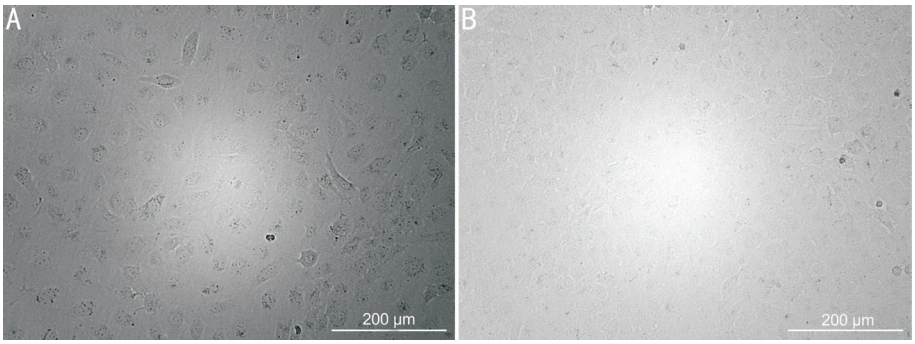

Figure 3 SRA01/04 cells under inverted microscope A: 24h after transmission; B: 48h after transmission.

cells could be walled and the cell density reached more than 80% within 24h to 48h after SRA01/04 cells were passaged. After adhering to the wall, the cells gradually extended from the unadhered round shape to the final cell shape of shuttle shape or polymorphism, with clear nucleus and transparent cytoplasm and good growth status.

UV Irradiation Damage Cell Model According to the literature, a UV lamp with a wavelength of 302 nm was placed on top of the incubator and irradiated SRA01/04 cells for 0, 10, 20, 30, 40, 50, and 60min. After that, cells were incubated for 24h. MTT test was implemented for cell viability assay. As shown in Figure 4, cell survival was significantly lower in all experimental groups compared with the control group (F=121.239, P<0.01). The cell survival rate showed a gradient downward trend between 10min and 50min groups. Cell survival rate decreased as irradiation time increased. There was no statistical significance between 50min group and 60min group (P=0.430). According to this result, we chose the cell survival rate around 80% (10min group) as the UV irradiation time for the next step of the experiment.

DAPI Staining of Cells SRA01/04 cells were treated with coumarin-loaded LBP-SeNPs for 24h and then stained with DAPI. As shown in Figure 5, nucleus with blue fluorescence in combination with dye can be seen under fluorescence microscope. The LBP-SeNPs loaded with coumarin fluorescent agent showed green fluorescent spots in the cytoplasm. This indicated that LBP-SeNPs had entered the cells in large quantities during this period.

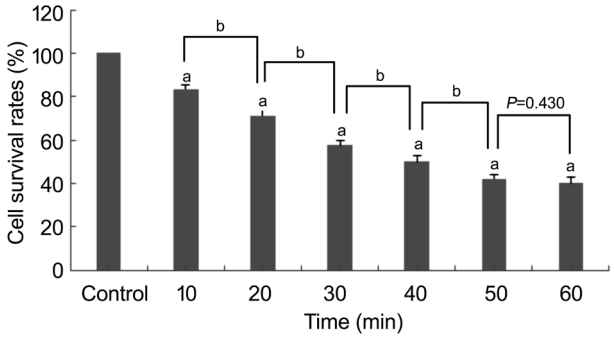

Figure 4 Cell survival rate under different time of UV irradiation [a]P<0.01 *vs* the control, [b]P<0.05.

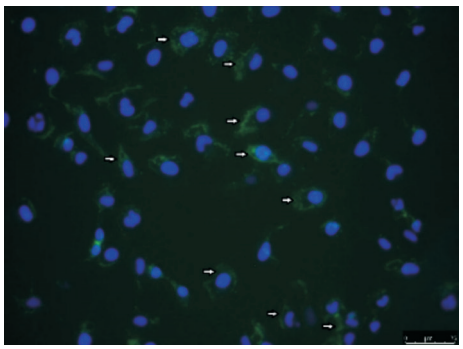

Figure 5 SRA01/04 cells treated with coumarin-loaded LBP-SeNPs after DAPI staining under fluorescence microscope.

Cytotoxicity Screening Six gradient concentrations (0.5, 1.0, 2.0, 4.0, 8.0, and 16.0 μmol/L) of four concentrations LBP-SeNPs (0.5, 1.0, 1.5, 2.0 mg/mL) were selected to interact with SRA01/04. Cell survival rates were detected by MTT (Table 1).

Table 1 Survival rate of SRA01/04 cells under different drug concentration gradients %

Drug	Concentrations (μmol/L)					
	0.5	1.0	2.0	4.0	8.0	16.0
0.5 mg/mL LBP-SeNPs	104.82	98.96	96.77	100.15	89.53	89.53
1.0 mg/mL LBP-SeNPs	96.42	94.77	92.87	91.61	87.65	82.65
1.5 mg/mL LBP-SeNPs	91.57	87.79	86.14	71.95	69.54	67.50
2.0 mg/mL LBP-SeNPs	98.07	87.79	86.14	71.95	69.54	67.50

The cell survival rates of the above experimental groups were lower than the control group (P<0.05) and decreased with increasing drug concentration (P<0.05; Figure 6). Based on this, we selected 0.5, 1.0, 2.0, and 4.0 μmol/L as the relatively safe drug concentrations for the next experimental step.

Drug Action Screening According to the previous steps, four gradient concentrations (0.5, 1.0, 2.0, 4.0 μmol/L) of four concentrations LBP-SeNPs (0.5, 1.0, 1.5, 2.0 mg/mL) were selected to interact with SRA01/04 damaged by UVB light. Cell proliferation rates were detected by MTT (Table 2; Figure 7). The cell proliferation rate of all the above experimental groups were greater than that of the control group (P<0.05), indicating its cytoprotective effect.

According to the results, after damaged with UV, SRA01/04 cells were incubated with 2.0 mg/mL LBP-SeNPs and photographed 24h later. The 2.0 mg/mL LBP-SeNPs showed a higher cell density at 0.5 μmol/L. As the concentration increased, cytoplasmic dehydration, cell crumbling and apoptotic vesicles appeared more, SRA01/04 cells density decreased (Figure 8).

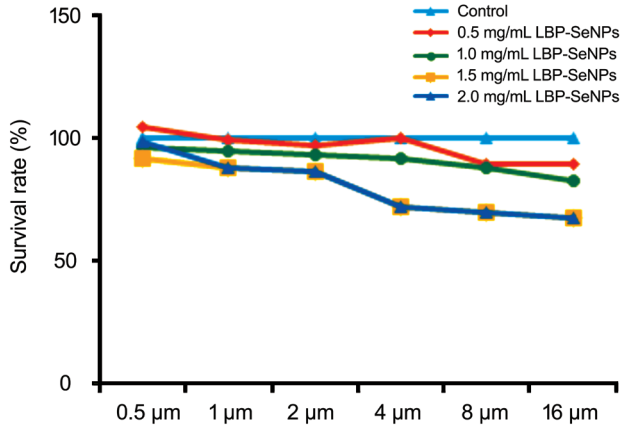

Figure 6 Cell survival rate of SRA01/04 after 24h effect of different concentrations of LBP-SeNPs.

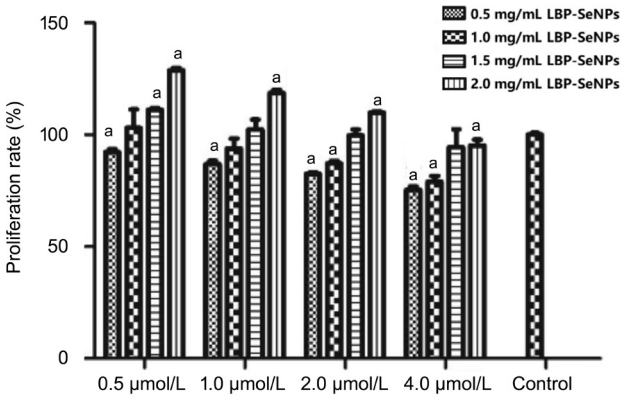

Figure 7 Proliferation rate of cells after 24h effect of different concentrations of LBP-SeNPs on UV-damaged cells ^{a}P<0.05 *vs* control group.

Table 2 Proliferation rate of on UV-damaged SRA01/04 cells under different drug concentration gradients %

Drug	Concentrations (μmol/L)			
	0.5	1.0	2.0	4.0
0.5 mg/mL LBP-SeNPs	91.75	88.45	82.23	74.78
1.0 mg/mL LBP-SeNPs	102.84	95.17	87.84	78.97
1.5 mg/mL LBP-SeNPs	111.17	101.12	99.43	93.86
2.0 mg/mL LBP-SeNPs	128.80	118.71	109.98	95.21

DISCUSSION

Studies had confirmed that LBP, as a bioactive compound, had good antioxidant properties[11-13] and similar effects on eye diseases. Published studies had associated LBP intake with a number of therapeutic effects, including antiaging, metabolic effects, neuroprotective effects in neurodegeneration and neurotoxicity[3,14-18]. Du *et al*[19] established an age-related macular degeneration model using blue light-induced oxidative stress damage in human retinal pigment epithelial cells, and found LBP could reduce cellular oxidative stress damage by enhancing mitochondrial membrane potential through LBP pretreatment. The latest LBP chemical component analysis demonstrated all glycopeptides in LBP act to eliminate lipid peroxidation[20-22]. Thus, LBP might decrease cell injury induced by oxygen free radicals and protect cell development and differentiation as an antioxidant.

Oxidative stress induced lens epithelial cell apoptosis plays an important role in cataract formation. It is very important to reduce the apoptosis of lens epithelial cells to control the occurrence and development of cataract. There were some studies on the use of selenium or SeNPs for the treatment of age-related cataracts. Zhu and Lu[23] believed selenium supplementation could slow the development of naphthalene cataract possibly by attenuating the oxidative stress in the lens. Post *et al*[24] found low serum selenium levels may constitute a potential risk factor of age-related cataract.

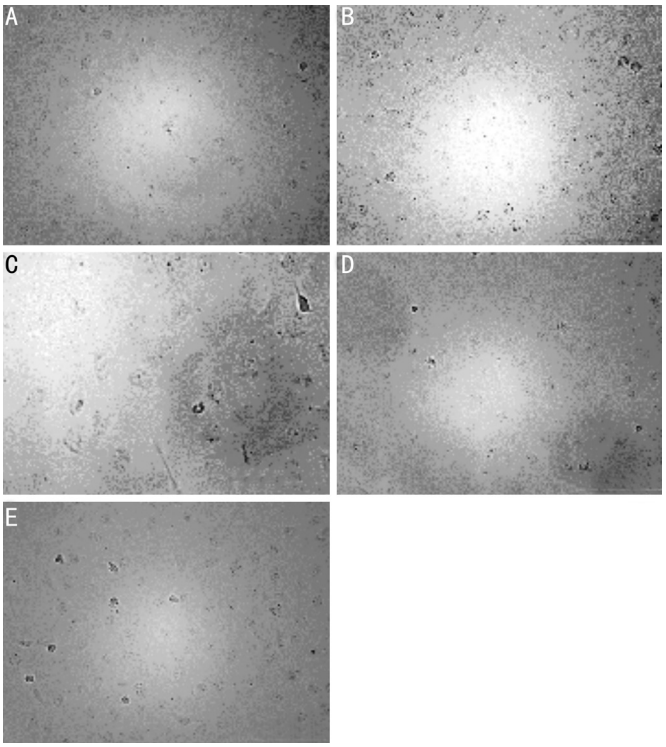

Figure 8 SRA01/04 cells treated with different concentrations of LBP-SeNPs 24h after UV-induced cell damage A: Control; B: 0.5 μmol/L; C: 1.0 μmol/L; D: 2.0 μmol/L; E: 4.0 μmol/L.

Based on those, we prepared LBP-SeNPs with suitable particle size and good stability and investigated whether LBP-SeNPs could protect SRA01/04 cells from UV irradiation. Damage model was HLECs (SRA01/04) in logarithmic growth phase with UV irradiation.

First, we established a safe concentration range of LBP-SeNPs. *In vitro*, proliferation rate of SRA01/04 cells decreased with higher concentrations of LBP-SeNPs. Although the concentration of selenium nanoparticles which showed drug toxicity was not consistent at different concentrations of LBP-SeNPs, each group showed different degrees of drug toxic effects at 8.0, 16.0 μmol/L. We selected the concentration regions of 0.5, 1.0, 2.0, 4.0 μmol/L as the relatively safe drug concentration range for the next experimental step.

Damaged HLECs (SRA01/04) were treated with LBP-SeNPs. It was verified that LBP-SeNPs could entered through the HLECs membrane into the cytoplasm. Cell proliferation rate was higher between the same concentration of LBP-SeNPs loaded with more LBP. Low concentration of LBP-SeNPs had a better protective effect compared with high concentration of LBP-SeNPs. The proliferation rate of SRA01/04 cells treated with 0.5 μmol/L of 2.0 mg/mL LBP-SeNPs was the highest.

We found that more cytoplasmic dehydration, cell crumpling, apoptotic vesicles appeared and cell density decreased when LBP-SeNPs concentration increased. This results is different with Qi *et al*[2]. who suggested that antioxidant and protection of human crystalline epithelial cells by LBP could increase with increasing concentration up to 400 mg/mL and decrease with increasing concentration beyond 400 mg/mL[2]. Maybe LBP combined with SeNPs changed LBP's original characteristics. But the ability of LBP-SeNPs to protect against oxidative damage is indisputable.

Further studies are needed to determine whether reducing the concentration of selenium nanoparticles and increasing the drug loading of LBP in LBP-SeNPs will improve cell survival. Protective effect of LBP-SeNPs on animal models will also be further carried out.

ACKNOWLEDGEMENTS

Foundations: Supported by the National Natural Science Foundation of China (No.81970806); Medical Scientific Research Foundation of Guangdong Province of China (No. A2019098).

Conflicts of Interest: Zhong JX, None; **Jin SS,** None; **Wu KS,** None; **Yu GC,** None; **Tu LL,** None; **Liu L,** None.

REFERENCES

1 Xiao J, Liong EC, Ching YP, Chang RC, Fung ML, Xu AM, So KF, Tipoe GL. Lycium barbarum polysaccharides protect rat liver from non-alcoholic steatohepatitis-induced injury. *Nutr Diabetes* 2013;3:e81.

2 Qi B, Ji QS, Wen YC, Liu L, Guo XL, Hou GH, Wang GF, Zhong JX. Lycium barbarum polysaccharides protect human lens epithelial cells against oxidative stress-induced apoptosis and senescence. *PLoS One* 2014;9(10):e110275.

3 Liu L, Sha XY, Wu YN, Chen MT, Zhong JX. *Lycium barbarum* polysaccharides protects retinal ganglion cells against oxidative stress injury. *Neural Regen Res* 2020;15(8):1526-1531.

4 Zhao QH, Li JJ, Yan J, Liu S, Guo YL, Chen DJ, Luo Q. Lycium barbarum polysaccharides ameliorates renal injury and inflammatory reaction in alloxan-induced diabetic nephropathy rabbits. *Life Sci* 2016;157:82-90.

5 Kou L, Du MZ, Zhang CP, Dai ZY, Li X, Zhang BH, Hu XY. Polysaccharide purified from Lycium barbarum protects differentiated PC12 cells against L-Glu-induced toxicity via the mitochondria-associated pathway. *Mol Med Rep* 2017;16(4):5533-5540.

6 Andley UP, Fritz C, Morrison AR, Becker B. The role of prostaglandins E2 and F_2 alpha in ultraviolet radiation-induced cortical cataracts *in vivo*. *Invest Ophthalmol Vis Sci* 1996;37(8):1539-1548.

7 Ayala MN, Michael R, Söderberg PG. Influence of exposure time for UV radiation-induced cataract. *Invest Ophthalmol Vis Sci* 2000;41(11):3539-3543.

8 Ottonello S, Foroni C, Carta A, Petrucco S, Maraini G. Oxidative stress and age-related cataract. *Ophthalmologica* 2000;214(1):78-85.

9 Ibaraki N, Chen SC, Lin LR, Okamoto H, Pipas JM, Reddy VN. Human lens epithelial cell line. *Exp Eye Res* 1998;67(5):577-585.

10 Liu L, Yu R, Shi Y, Dai Y, Zeng Z, Guo X, Ji Q, Wang G, Zhong J. Transduced protein transduction domain linked HSP27 protected LECs against UVB radiation-induced damage. *Exp Eye Res* 2014;120:36-42.

11 Ding Y, Yan YM, Peng YJ, Chen D, Mi J, Lu L, Luo Q, Li XY, Zeng XX, Cao YL. *In vitro* digestion under simulated saliva, gastric and small intestinal conditions and fermentation by human gut microbiota of polysaccharides from the fruits of Lycium barbarum. *Int J Biol Macromol* 2019;125:751-760.

12 Yu N, Song N, Liu CY, Yang GL. The estrogen-like protective effect of Lycium barbarum polysaccharides in reducing oxidative stress on myocardial cells from ovariectomized rats. *Mol Med Rep* 2019;19(3):2271-2278.

13 Zheng GZ, Ren HJ, Li HQ, Li XH, Dong TC, Xu SM, Yan YL, Sun BK, Bai JW, Li YS. Lycium barbarum polysaccharide reduces hyperoxic acute lung injury in mice through Nrf2 pathway. *Biomedecine Pharmacother* 2019;111:733-739.

14 Deng HB, Cui DP, Jiang JM, Feng YC, Cai NS, Li DD. Inhibiting effects of Achyranthes bidentata polysaccharide and Lycium barbarum polysaccharide on nonenzyme glycation in D-galactose induced mouse aging model. *Biomed Environ Sci* 2003;16(3):267-275.

15 Luo Q, Cai YZ, Yan J, Sun M, Corke H. Hypoglycemic and hypolipidemic effects and antioxidant activity of fruit extracts from Lycium barbarum. *Life Sci* 2004;76(2):137-149.

16 Ho YS, Yu MS, Lai CS, So KF, Yuen WH, Chang RC. Characterizing the neuroprotective effects of alkaline extract of Lycium barbarum on beta-amyloid peptide neurotoxicity. *Brain Res* 2007;1158:123-134.

17 Ho YS, Yu MS, Yik SY, So KF, Yuen WH, Chang RC. Polysaccharides from wolfberry antagonizes glutamate excitotoxicity in rat cortical neurons. *Cell Mol Neurobiol* 2009;29(8):1233-1244.

18 Chiu K, Chan HC, Yeung SC, Yuen WH, Zee SY, Chang RC, So KF. Modulation of microglia by Wolfberry on the survival of retinal ganglion cells in a rat ocular hypertension model. *J Ocul Biol Dis Infor* 2009;2(2):47-56.

19 Du XJ, Dong WH, Bi HS, Xie XF. Effects of Lycium barbarum polysaccharides on apoptosis and mitochondrial membrane potential of human retinal pigment epithelial cells injured by blue light. *Chinese Journal of Practical Ophthalmology* 2013;31(4):489-493.

20 Varoni MV, Pasciu V, Gadau SD, Baralla E, Serra E, Palomba D, Demontis MP. Possible antioxidant effect of Lycium barbarum polysaccharides on hepatic cadmium-induced oxidative stress in rats. *Environ Sci Pollut Res Int* 2017;24(3):2946-2955.

21 Chen L, Li W, Qi D, Wang DX. Lycium barbarum polysaccharide protects against LPS-induced ARDS by inhibiting apoptosis, oxidative stress, and inflammation in pulmonary endothelial cells. *Free Radic Res* 2018;52(4):480-490.

22 Schoppet M, Tailhades J, Kulkarni K, Cryle MJ. Precursor manipulation in glycopeptide antibiotic biosynthesis: are β-amino acids compatible with the oxidative cyclization cascade? *J Org Chem* 2018;83(13):7206-7214.

23 Zhu XJ, Lu Y. Selenium supplementation can slow the development of naphthalene cataract. *Curr Eye Res* 2012;37(3):163-169.

24 Post M, Lubiński W, Lubiński J, Krzystolik K, Baszuk P, Muszyńska M, Marciniak W. Serum selenium levels are associated with age-related cataract. *Ann Agric Environ Med* 2018;25(3):443-448.