Dear reviewers:

Thank you for your decision and constructive comments on my manuscript. We have carefully considered the suggestion of Reviewers and make some changes. We have tried our best to improve and made some changes in the manuscript.

The yellow part that has been revised according to your comments. Revision notes, point-to-point, are given as follows:

Reviewer #1:

Point 1: Title: the title is not appropriate. I suggest making up it.

Response 1: We changed our title according to the reviewer's comment and the new title is: ADSC-exos present better effects on ameliorating RGC injury induced by hydrostatic pressure through upregulating nerve growth factors than BMSC-exos. Please check if they meet the requirements, thank you!

Point 2: Some references missing. For example, "The mechanism of RGC apoptosis in glaucoma is rather complicated in which the molecular mechanism has not been fully studied." and etc. The following reference may increase the reader's comprehension: Sheykhhasan M, Amini R, Soleimani Asl S, Saidijam M, Hashemi SM, Najafi R. Neuroprotective effects of coenzyme Q10-loaded exosomes obtained from adipose-derived stem cells in a rat model of Alzheimer's disease. Biomed Pharmacother. 2022 Aug;152:113224. doi: 10.1016/j.biopha.2022.113224. Epub 2022 Jun 6. PMID: 35679720.

Response 2: We sincerely appreciate the valuable comments. We have checked the literature carefully and added more references on the mechanism of RGC

apoptosis into the INTRODUCTION part of the revised manuscript. The details are as follows: “The mechanisms of RGC apoptosis in glaucoma include oxidative injury[1], inflammatory response[2], and glutamate toxicity[3].” Please see the references:

1. Liu L, Sha XY, Wu YN, Chen MT, Zhong JX. Lycium barbarum polysaccharides protects retinal ganglion cells against oxidative stress injury. Neural regeneration research 2020; 15: 1526-1531 [PMID: Pmc7059572 DOI: 10.4103/1673-5374.274349

2 Li Q, Cheng Y, Zhang S, Sun X, Wu J. Trpv4-induced müller cell gliosis and tnf-α elevation-mediated retinal ganglion cell apoptosis in glaucomatous rats via jak2/stat3/nf-κb pathway. 2021; 18: 271 [PMID: DOI: 10.1186/s12974-021-02315-8

3 Kanamoto T, Okumichi H, Rimayanti U, Kiuchi Y. Cullin5 reduces retinal cell death induced by glutamate toxicity. Current eye research 2011; 36: 66-70 [PMID: DOI: 10.3109/02713683.2010.514658

Moreover, considering the review’s suggestion, the article “Neuroprotective effects of coenzyme Q10-loaded exosomes obtained from adipose-derived stem cells in a rat model of Alzheimer's disease” has been added to the third paragraph of the INTRODUCTION. The details are as follows: “Moreover, Mohsen et al[4] demonstrated that ADSC-exo and CoQ10 administration could ameliorate memory deficits by modulating SOX2 and BDNF expression in a rat model of Alzheimer's disease. Numerous preclinical studies have confirmed the therapeutic potential of ADSCs-

derived exosomes in the neurodegenerative diseases[5, 6]. Glaucoma is a neurodegenerative disease in which the role of the ADSC-exos has not been studied yet." please check if they meet the requirements, thank you!

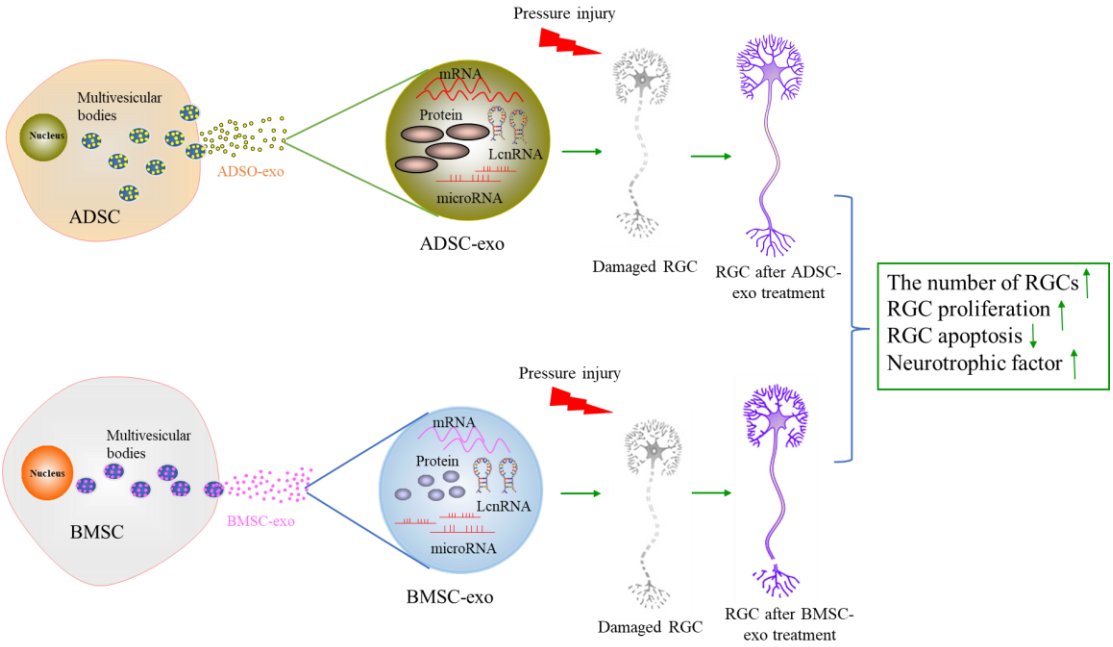
Point 3. The materials and Methods section requires more information: To confirm the success of mesenchymal stem cells derived from human adipose and bone marrow tissues isolation, in addition to the electron microscope method, it is necessary to confirm using trilineage differentiation potential by Alcian Blue, Alizarin Red staining and Oil Red O staining and flow cytometry. As a result, it is better to include the results of flow cytometry method and Alcian Blue, Alizarin Red staining and Oil Red O staining and flow cytometry in the result section of the present paper. Furthermore, to confirm the success of exosome isolation, in addition to the electron microscopy and western blotting methods, it is necessary to confirm using the size-based method, including DLS. As a result, it is better to include the results of DLS method in the result section of the present paper.

Response 3: Thanks to the reviewers for constructive comments on the Materials and Methods section. The BMSCs and ADSCs were a kind gift from Kunming Cell Bank of Chinese Academy of Sciences (Kunming, China). Therefore, the trilineage differentiation potential has not been done. Moreover, due to the limited conditions of our laboratory, we were unable to perform DLS.

Point 4. In order to make the paper more interesting to read, I suggested that the authors

could add a graphical abstract to the manuscript.

Response 4: A graphical abstract has been made as follow and added in the revised manuscript to make the paper more interesting to read. please check if they meet the requirements, thank you!



Reviewer #2:

Specific Comments to Authors: Comment: The authors did not pay attention to definitions of scientific terminology. E.g., “After SD rats were anesthetized with 4% chloral hydrate (0.1 g/L) by intraperitoneal injection, rat bone marrow blood was collected for BMSC extraction, and inguinal adipose tissue was collected for ADSC extraction. The procedure of BMSC extraction was as follows: The collected rat bone marrow blood was placed in a 15 mL centrifuge tube,” What did they use, “rat bone marrow blood” – either rat bone marrow or blood – did they mix both up? No QC for either. Another example was the retinal ganglion cell injury: Neither in vivo nor

functional data was present but claimed its impact. Some essential elements were missing, such as the IACUC and reference structure. The entire manuscript was not written logically. Neither did the authors narrate why nor how, but they overstate their conclusion without data support. The following 20 specifics should be observed for clarity. Specific comments:

Point 1. The current version of the title "Mesenchymal stem cell-derived exosomes from different sources significantly ameliorate the retinal ganglion cell injury induced by high-pressure" did not capture the content accurately or entirely. Thus, it misled the reader.

Response 1: We changed our title according to the reviewer's comment and the new title is: ADSC-exos present better effects on ameliorating RGC injury induced by hydrostatic pressure through upregulating nerve growth factors than BMSC-exos. Please check if they meet the requirements, thank you!

Point 2. "The RGC injury model was constructed by RGC damage under different pressures (40, 80, 120 mmHg)." How did these conditions differ from the in vivo pathological progression changes on RGCs? Any reference to the conditions?

Response 2: Hydrostatic pressure is known to have an impact on various aspects of cellular anatomy and physiology. Morphological changes in cell shape, alignment and processes and cytoskeletal actin redistribution have been demonstrated in rat RGCs[7, 8]. Agaretal et.al showed that neurons may undergo apoptosis in direct response to increased pressures at clinically relevant levels[9]. Cell cultures were subjected to

elevated hydrostatic pressures in an in vitro system based on established pressure chamber models[10, 11]. Experimental pressure conditions were selected to be relevant to intra-ocular pressures seen in clinical settings, with levels of 100mm Hg analogous to acute glaucoma, 30mm Hg for chronic glaucoma and 15mm Hg for the so-called 'normal' IOP. Therefore, hydrostatic pressures of 40, 80, and 120 mmHg were chosen for this study. Part of this paragraph was added in the discussion section.

Point 3. The methodology was not written logically.

Response 3: This has duly been rewritten for clarity. Please check if they meet the requirements, thank you!

Point 4. Lack of QC for BM-MSCs or ADSC-MSCs?

Response 4: The BMSCs and ADSCs were a kind gift from Kunming Cell Bank of Chinese Academy of Sciences (Kunming, China). Unfortunately, we were unable to obtain QC for BM-MSCs or ADSC-MSCs. We sincerely apologize for our negligence on this problem.

Point 5. How did they isolate ADSC-MSCs? QC reports? Yields?

Response 5: The ADSCs were a kind gift from Kunming Cell Bank of Chinese Academy of Sciences (Kunming, China). Unfortunately, we were unable to obtain QC for BM-MSCs or ADSC-MSCs. We sincerely apologize for our negligence on this problem.

Point 6. Rat RGC-5 cells: QC reports?

Response 6: According to previous studies [12, 13], we extracted RGC cells according to the following procedure and characterised them by immunofluorescence. “SD rats 1-2 days old were used to be executed by intraperitoneal injection of 4.3% chloral hydrate, and the eyeballs were removed under aseptic conditions and rinsed three times. Under the microscope, the retinal neuroepithelial layer tissue was isolated. The tissue was rinsed 3 times and digested in 0.5 g/L trypsin for 30 min at 37°C. The digestion was terminated by the addition of DMEM containing 10% FBS and filtered through a 40-μm filter. Centrifuge at room temperature for 5 min, discard the supernatant, and add DMEM complete medium. After adjusting the cell density, the cells were inoculated into cell culture flasks. Place in a 5% CO_2 incubator at 37°C for 7-10 days. The obtained cells were taken and identified by immunofluorescence staining method using Thy1.1 antibody.” has been added in the Materials and Methods section.

Point 7. “The other tube of the tee tube was used to connect the pressure gauge and the culture bottle, so that the pressure reached the expected value (40 mmHg, 80 mmHg, 120 mmHg),” What was the device? For how long? QC reproducibility of the measurement and references?

Response 7: We thank the reviewer for raising this issue, this point is very important for supporting our study results. In this study, pressure chamber was chosen according to previous studies [14, 15], and the incubating gas mix was pressurised. The cells were exposed to conditions of elevated ambient hydrostatic pressure (40 mmHg, 80 mmHg, 120 mmHg), over and above atmospheric, for a period of 2h. The pressure conditions

were then restored to atmospheric, and the culture dishes removed from the chamber. Under normal cell culture conditions (37℃, 5% CO_2), the culture was continued for 24 h.

Point 8. "The RGC damage induced by different pressures (40, 80, 120 mmHg) was significantly reduced by ADSC-expos and BMSC-expos treatment. At the same time, the proliferative activity was increased, and the apoptosis was inhibited of RGCs." Neither logical nor observable in the context of the logical flow.

Response 8: Thank you very much for the compliments for the detailed comments. We have rewritten this passage in the revised version: The gibbosity of RGCs was less and the cells were irregularly ellipsoidal under pressure and the addition of ADSC-exos and BMSC-exos significantly restored RGC morphology. Furthermore, the proliferative activity of RGCs was increased and the apoptosis of RGCs was inhibited. Try to make our manuscript more logical.

Point 9. How did they define "damaged RGCs?"

Response 9: The cell morphology, cell proliferation, the levels of apoptosis-related factors, and neurotrophic factors are considered to be indicators of the damaged RGCs. In the study, we mainly focused on apoptosis-related indicators. We observed morphological changes in RGCs through phase contrast microscopy and TEM. The CCK-8 and flow cytometry were used to detect the viability and apoptosis of RGCs. The degree of RGC neuron damage evaluated by the Lactate Dehydrogenase release test. Moreover, apoptosis-related mRNA and protein were detected by qRT-PCR and

Western blot.

Point 10. “These findings indicated that ADSC-expos and BMSC-expos could ameliorate optic nerve injury caused by pressure by inhibiting apoptosis and increasing the secretion of neurotrophic factors.” Which is not supported by its data.

Response 10: Thanks for your critical appraisal. The CCK-8 and flow cytometry were used to detect the viability and apoptosis of RGCs. Neurotrophic factors (CNTF, BDNF, NGF) were detected by qRT-PCR and Western blot. The conclusions are well supported by the data presented.

Point 11. Fig 1A, B, missing scale bars. Neither ADSCs nor BMSCs came with Quality control parameters support and yields. Fig 1C, size range? Fig D, negative controls?

Response 11: We are very sorry that because of our personal negligence. The scale bars have been added in Fig 1. Due to the limited conditions of our laboratory, we were unable to perform NLS. Calnexin is used as a negative marker in Fig D. Please check if they meet the requirements, thank you!

Point 12. Fig 2. That is not a sufficient description. How did they determine damages quantitively in how many viewfields? Fig 2A missing scale bars embedded.

Response 12: For each condition, we randomly chose at least 10 fields-of-view and took images. Scale bars have been embedded in Fig 2A.

Point 13. Fig 3. How did they quantify?

Response 13: We calculated the fluorescent intensity with ImageJ Software to quantify.

Point 14. Fig 4: how did they ensure the time intervals were physiologically relevant?

Response 14: In general, CCK8 is detected at 24, 48 and 72 hours according to the instructions.

Point 15. Fig 5 missing scale bars embedded. How did they quantify?

Response 15: Thank the reviewer for his/her thoughtful guidance. The ruler had been added in Fig 5. The number of RGCs was counted by imageJ software.

Point 16. Fig 6. How did this pattern relate to the above data sets?

Response 16: LDH is present in all cells. When RGC-5 is damaged, it is released rapidly. When LDH is elevated, it indicates that RGC-5 is damaged or dysfunctional, which leads to apoptosis. Therefore, this study explored the effects of exosomes from different sources on LDH in RGCs. Furthermore, the anti-apoptotic protein (Bcl-2), the pro-apoptotic protein (Bax, caspase3, caspase9, CREB), and neurotrophic factor (BDNF, NGF, CNTF, TRKA, TRKB) were detected by Western blot and qRT-PCR. These pathological changes are important events to induce RGC apoptosis.

Point 17. Missing the names of the Journals and the authors: 28 Wang Y, Lv J. Human umbilical cord-mesenchymal stem cells survive and migrate within the vitreous cavity and ameliorate retinal damage in a novel rat model of chronic glaucoma. 2021; 2021: 8852517 [PMID: DOI: 10.1155/2021/8852517 [correct: Stem Cells Int . 2021 Oct 25:2021:8852517. doi: 10.1155/2021/8852517. eCollection 2021.] 29 Seyedrazizadeh SZ, Poosti S, Nazari A, Alikhani M, Shekari F, Pakdel F, Shahpasand K, Satarian L, Baharvand H. Extracellular vesicles derived from human es-mscs protect retinal

ganglion cells and preserve retinal function in a rodent model of optic nerve injury. 2020; 11: 203 [PMID: DOI: 10.1186/s13287-020-01702-x [Journal?]

Response 17: We appreciate the reviewer`s meticulous comment. The Journal name of the references has been added.

Point 18. Many grammar errors crawl around the pages. For example, “After digestion, add 7.5mL of DMEM containing FBS (low sugar 10%) to terminate the digestion, and filter through a strainer. Finally, the digested solution was collected and centrifuged at 1500 r/min for 5 min,” – inconsistent in the tense usage. “FBS (low sugar 10%)” – FBS came with sugar?

Response 18: Thank reviewer for pointing out our mistakes. According to the reviewer and Journal's request, our revised draft was submitted to a professional translation company for retouching in the English language. They helped us make some language corrections to this revised version of the manuscript. Moreover, we have removed details of the process of extraction of ADSC and BMSC as the cells are a kind gift from Kunming Cell Bank of Chinese Academy of Sciences (Kunming, China)

Point 19. The discussion was not tied to its data but drifted around without proper references. E.g., “There is evidence that ADSCs are most conducive to clinical utilization. Besides, adipose tissue is relatively abundant in the human body compared with other tissues. ADSCs can be isolated from adipose tissue. In addition, 500 times more stem cells were obtained from adipose tissue than from the same amount of bone marrow. Moreover, ADSCs are easier to obtain from the adipose tissue due to their subcutaneous location than BMSCs. Patients tend to choose less traumatic sites for

collecting tissue." (who did what and how and why?)

Response 19: Thank you to the reviewer for this question. According to your suggestion, we have added proper references in the part of discussion.

Point 20. The authors need to observe the format of manuscript structures.

Response 20: Thank you. Sorry about that. we revised the manuscript carefully according to the required format of the journal.

Reviewer #3:

Point 1. This is a comparative study evaluating the effect bone marrow stem cell-derived exosomes and adipose stem cell-derived exosomes on retinal ganglion cell exposed to pressure injury (40, 80, and 120 mmHg), please consider these information in the title.

Response 1: Thank you to the reviewer for pointing out this issue. Since the title of the article should not be too long according to the rules, we summarise the title accurately and the title of this article has been changed to "ADSC-exos present better effects on ameliorating RGC injury induced by hydrostatic pressure through upregulating nerve growth factors than BMSC-exos", please check if they meet the requirements, thank you!

Point 2. The title, aim and conclusion should be consistent, regarding hypothesis (null or alternative).

Response 2: The title of this article has been reworded to "ADSC-exos present better

effects on ameliorating RGC injury induced by hydrostatic pressure through upregulating nerve growth factors than BMSC-exos", the aim "This study aimed to investigate the ameliorate effect of exosomes derived from different mesenchymal stem cells on retinal ganglion cell (RGC) injury induced by hydrostatic pressure.", and the conclusion "These findings indicated that ADSC-exos and BMSC-exos could ameliorate RGC injury caused by hydrostatic pressure by inhibiting apoptosis and increasing the secretion of neurotrophic factors." They're all consistent with each other. please check if they meet the requirements, thank you!

Point 3. The method of obtaining BMSCs should be clearly described and referenced.

Response 3: I apologize for our carelessness. In fact, the BMSCs and ADSCs were a kind gift from Kunming Cell Bank of Chinese Academy of Sciences (Kunming, China). This information has been added in the Material and Methods section. At the same time, we deleted the paragraphs related to extraction, isolation, and culture of BMSC and ADSC in the Material and Methods section to make our manuscript more succinct. Thank you for your comments.

Point 4. The concentration of exosomes were 20 μg/mL, depending on what?

Response 4: The concentration of exosomes were 20 μg/mL according to previous studies[16, 17]. The reference has been added in the revised manuscript.

Point 5. Pancreatic enzyme, whish one was used, please specify.

Response 5: Pancreatic enzyme is Trypsin-EDTA Solution (Beyotime, Shanghai,

China), which has been clearly stated in the revised manuscript.

Point 6. Method of characterization of stem cells is not sufficient, positive and negative markers should be provided.

Response 6: Thank you for this comment. In fact, the BMSCs and ADSCs were a kind gift from Kunming Cell Bank of Chinese Academy of Sciences (Kunming, China). So we did not perform detailed analyses of characterization of stem cells. However, we detected stem cell positive markers SOX2 by immunofluorescence.

Point 7. Exosome marker molecules CD9, CD63, and CD81, which one is positive and which one is negative.

Response 7: Suitable markers for exosome marker molecules are known to be CD9, CD63, and CD81 positive, and Calnexin is used as a negative marker (see literature below).

1. Deng F, Miller J. A review on protein markers of exosome from different bio-resources and the antibodies used for characterization. Journal of histotechnology 2019; 42: 226-239 [PMID: DOI: 10.1080/01478885.2019.1646984

2. Lötvall J, Hill AF, Hochberg F, Buzás EI, Di Vizio D, Gardiner C, Gho YS, Kurochkin IV, Mathivanan S, Quesenberry P, Sahoo S, Tahara H, Wauben MH, Witwer KW, Théry C. Minimal experimental requirements for definition of extracellular vesicles and their functions: A position statement from the international society for extracellular vesicles. Journal of extracellular vesicles 2014; 3: 26913 [PMID: Pmc4275645 DOI: 10.3402/jev.v3.26913

Point 8. Please report the significance of using TEM in the characterization of the stem cells (morphology/structure) and their derived exosomes (size).

Response 8: Exosomes are small membranous vesicles containing complex RNA and proteins, specifically discoidal vesicles with a diameter of 40-100 nm, which originate mainly from the formation of multivesicular bodies by the invagination of intracellular lysosomal particles, and are released into the extracellular matrix after fusion of the outer membrane of the multivesicular body with the cell membrane. Transmission electron microscopy projects an accelerated and aggregated electron beam onto a very thin sample, where the electrons change direction by colliding with atoms in the sample, resulting in steric angle scattering. The magnitude of the scattering angle is related to the density and thickness of the sample, so that light and dark images can be formed. Transmission electron microscopy has a resolution of 0.1-0.2nm and a magnification of tens of thousands to millions of times. It is used to observe ultrastructure, i.e., structures smaller than 0.2μm, which are not visible under an optical microscope, also known as "sub-microscopic structures", and is therefore an important method for characterising nanoparticles in terms of their size and morphology, and is more suited to observing the morphology of exosomes. Therefore, TEM is an important method to characterise the size and morphology of nanoparticles and is more suitable for observing the morphology of exosomes.

Moreover, TEM in the characterization of the stem cells (morphology/structure) and their derived exosomes (size) will make the results more convincing.

Point 9. The abbreviation NC referred to normal control or what?

Response 9: It is really true as Reviewer suggested that the abbreviation NC referred to normal control. We have made an explanation in the "Primary RGCs extraction, culture, and grouping" part of the Material and Methods section.

Point 10. Quantification and the purity of the exosomes should be considered in the methodology.

Response 10: We agree with the reviewer's suggestions. Quantification and the purity of the exosomes have been added in the Material and Methods section.

Point 11. Figure 3 report the results of CREB and pCREB not β III-tubulin.

Response 11: We are very sorry for our negligence of misplacing Figure 3. We have checked all the figures and put them into the correct locations. Thank you for your correction.

Point 12. Figure 5 report the results of β III-tubulin not CREB and pCREB.

Response 12: Response 11: We are very sorry for our negligence of misplacing Figure 5. We have checked all the figures and put them into the correct locations. Thank you for your correction.

References

1 Liu L, Sha XY, Wu YN, Chen MT, Zhong JX. Lycium barbarum polysaccharides protects retinal ganglion cells against oxidative stress injury. Neural regeneration research 2020; 15: 1526-1531 [PMID: Pmc7059572 DOI: 10.4103/1673-5374.274349

2 Li Q, Cheng Y, Zhang S, Sun X, Wu J. Trpv4-induced müller cell gliosis and tnf-α elevation-mediated retinal ganglion cell apoptosis in glaucomatous rats via jak2/stat3/nf-κb pathway. 2021; 18: 271 [PMID: DOI: 10.1186/s12974-021-02315-8

3 Kanamoto T, Okumichi H, Rimayanti U, Kiuchi Y. Cullin5 reduces retinal cell death induced by glutamate toxicity. Current eye research 2011; 36: 66-70 [PMID: DOI:

10.3109/02713683.2010.514658

4 Sheykhhasan M, Amini R, Soleimani Asl S, Saidijam M, Hashemi SM, Najafi R. Neuroprotective effects of coenzyme q10-loaded exosomes obtained from adipose-derived stem cells in a rat model of alzheimer's disease. Biomedicine & pharmacotherapy = Biomedecine & pharmacotherapie 2022; 152: 113224 [PMID: DOI: 10.1016/j.biopha.2022.113224

5 Li Q, Wang Z, Xing H, Wang Y, Guo Y. Exosomes derived from mir-188-3p-modified adipose-derived mesenchymal stem cells protect parkinson's disease. Mol Ther Nucleic Acids 2021; 23: 1334-1344 [PMID: Pmc7920810 DOI: 10.1016/j.omtn.2021.01.022

6 Jiang M, Wang H, Jin M, Yang X, Ji H, Jiang Y, Zhang H, Wu F, Wu G, Lai X, Cai L, Hu R, Xu L, Li L. Exosomes from mir-30d-5p-adscs reverse acute ischemic stroke-induced, autophagy-mediated brain injury by promoting m2 microglial/macrophage polarization. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology 2018; 47: 864-878 [PMID: DOI: 10.1159/000490078

7 Liu B, Ma X, Guo D, Guo Y, Chen N, Bi H. Neuroprotective effect of alpha-lipoic acid on hydrostatic pressure-induced damage of retinal ganglion cells in vitro. Neuroscience letters 2012; 526: 24-28 [PMID: DOI: 10.1016/j.neulet.2012.08.016

8 Ju WK, Kim KY, Lindsey JD, Angert M, Patel A, Scott RT, Liu Q, Crowston JG, Ellisman MH, Perkins GA, Weinreb RN. Elevated hydrostatic pressure triggers release of opa1 and cytochrome c, and induces apoptotic cell death in differentiated rgc-5 cells. Molecular vision 2009; 15: 120-134 [PMID: Pmc2629709,

9 Agar A, Yip SS, Hill MA, Coroneo MT. Pressure related apoptosis in neuronal cell lines. Journal of Neuroscience Research 2000; 60: [PMID,

10 An in vitro system for studying the effect of ambient hydrostatic pressure on growth, morphology, and biochemical aspects of ganglion cells Proceedings of the Annual Meeting of the; 2003,

11 Mattana J, Singhal PC. Applied pressure modulates mesangial cell proliferation and matrix synthesis. [PMID:

12 Chan-Juan H, Sen L, Li-Qianyu A, Jian Y, Rong-Di Y. Microrna-30b regulates the polarity of retinal ganglion cells by inhibiting semaphorin-3a. Molecular vision 2019; 25: 722-730 [PMID: Pmc6857778,

13 Xu C, Lu H, Li F, Su G. Protein expression profile on differentiation of bone marrow mesenchymal stem cells into retinal ganglion-like cells. Journal of computational biology : a journal of computational molecular cell biology 2020; 27: 1329-1336 [PMID: DOI: 10.1089/cmb.2019.0024

14 Coroneo MT, Li S, Agar A, Hill M. Pressure related apoptosis in human & neuronal cell lines. Investigative ophthalmology & visual science 2001; 42: S23-S23 [PMID,

15 Agar A, Li S, Agarwal N, Coroneo MT, Hill MA. Retinal ganglion cell line apoptosis induced by hydrostatic pressure. Brain Research 2006; 1086: 191-200 [PMID,

16 Ren S, Chen J, Guo J, Liu Y, Xiong H, Jing B, Yang X, Li G, Kang Y, Wang C, Xu X, Liu Z, Zhang M, Xiang K, Li C. Exosomes from adipose stem cells promote diabetic wound healing through the ehsp90/lrp1/akt axis. 2022; 11: [PMID: DOI: 10.3390/cells11203229

17 Yang W, Huang C, Wang W, Zhang B, Chen Y, Xie X. Bone mesenchymal stem cell-derived

exosomes prevent hyperoxia-induced apoptosis of primary type ii alveolar epithelial cells in vitro. PeerJ 2022; 10: e13692 [PMID: Pmc9443791 DOI: 10.7717/peerj.13692

Thanks very much for your attention to our paper.

Sincerely yours,

Min Dai,

Correspondence to: Department of Ophthalmology, Affiliated Hospital of Yunnan University, No. 176, Qingnian Road, Wuhua District, Kunming City, Yunnan Province, China. E-mail address: dm9024@163.com.

Dear reviewers:

Thank you for your decision and constructive comments on my manuscript. We have carefully considered the suggestion of Reviewers and make some changes. We have tried our best to improve and made some changes in the manuscript.

The yellow part that has been revised according to your comments. Revision notes, point-to-point, are given as follows:

1) This uploaded file was wrongly placed: “

Download	Answering Reviewers	88018-Answering Reviewers-revision.mp3	0.96MB	2023-11-17 02:54	min dai	Author

Response 1: Due to our carelessness, we apologised for uploading the wrong file. This time we have uploaded the “88018-Answering Reviewers” file, please check f it meets the requirements, thank you!

2) What was the biomarker profile by the authors? (DEG analysis identified subsets of RGCs with markers like MAP2, RBPMS, TUJ1, BRN3A, SOX4, TUBB3, SNCG, PAX6 and NRN1) [PMID: 34946963]. RGC-specific in rats, mice, and macaques is BRN3A [PMID: 36594396 PMCID: PMC9841181 DOI: 10.24272/j.issn.2095-8137.2022.308]. In the Manuscript, they used Thy1.1 as the ID of RGCs; however, “Thy1.1 has also been used as a marker for RGC loss, but after optic nerve crush (ONC) a decrease in Thy1.1 expression precedes the loss of RGCs”[PMID: 24526440 DOI: 10.1167/iovs.13-12986].

Response 2: Thy-1.1 antigen expression in RGCs begins at 19 d of the embryo and is only expressed in RGCs and protrusions, and only increases at 14 d of life, parallel to the increase in the thickness of the inner plexiform layer, which is thought to correlate with axon elongation, dendritic growth, and establishment of synaptic connections with the target superior colliculus of the target tissue [1]. Bamstable et al. [2] found that Thy-1.1 antigen is a specific marker for rodent RGCs. Takahash et al [3] also identified RGCs with anti-mouse Thy-1.1 mAb. Therefore, we chose Thy-1.1 antibody to identify RGCs.

3) The R1 title was not clear. "ADSC-exos present better effects on ameliorating RGC injury induced by hydrostatic pressure through upregulating nerve growth factors than BMSC-exos" could be modified to “ADSC-exos

outperform BMSC-exos in alleviating hydrostatic pressure-induced injury to retinal ganglion cells by upregulating nerve growth factors."

Response 3: As requested by the Reviewer we have amended the title to: ADSC-exos outperform BMSC-exos in alleviating hydrostatic pressure-induced injury to retinal ganglion cells by upregulating nerve growth factors.

4) Fig 1 C & D did not show differences between ADSC-exos and BMSC-exos in those biomarkers. How did they explain the differences between their effects on RGCs?

Response 4: Fig 1C & D are generic biomarkers for exosomes and they are common to all exosomes. Previous studies have already identified some markers of exosomes: CD9, CD63, and CD81[4, 5]. Expression of these markers demonstrated successful isolation and extraction of exosomes. However, significant discrepancies are observed in cellular exosomes from different sources, including the type and content of mRNAs, miRNAs, and proteins[6]. This explains the different effects of exosomes from different sources on RGCs.

5) Fig 2: "The RGC damage models under 40, 80, and 120 mmHg pressure were constructed," – What assays did they use for quality controls of their models as the standardized starting points?

Response 5: We thank the reviewer for raising this issue, this point is very important for supporting our study results. In this study, pressure chamber was chosen according

to previous studies[7, 8], and the incubating gas mix was pressurised. The cells were exposed to conditions of elevated ambient hydrostatic pressure (40 mmHg, 80 mmHg, 120 mmHg), over and above atmospheric, for a period of 2h. The pressure conditions were then restored to atmospheric, and the culture dishes removed from the chamber. Under normal cell culture conditions (37℃, 5% CO2), the culture was continued for 24 h.

Thanks very much for your attention to our paper.

Sincerely yours,

Min Dai,

Correspondence to: Department of Ophthalmology, Affiliated Hospital of Yunnan University, No. 176, Qingnian Road, Wuhua District, Kunming City, Yunnan Province, China. E-mail address: dm9024@163.com.

1. Leifer D, Lipton SA, Barnstable CJ, Masland RH: **Monoclonal antibody to Thy-1 enhances regeneration of processes by rat retinal ganglion cells in culture**. *Science* 1984, **224**(4646):303-306.
2. Barnstable CJ, Dräger UC: **Thy-1 antigen: a ganglion cell specific marker in rodent retina**. *Neuroscience* 1984, **11**(4):847-855.
3. Caprioli CJ: **Rat retinal ganglion cells in culture**. *Experimental Eye Research* 1991.
4. Fan Y, Pionneau C: **Differential proteomics argues against a general role for CD9, CD81 or CD63 in the sorting of proteins into extracellular vesicles**. 2023, **12**(8):e12352.
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