The anti-oxidative Effect of Chrysanthemum Extract on Retinal- light Damage of Mice

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Research

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Abstract

Background:
Apoptosis of photoreceptor cells and oxidative stress of RPE in age-related macular degeneration (AMD) could be promoted by photopic oxidative stress. In our study we are aim to study the protective effect of chrysanthemum extract on light damaged retina of mice.

Methods:
In vitro, ARPE-19 cells are incubated and divided into four groups: the control, the light damaged, the low and high dose-chrysanthemum extract groups. The last three groups were dropped in zero, low and high concentration of chrysanthemum extract separately before exposing to light. Cellular viability and Reactive Oxygen Species(ROS) production were measured by MTT and immunofluorescence. In vivo, C57BL/6J mice were divided into four groups as above mentioned. Low and high concentration of chrysanthemum extract were given by continuous intragastric administration before being exposed to white light. Retinal function was evaluated by electroretinogram. Optical coherence tomography and Fluorescein fundus angiography were used to observe the morphology and vessels. HE staining and TUNEL immunofluorescence for presenting morphology and apoptosis of isolated retina.

Results:
Viability of ARPE-19 cells decreased and ROS production increased after the light damaged. However, treatment with chrysanthemum extract, viability improved and ROS declined. After light injury, dysfunctional retina, destroyed morphology and increased apoptosis rate were observed in mice especially in RPE and photoreceptor layer. Treatment with chrysanthemum extract, retina function improved as well as structure of RPE and photoreceptor layers. Rate of apoptosis decreased via the raised concentration of anti-oxidative enzyme superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px).

Conclusions:
Preventive administration of chrysanthemum extract reduces the oxidative-stress induced by light damage, which indicating Chrysanthemum have a potential of preventive measure for AMD.

1. Introduction
Age-related degeneration (AMD) has been a common disease in developed countries and has a tendency to occur in more young people as the aging population raising in the world. According to a study of meta-analysis, the number of patients with AMD could reach to 0.2 billion in 2020 and add to 0.3 billion until 2040[1]. AMD is characterized by the aggregation of drusen and the destroy of RPE layer in the early period, develop to wet type with neovascular or dry type with geographic atrophy (GA) in the late gradually[2, 3]. The mechanism of AMD is not completely clear now, some studies show that the inducing factor could be relative with exposing to high intensity of light and lacking of anti-oxidative food in daily diet, which both lead to ROS produce in excess[4–6]. Chrysanthemum considered as a common herbal in Chinese Medicine for thousands of years, chrysanthemum contains anti-oxidative materials especially the micro molecules of avonoids, which are useful in anti-oxidative stress caused by a variety of color lights and scavenging oxygen free radicals[7–9]. The main purpose of this study is to observe the protective effect of chrysanthemum extract on the lighted injured RPE cells and retinal of mice. Discuss the possible mechanism of anti-oxidative stress.

2. Material And Methods

2.1. Extraction of Chrysanthemum:
The chrysanthemum of experiment is produced in Hangzhou, China, and the petals were used to prepare for water extraction. After soaking chrysanthemum petals in water that 8 times weight of materials and boiling for 0.5 hour. Keep the liquid and residue was boiled for another time. Repeat the process for 3 times and then combine all the liquid obtained. Concentrate the collection to low and high concentration of extract. (1g chrysanthemum extract obtained from 4.255g materials)[10]. The content of total flavonoids in chrysanthemum was 5.0%, according to Technical specification for inspection and evaluation of health food.

2.2. Cell experiment:

2.2.1. Cell culture:
ARPE-19 cells (China Center for Type Culture Collection) were cultured in DMEM / F12 medium (Hyclone, the USA) containing 10% fetal bovine serum, 100U/mL penicillin and 100U mg/mL streptomycin, and incubated in 5% CO2 under constant temperature of 37 °C and humidity. When the cells grow exponentially, it will be used in the follow-up experiment.

2.2.2. The toxicity of chrysanthemum extract to ARPE-19 cells:
The safe concentration and toxicity of chrysanthemum extract to ARPE-19 cells were detected by MTT. AREP-19 cells approximate number of 10 ×103 cells were inoculated into 96-well plates with 100 μL per well. When the cells grew exponentially, the concentrations were 0.2mg/mL, 0.4mg/mL, 0.8mg/mL, 1.0mg/mL, 1.2mg/mL, 1.5 mg/mL, 2.00mg/mL and 5.00mg/mL respectively. of chrysanthemum extract were added to 96-well plate separately and cultured...
for 24 hours. After the end of culture, 20 μL MTT (5mg/mL) solution was added to each well and cultured in CO2 incubator for 4 hours. MTT was discarded and 150 μL DMSO solution was added. After being fully dissolved, the cell activity was evaluated by measuring absorbance (A value) at wavelength of 492 nm. According to the results, the safe concentration was applied for the follow-up experiment.

2.2.3. Light induced damage of RPE cell:

After the cells were digested by trypsin, the cells were made into cell suspension and inoculated in the culture plate. The cells were irradiated directly with LED white light with the intensity of (2500 ± 500) Lux for 24 hours, after the cells grew exponentially[11].

2.3. Animals:

2.3.1. Animal ethics:

Totally 32 mice of C57BL/6J of male with the age of 6 weeks were obtained from the SPF Biotechnology (Beijing, Co.,Ltd), the mice were raised in 12/12h light and dark with the temperature of 23 ± 2℃, relative humidity of 55% in the Tianjin Eye Institute, The food and water could take freely. All the procedures were coherence with international standard of laboratory animal use and care and in accordance with the principles and guidelines of the Chinese Council Animal Care, the experiment was approved by the Institutional Animal Care and Use Committee of Tianjin Medical University. Mice were experimented or sacrificed by inhaling the isoflurane, all the steps to minimize suffering.

2.3.2. The groups division and chrysanthemum extract intervention:

Mice were randomly divided into control group (n = 8), light damaged group (LD, n = 8), low-dose chrysanthemum group (LC, n = 8) and high-dose chrysanthemum group (HC, n = 8). After concentrated and dried by water extraction, the extracts were crushed into powder and suspended with 0.9% saline when it was given to intragastric administration. In the range of effective concentration, 0.23g/kg/d for the low dose group, and 0.38g/kg/d for the high dose. 0.2ml/ per day for 8 weeks. The dose concentration was also similar with the study by Dong Lumeng et al. about the protective effect of chrysanthemum on Parkinson's mice[12].

2.3.3. Light damage method:

One day after intragastric administration, the light injury mode were as follows: light damage group(LD), low and high dose of chrysanthemum group (LC and HC) were treated with continuous white light of 9500±500lux for 7 days, 4 h /d after pupil dilated , accumulating 28 hours in total, each mouse was separated by transparent box during light exposing in order to avoid crowding. The function and morphology of retina were examined 7 days after photic injury.

2.3.4. Electroretinogram (ERG):

12 hours after dark adaptation, mice were anesthetized by inhaling 2% isoflurane and fixed on the animal experiment table. Dilate both of eyes and certain gel to keep the cornea transparent. Connecting reference electrode to the head, annular corneal electrode to the eyeball, and the ground electrode to the tail of mouse. Visual electrophysiological apparatus (Roland Germany) was used to record the amplitude of wave a, b and Ops under the stimulating light of 0.01cds/m² and 3.0 cds/m² in dark. After 10 minutes of adaptation of photopic, amplitude of a and b under 3.0 cds/m² was recorded.

2.3.5. Optical Coherence Tomography (OCT):

Mouse was lied prone on the animal experimental platform after being anesthetized by inhaling 2% isoflurane, 0.5% tropicamide phenylephrine eye drops (Santen) were used to dilate pupils and carbomer eye gel keeping the cornea transparent. Phoenix eye testing equipment for animals (Phoenix research labs, model: Micron IV) was used to scan the retina in vivo and gain morphological imaging of each layer.

2.3.6. Fluorescein fundus angiography (FFA):

2% sodium fluorescein was injected intraperitoneally immediately after finish the OCT examination. The state of filling of the retinal vessels at arterial phase, venous phase and arteriovenous phase was observed at the same gain value. Application of angio-tool 0.6a that a software download free was to analyze the relative parameters consist of vessel area, vessel area percentage and the total number of junctions.

2.3.7. HE staining and TUNEL:

The mice were sacrificed 3 days later by Inhaling isoflurane, ophthalmic vessels blood collected, and the eyeballs were taken out at the same time. 12 hours after formalin fixation of the eyeball, part of cornea was cut off and fixed in fresh formalin for another 8 hours. After dehydration and paraffin liquid immersing at 40℃ for 6 hours, eyeballs were completely embedded Paraffin block which would be sliced into 4μm of thickness. HE and Tunel
immunofluorescence staining (Tunel fluorescence kit provide by Dalian meiliun biotechnology Co.,LTD.) were performed to analyze the apoptosis rate of each retinal layer for the all groups.

2.3.8. Anti-oxidative enzyme assay:
Superoxide dismutase (SOD), Catalase (CAT) and Glutathione (GSH-Px ) assay kit: Support by Nanjing Jiancheng Bioengineering Institute. Collected blood from ophthalmic artery was centrifugated at 4℃, 3000r/min for 10 minutes in order to obtain the serum. Keeping them at -80℃ to measure the enzyme activation of SOD, CAT and GSH-Px.

2.4. Statistical methods:
All the parameters including the amplitude of a and b wave in the examination of ERG, the vessel area, the vessel percentage and the total number of junctions measured and analyzed in FFA and angio-tool, the apoptosis rate in each layer of retina are presented in the term of \( \bar{x} \pm s \), one-way ANOVA of SPSS22.0 software was to evaluate the parameter. \( P<0.05 \) has been considered as statistically significant.

3. Result

3.1. Cell examinations:
3.1.1: The toxicity of chrysanthemum to ARPE-19 cells:
The results of MTT assay showed that the cell viability of the control group was 99.98% as the reference. The scores of cell viability were 97.64%, 98.52%, 96.18%, 96.24%, 94.42%, 90.65%, at 0.2 mg/mL, 0.4 mg/mL, 0.8 mg/mL, 1.0mg/mL, 1.2mg/mL, 1.5mg/mL respectively. When the concentration was 2.00 mg/mL, chrysanthemum extract could significantly inhibit the viability of ARPE-19 cells and the cell viability was decreased to 88.15% of the normal (\( P<0.05 \)) significantly and decreased to 64.66% of the normal (\( P<0.001 \)) if the concentration was 5 mg/mL. Therefore, the concentration of chrysanthemum extract was selected no more than 1.2 mg/mL for the follow-up experiment. The LC groups with 0.4mg/mL and HC with 1.0mg/mL.

3.1.2: The effect of chrysanthemum extract on the activity and ROS production of ARPE-19 cells after the light damage:
The cell viability of the LD group decreased to 61.43% (\( P<0.001 \)) after 24 hours of light exposure of 2500 lux. Treatment with chrysanthemum extract before the light damage, the cell viability of LC and HC groups increased to 69.22% and 76.04% respectively compared with the LD group (\( P<0.05, P<0.001 \)), the production of ROS in LD (84.94±3.29%) were increased by 45.92% (\( P<0.001 \)) compared with the control group (58.21±6.46%), the LC (76.62±4.40%) and HC (61.56±2.02%) groups were decreased by 10.5% (\( P<0.05 \)) and 27.53% (\( P<0.0001 \)) compared with LD group (Figure1A.B.C).

3.2. Animal examinations:
3.2.1. OCT and HE staining:
OCT and HE staining: As the imaging of OCT show, when the retinal structure was observed in vivo, it could be seen clearly of each layer(Figure 2A), the signal of high refection that shaping of arched had been found between the RPE and interdigitation zone (IZ) layer in LD group, which also make the outer segments of photoreceptors (OSP), the ellipsoid zone (EZ) and myoid zone (MZ) layer to be deformed. (Figure 2B). At mean while the HE staining expressed the same structure and change with OCT imaging. The obviously damaged presented in OCT and HE staining that were similar with the AMD of early phase (Figure2.3).

3.2.2. The vessel change measured by FFA:
3.2.2. The Outcomes of ERG:
The wave of a, b and Ops shaped in Figure 6, under the 0.01cds/m² dark reaction in LD group, the amplitude of b-wave in LD group was 41.6% lower than control group, and the amplitude of b-wave in the LC and HC groups was 83.5% and 120.6% higher than LD group under the stimulate of 0.01cds/m² in the scotopic reaction (\( P<0.05 \) in LC , \( P<0.01 \) in HC. Under 3.0 cds/m² dark reaction, the a-wave amplitude in LD group was 22% lower than control group, in LC group increased by 50.8% and HC and HC 118.5%. Compared with LD group, the amplitude of a wave in HC group was significantly higher than LD group (\( P<0.05 \)) in the stimulation of 3.0cds/m² in photopic reaction (Figure 7). The data with amplitude and implicit time are displayed in Table 2.

3.2.3. The vessel change measured by FFA:
The central vascular with the range of diameter between 2 to 4 mm (Figure 8), the LD group increased by 35% compared with the control group, while the LC and HC groups decreased by 20.8% and 15.7% respectively. In the total number of junctions, LD group increased by 64.4% than the control group, while the LD and HD groups decreased by 34.2% and 23.3% than the LD respectively. There was no statistical difference among each group, however there is a significant change between the LD and Control groups according to t-test of independent samples (P<0.05) as presented in Figure 9.

3.2.4. The apoptosis rate of retina measured by TUNEL assay:

There was outstanding increase of apoptosis cells in the LD group however much fewer in LC and HC groups (Figure 10). The quantity of apoptotic cells counted by image J in LD group increased statistically significant. The apoptosis rate of RGC, INL and ONL layers were calculated separately (Figure 11). The outcomes show significant differences of RGC, INL and total apoptosis rate among the four groups. Apoptosis rate of RGC cells in LD was 9.04% higher than the control group. LC group was 73.18% lower than LD group (P<0.01), HC group was 57.28% (P<0.01).

3.2.5. The enzyme activation of SOD, CAT and GSH-Px:

In the mouse fed up with continuous intragastric administration of chrysanthemum extract, the enzyme activity of SOD was increased with the mean value of 75.50±3.2U/ml and 75.57±6.33 U/ml in the LC and HC group respectively. CAT and GSH-Px show a more certain tendency of activity with the mean value of 1048.61±85.18 U/ml (P<0.01) and 1005.56±65.73 U/ml (P<0.01) in LC and HC groups separately. As depicted in figure 12, the CAT in HC group and the GSH-Px in LC and HC group are even more activating than Control group but with no statistically significant (Table 3).

4. Discussion

4.1. The relative between ROS and AMD:

The light damage as a method to establish model of oxidative stress of mice for more than 40 years, which could lead to photoreceptor destroyed[13]. Acute or chronic way is applied commonly by researchers, however studies by Chulbul M et al have shown that the generation of AMD is related to subacute inflammation[14–16]. Therefore, the use of strong light irradiation for a moderate time may be helpful for modeling. As we know that too powerful or long-time of light would lead to retinal phototoxic reaction and produce quantity of reactive oxygen species (ROS) that considered as the main metabolite of oxidative stress and attribute to oxidative damage reaction[17, 18]. Recent study in clinical found that IS/OS is less detailed to divide the retina layer before. The new more detailed zone named myoid zone (MZ) and ellipsoid zone (EZ), especially the EZ illustrated are corelate with the inner segment ellipsoid (ISE) that consist of large intensity of mitochondria[19]. When too much ROS can't be solved by mitochondria, aging or destroyed RPE cells accumulate some substances of lysosome non-degradable and then lipofuscin formed. These substances include dysfunctional mitochondria and the outer segments of swollen photoreceptor cells[18]. The deposition of lipofuscin in the early stage does not cause abnormal cell metabolism. However, on account of RPE cells act as permanent cells in the body, and the accumulated lipofuscin cannot be diluted by proliferation. Long-term involvement of intracellular lipofuscin affecting the function of mitochondria and lysosomes even the proteolysis system[20, 21]. The decreased proteolytic capacity leads to the accumulation of extracellular oxidized proteins, which resulting in drusen [22] and may cause the apoptosis raised in retina. At present, some studies have found that abnormal autophagy and DNA damage response (DNA damage response, DDR) is an important mechanism of AMD, and the main reason is also related to the increase of ROS level[23]. Therefore, oxidative damage is not only an important factor in cell senescence, but also forms a malignant pathological circulation mechanism of AMD. In our study we observed the damaged of EZ, OSP, and the RPE cell layer from OCT imaging in vivo and HE staining in vitro. All above we could come to conclude that process of anti-oxidative is important for prevent the retina from AMD.

4.2 The effect of chrysanthemum extract on oxidative stressed retina

Among the remarkable achievement of Traditional Chinese medicine in the study of anti-oxidant damage, chrysanthemum is a plant of asteraceae specie, natured as sweet, bitter and slightly cold for the body and attribute to the lung and liver meridians. They have good function to dissipate heat, protect eye from decreasing vision and fatigue. Chrysanthemum extract is rich in flavonoids and polysaccharides[24]. Yao Xiang-chao et al found that wild chrysanthemum of eye drops had a protective function on retinal light injury in rats[25]. Hollyfield et al in order to prove the key role of oxidative stress in AMD, the inflammatory response and retinal phenotypic changes caused by oxidative stress, such as CEP, MDA, are very similar to those of AMD[26].

In the experiment, we found that the electrophysiological function indicated by ERG and the morphology of retina displayed by OCT and HE staining in vivo and in vitro were significantly improved after the intervention of chrysanthemum extract. This is similar to the study of cell by Kim, I. S, which consistent with the conclusion that chrysanthemum can improve the loss of neuronal vitality induced by MPP+, reduce the apoptosis rate and increase the expression of Bcl-2[27]. Suyao sun et al also found that wild chrysanthemum extract can reduce the level of ROS in cells and has the effect of anti-ultraviolet damage and skin photoaging in vitro[28]. On the opposite, result of FFA, the micro vessels diameter 2–4 mm analyzed shows that the vessel area, the vessel area percentage and the total number of junctions between the vascular are largely increased in the LD group compared with the control group. However, LC and HC groups have no significantly improved.

So, we further measure apoptosis in our study, in LD group, the apoptosis of retinal ganglion cells, inner nuclear layer cells and outer nuclear layer cells increased, especially the first two, and the apoptosis rate of ganglion cell layer decreased significantly in LC and HC mice. In biochemical of blood test, the activation of antioxidant enzyme of CAT and GSH-Px increased significantly after intervention with chrysanthemum extract. The CAT and the GSH-Px were even better than the control group. However, the SOD increased but with no statistically significant which may have the relationship with the number of samples.
Therefore, we can speculate that the functional and morphological protection of chrysanthemum on retina may play a role by antagonizing oxidative stress and reducing the pathway of mitochondrial apoptosis. The mechanism of the protective function needs more research.

5. Conclusion

In our study, preventive administration of chrysanthemum extract improves both function and morphology of retina injured by light exposing and reduces the oxidative-stress by increasing SOD, CAT and GSH-Px, which indicating Chrysanthemum have a potential of preventive measure for AMD.

Abbreviations

age-related macular degeneration (AMD)
Reactive Oxygen Species (ROS)
superoxide dismutase (SOD),
catalase (CAT)
glutathione peroxidase (GSH-Px)
Electroretinogram (ERG)
Optical Coherence Tomography (OCT)
Fluorescein fundus angiography (FFA)
interdigitation zone (IZ)
outer segments of photoreceptors (OSP)
ellipsoid zone (EZ)
myoid zone (MZ)
inner nuclear layer (INL)
outer nuclear layer (ONL)

Declarations

1. Ethic approval and consent participate:
Not applicable.

2. Consent for publication:
Not applicable.

3. Competing interests:
The author declare that they have no competing interests.

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5. Authors’ contributions:
Yibo Gong and Xuechun Wang performed the experiments and analyzed the data and writing the manuscript; Yatu Guo and Wei Zhang were the major designer of the research and revise the manuscript. Yuchuan Wang and Peng Hao gave instructions of experiment. All authors read and approved the final manuscript.

6. Acknowledgement:
7. Availability of data and materials:

The datasets analysed during the current study are available from the corresponding author on reasonable request.

References

1. Wong WL, Su X, Li X, Cheung CMG, Klein R, Cheng C-Y, Wong TY: Global prevalence of age-related macular degeneration and disease burden projection for 2020 and 2040: a systematic review and meta-analysis. *The Lancet Global Health* 2014, 2(2):e106-e116. https://doi.org/10.1016/s2214-109x(13)70145-1

2. Lim LS, Mitchell P, Seddon JM, Holz FG, Wong TY: Age-related macular degeneration. *The Lancet* 2012, 379(9787):1728-1738. https://doi.org/10.1016/s0140-6736(12)60282-7

3. Jager RD, Mieler WF, Miller JW: Age-related macular degeneration. *N Engl J Med* 2008, 358(24):2506-2517. https://doi.org/10.1056/NEJMra0801537

4. Cano M, Thimmalappula R, Fujihara M, Nagai N, Sporn M, Wang AL, Neufeld AH, Biswal S, Handa JT: Cigarette smoking, oxidative stress, the anti-oxidant response through Nrf2 signaling, and Age-related Macular Degeneration. *Vision Res* 2010, 50(7):652-664. https://doi.org/10.1016/j.visres.2009.08.018

5. Chakravarthy U, Wong TY, Fletcher A, Piault E, Evans C, Zlateva G, Buggage R, Pleil A, Mitchell P: Clinical risk factors for age-related macular degeneration: a systematic review and meta-analysis. *BMC Ophthalmol* 2010, 10:31. https://doi.org/10.1186/1471-2415-10-31

6. Jonasson F, Fisher DE, Eiriksdottir G, Sigurdsson S, Klein R, Launer LJ, Harris T, Gudnason V, Cotch MF: Five-year incidence, progression, and risk factors for age-related macular degeneration: the age, gene/environment susceptibility study. *Ophthalmology* 2014, 121(9):1766-1772. https://doi.org/10.1016/j.ophtha.2014.03.013

7. Zheng L, Van Labeke MC: Chrysanthemum morphology, photosynthetic efficiency and antioxidant capacity are differentially modified by light quality. *J Plant Physiol* 2017, 213:66-74. https://doi.org/10.1016/j.jplph.2017.03.005

8. Kuang CL, Hu, Gao W, Shen GH, Zhang ZQ: Research of the antioxidant activity of Chrysanthemum Morifolium’s extracts with different solvents. *College of Food Science* 2015:36(21):83-87+92. https://doi.org/10.13325/j.cnki.acta.nutr.sin.2017.03.014

9. He ZR HY, Liu L, Zhao EL: Research progress on antioxidant activity of Chrysanthemum morifolium. *Journal of Green Science and Technology* 2019:128-130. https://doi.org/10.16663/j.cnki.lskj.2019.02.052

10. JJ M: Study on the extraction, purification and separation of flavonoids from Hawthorn Leaves. Master of Shandong agriculture university 2016. https://kns.cnki.net/kcms/detail/detail.aspx?dbcode=CMFD&dbname=CMFD201801&filename=1017101475.nh &v=EfwmnzTAJVeUC3dUsRQ5c2y4AOteS8lpPkmGo1CXXM%25mmd2Brx0l6mIUjn6pT

11. Tanaka J, Nakanishi T, Ogawa K, Tsuruma K, Shimazawa M, Shimoda H, Har a H: Purple rice extract and anthocyanidins of the constituents protect against light-induced retinal damage in vitro and in vivo. *J Agric Food Chem* 2011, 59(2):528-536. https://doi.org/10.1021/jf103186a

12. Dong LM WR, Chen Y, Yin TP, Li XB, Du LF, Chen CY, Xin XM: Anti-Parkinsonian activity of chrysanthemum morifolium ramat extractin mice. *Acta Nutrimenta Sinica* 2017:39(03):294-298. https://doi.org/10.13325/j.cnki.acta.nutr.sin.2017.03.014

13. Yamashita H, Horie K, Yamamoto T, Nagano T, Hirano T: Light-induced retinal damage in mice. Hydrogen peroxide production and superoxide dismutase activity in retina. *Retina* 1992, 12(1):59-66. https://pubmed.ncbi.nlm.gov/1565873/

14. CM A, MR B, H L, P H, CJ I, AS L: Repurposing an orally available drug for the treatment of geographic atrophy. *Molecular vision* 2016, 22:294-310. https://pubmed.ncbi.nlm.gov/27110092/

15. D S, Y S, M H, Y Z, JL D: Systemic administration of the iron chelator deferiprone protects against light-induced photoreceptor degeneration in the mouse retina. *Free radical biology & medicine* 2012, 53(1):64-71. https://doi.org/10.1016/j.freeradbiomedi.2012.04.020

16. D S, J S, C W, Y L, JL D: Berberine protects against light-induced photoreceptor degeneration in the mouse retina. *Experimental eye research* 2016, 145:1-9. https://doi.org/10.1016/j.exer.2015.10.005

17. Hanus J, Anderson C, Wang S: RPE necroptosis in response to oxidative stress and in AMD. *Ageing Res Rev* 2015, 24(Pt B):286-298. https://doi.org/10.1016/j.ager.2015.09.002

18. Mitter SK, Song C, Qi X, Mao H, Rao H, Akin D, Lewin A, Grant M, Dunn W, Jr., Ding J: Dysregulated autophagy in the RPE is associated with increased susceptibility to oxidative stress and AMD. *Autophagy* 2014, 10(11):1989-2005. https://doi.org/10.4161/auto.36184

19. LW T, Z W, RH G, CD L: Ellipsoid zone on optical coherence tomography: a review. *Clinical & experimental ophthalmology* 2016, 44(5):422-430. https://doi.org/10.1111/coe.12685

20. NP B, DH M, CB C, LR B, CC C, Z A, RK C, Y K: Lipofuscin and N-retinylidene-N-retinylethanolamine (A2E) accumulate in retinal pigment epithelium in absence of light exposure: their origin is 11-cis-retinal. *The Journal of biological chemistry* 2012, 287(26):22276-22286. https://doi.org/10.1074/jbc.M111.329234

21. N S, M H, T G, A L, WD D, T VZ, KJ D: Proteasome inhibition by lipofuscin/eroid during postmitotic aging of fibroblasts. *FASEB Journal : official publication of the Federation of American Societies for Experimental Biology* 2000, 14(11):1490-1498. https://doi.org/10.1096/fj.14.11.1490

22. Ferrington DA, Sinha D, Kaamiranta K: Defects in retinal pigment epithelial cell proteolysis and the pathology associated with age-related macular degeneration. *Prog Retin Eye Res* 2016, 51:69-89. https://doi.org/10.1016/j.preteyeres.2015.09.002

23. Hytinen JMT, Blasiak J, Nittykoski M, Kinnunen K, Kauppinen A, Salminen A, Kaamiranta K: DNA damage response and autophagy in the degeneration of retinal pigment epithelial cells—Implications for age-related macular degeneration (AMD). *Ageing Res Rev* 2017, 36:64-77. https://doi.org/10.1016/j.arr.2017.03.006
24. Commission CP: Chinese Pharmacopoeia. 2015 edn. Beijing: China Medical Science and Technology Press; 2015.

25. Yao XQ WY, Huang CL: Observation on the Therapeutic effect of Wild Chrysanthemum Eye Drops on Experimental Retinal Light injury in Rats. Pharmacy Today 2014, 24(05):323-325. https://kns.cnki.net/kcms/detail/detail.aspx?dbcode=CJFD&dbname=CJFD2014&filename=YAXU201405008&v=eBE%25mmd2B88H19OY3J8hh5QYA0ySnkewNFuk8e9GF4MqRtERc0HX0pi0NVO%

26. K R, J G, ME R, JS C, RG S, RJ C, MA K, C R, JG H, JW C: CEP biomarkers as potential tools for monitoring therapeutics. PloS one 2013, 8(10):e76325. https://doi.org/10.1371/journal.pone.0076325

27. Kim IS, Koppula S, Park PJ, Kim EH, Kim CG, Choi WS, Lee KH, Choi DK: Chrysanthemum morifolium Ramat (CM) extract protects human neuroblastoma SH-SY5Y cells against MPP+-induced cytotoxicity. J Ethnopharmacol 2009, 126(3):447-454. https://doi.org/10.1016/j.jep.2009.09.017

28. Sun S, Jiang P, Su W, Xiang Y, Li J, Zeng L, Yang S: Wild chrysanthemum extract prevents UVB radiation-induced acute cell death and photoaging. Cytotechnology 2014, 68(2):229-240. https://doi.org/10.1007/s10616-014-9773-5

Tables

Due to technical limitations, table 1, table 2 and table 3 are only available as a download in the Supplemental Files section.

Figures

**Figure 1**

ROS production (A,B) and Viability(C) of ARPE-19 cells. A: Imagine J were used to measure the intensity of the fluorescence, the production of ROS in LD (84.94±3.29%) were increased by 45.92% (P<0.001) compared with the control group (58.21±6.46%), the LC (76.62±4.40%) and HC (61.56±2.02%) groups were decreased by 10.5% (P<0.05) and 27.53% (P<0.0001) compared with LD group. C. Treatment with chrysanthemum extract before the light damage, the cell viability of LC and HC groups increased to 69.22% and 76.04% respectively compared with the LD group (P<0.05, P<0.001), the data was analyzed by one way-ANOVA, *P<0.05, **P<0.01, ***P<0.0001
Retina structure of the control(A) and the LD(B) mice

Figure 2
Retina structure of the control(A) and the LD(B) mice by OCT. A: NFL: nerve fiber layer. IPL: inner plexiform layer. INL: inner nuclear layer. OPL: outer plexiform layer. ONL: outer nuclear layer ELM: external limiting membrane MZ: myoid zone. EZ: ellipsoid zone. OSP: outer segments of photoreceptors. IZ: interdigitation zone. RPE: retinal pigment epithelium. B: The yellow arrow exhibits the signal of high reflection between the RPE and the IZ layer, corresponding to the destroyed PRE, IZ, OSP and EZ layer showed in HE staining. The imaging of OCT and HE staining we record are similar to AMD of early phase.

Figure 3
Image of HE staining of the four groups. HE: The destroyed RPE and photoreceptors of retina in LD group marked by red arrow. LC and HC groups have no outstandingly deformed after the light damaged.
OCT and Fundus photos of every groups. OCT: Compared with the LD group, the structure of LC and HC retina kept relative integrity and had no signals of high reflection in RPE layer. The red arrow showed the change position caused by light injury.
The thickness of INL and ONL. There were significant thinner in LD group when analyze the thickness on the zone of peri-retina from 300 to 600μm distance to optic nerve of INL. The tendency become more obviously from -300 to -600μm and from 300 to 600μm distance from the optic nerve. The detailed data could be seen in table1.

Figure 6
The shape of full-field ERG waves in scotopic and photopic. Figure5 legend: The first trough considered as a wave, the first peak considered as b wave, the amplitude of b was valued from the trough point to the peak. Ops was measured under the stimulation of 3.0cds/m2, and filter out from 20 to 40Hz. The ruler of the amplitude and implicit time was 12.5μv and 5ms respectively.
Figure 7

Amplitude of a and b wave under the scotopic situation. Figure6 legend: A: The amplitude of b wave under the scotopic situation of 0.01, 3.0 and 10.0cds/m², LC was significantly higher than LD group at the stimulation of 0.01 c/ds/m² (P<0.05), so did the HC group (P<0.01). The amplitude of a wave under the scotopic situation of 3.0 and 10.0cds/m², LC group was significantly higher than LD group at the stimulation of 3.0 c/ds/m²(*P<0.05), so did the HC group (**P<0.01).

FFA of the central fundus in the artery-venous period
Figure 8

FFA of the central fundus in the artery-venous period. Figure7 legend: Take the optic disk as the center position, imaging of FFA recorded at the arteriovenous phase, intensity of micro vessels between 2-4mm diameters were obvious increased in LD group. The amplified detail circled red showed the vessel area percentage and the total number of junctions.

![Comparison of micro-vessels analyzed by the angio-tool software](image)

Figure 9

Comparison of micro-vessels analyzed by the angio-tool software. After processing the data by angio-tool, the parameter collected and analyzing by SPSS, there were no significant statistically between the four groups by ANOVA, but testing by t-test of independent samples, the LD group had a remarkably rising compared with the control group. (*P<0.05). A: vessel area, B: vessel area percentage, C: total number of junctions.
Apoptosis assay by TUNEL

Figure 10
Apoptosis assay by TUNEL Figure9 legend: The number of apoptosis cells increased distinctly in LD groups of each cell layers of retina. The level of apoptosis decreased obviously in the LC and HC groups especially of RGC layer.

Comparison of apoptosis of cell layers

Figure 11
Comparation of apoptosis of cell layers. Analyze by image J, RGC, INL, ONL, and the total number of apoptotic cells and normal cells were counted. SPSS used to analyze the apoptosis rate of RGC(A), INL(B), ONL(C) and total number(D) of each group. Apoptotic cells of RGC in LC and HC groups were much lower than the LD groups(**P<0.01), so did the INL(**P<0.01). There was no significant change in ONL of apoptosis rate. The total number of apoptosis rate has decreased largely in LC and HC groups(**P<0.01).

The anti-oxidative enzyme of SOD, CAT, GSH-Px

Figure 12

The anti-oxidative enzyme of SOD, CAT, GSH-Px Figure11 legend: SOD(A), CAT(B) and GSH-Px(C), LC and HC group had a quite large increase in the enzyme activation of SOD, CAT and GSH-Px, and even more activating than the control group of last two.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- OnlineTABLE1.png
- OnlineTABLE2.png
- OnlineTABLE3.png