

Dear Editor and Reviewers,

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "6-Gingerol protects nucleus pulposus-derived mesenchymal stem cells from oxidative injury by activating autophagy (Journal: World Journal of Stem Cells, Manuscript NO. 55783)". Those comments are all valuable and very helpful for improving our study and revising our paper. We have studied the comments carefully and made necessary correction.

The corrections in the paper were marked in red in the revised manuscript and the responds to the reviewer's comments are as following:

Key points of revising the manuscript

(1) **Scientific quality:** Please resolve all issues in the manuscript based on the peer review report and make a point-by-point response to the issues raised in the peer review report.

Responses: We have resolved the issues in the manuscript based on the peer review report and made a point-by-point response to the issues raised in the peer review report.

(2) **Language quality:** Please resolve all language issues in the manuscript based on the peer review report. Please be sure to have a native-English speaker edit the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability, so that the manuscript's language will meet our direct publishing needs.

Responses: We have resolved all language issues in the manuscript based on the peer review report. A native-English speaker helped us re-edited the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability edit the manuscript for grammar.

(3) **Special requirements for figures:** Figures must be presented in the order that they appear in the main text of the manuscript (numbered as 1, 2, 3, *etc.*). The requirements for the figures and figure legends include: (A) All submitted figures, including the text contained within the figures, must be editable. Please provide the text in your figure(s) in text boxes; (B) For line drawings that were automatically generated with software,

please provide the labels/values of the ordinate and abscissa in text boxes; (C) Please prepare and arrange the figures using PowerPoint to ensure that all graphs or text portions can be reprocessed by the editor; and (D) In consideration of color-blind readers, please avoid using red and green for contrast in vector graphics or images.

Responses: We have resolved the issues mentioned above.

(4) *Special requirements for tables:* Tables must be presented in the order that they appear in the main text of the manuscript (numbered as 1, 2, 3, *etc.*). Please verify that the tables are referred to in the text by their respective Roman numerals and that the numbering order is correct and format the tables. Please verify that there are no missing or multiple spaces in the text and tables, *e.g.* before or after parentheses, between words, or before or after symbols like +, ×, ±, <, >, ≥, and ≤. Please verify that special words or letters in the text and tables are correct, *e.g.* *P* (uppercase), *n* (lowercase), *via*, *vs* (lowercase, no punctuation), *in vivo*, *in vitro*, and *et al* (no punctuation) are italicized.

Responses: We have resolved the issue mentioned above.

(5) *Special requirements for references:* Please provide the PubMed numbers and DOI citation numbers to the reference list and list all authors of the references. Please revise throughout. NOTE: The PMID is required, and NOT the PMCID; the PMID number can be found at <https://pubmed.ncbi.nlm.nih.gov>. (Please begin with PMID:) The DOI number can be found at <http://www.crossref.org/SimpleTextQuery/>. (Please begin with DOI: 10.**).

Please verify that the references are cited by Arabic numerals in square brackets and superscripted in the text, and that the numbering order is correct. There should be no space between the bracket and the preceding word or the following punctuation. When references in the text and tables are cited with author name, it is necessary to manually verify that the name is consistent with the first author's family (sur)name in the corresponding reference list, *e.g.* Wang *et al*^[27], Vanhoos *et al*^[53].

Responses: We have re-edited the corresponding part as requested.

(5) *Special requirements for Article Highlights:* If your manuscript is an original study (basic study or clinical study), meta-analysis, or systemic review, the “Article

Highlights” section should be provided. Detailed writing requirements for “Article Highlights” can be found in the Guidelines and Requirements for Manuscript Revision.

Responses: We have added the “Article Highlights”.

(6) Ethical documents: Please double check the accuracy of all ethical documents and verify the completeness of the documents according to the type of manuscript.

Responses: We have resolved the issue mentioned above.

(7) Approved grant application form(s) or funding agency copy of any approval document(s): If your manuscript has supportive foundations, the approved grant application form(s) or funding agency copy of any approval document(s) must be provided.

Responses: We have added the related approval document(s).

Manuscript revision deadline

We request that you submit your revision in no more than **14 days**.

Responses: We applied for an extension of manuscript revision deadline via e-mail.

Peer-review report(s)

Authors must resolve all issues in the manuscript that are raised in the peer-review report(s) and make point-by-point responses to the issues raised in the peer-review report(s), which are listed below:

Responses: The point-by-point responses responds to the reviewer's comments are as following:

Reviewer #1:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: This manuscripts is presenting that the protective effect of 6-GIN on oxidative stress-induced injury of NPMSC by controlling the autophagy signaling regulation for the first time.

In Figure 6, the protein expressional changes of Beclin-1 did not seem to be appropriately changed with 6-GIN treatment. Additionally, the beta-actin expression

also presented with significant changes. Furthermore, the phosphorylated AKT expression also showed no significant changes with 6-GIN treatment in Figure 7. Authors need to make these protein expressional changes concrete as well as make clear which residue of AKT was phosphorylated. Although it seems that it is well-organized manuscript, still it needs to be further taken the English editing including typos and errors. Besides aforementioned issue, authors need to correct and add more explanation in detail list below, which were also marked in yellow highlight with comments in attached file.

Responses: We are sorry for causing this misunderstanding. We confirmed the final result of the Figure 6B and Figure 7 after repeated study of that part. Thus, we exchanged with higher quality figures to make these protein expressional changes concrete in Figure 6 and Figure 7.

A native-English speaker helped us re-edited the manuscript for grammar, sentence structure, word usage, spelling, capitalization, punctuation, format, and general readability edit the manuscript for grammar. We have corrected and added explanation in detail list below, which were in attached file.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: The results presented in the manuscript entitled “6-Gingerol protects nucleus pulposus-derived mesenchymal stem cells from oxidative injury by activating autophagy” are in a logical sequence with appropriate analysis with figures and table to that contain data to inform the readers. The manuscript builds upon previous important research that is appropriately referenced. The data from this manuscript does move the canon of knowledge forward and may be considered by the Top 10 % of the research field. The manuscript is sufficiently novel and interesting to warrant publication in “World Journal of Stem Cells” after revision.

1) Why is the “Abstract section” so long? Please re-write it briefly.

Responses: Thank you for your valuable comments. The reason for the long “Abstract section” is 350 words at least is required in the “Guidelines for Manuscript Preparation” of this journal. However, we have revised and shortened the “Abstract section” as short as possible.

2) Which passage of cells were used? Please specify in the text of manuscript.

Responses: Third passage (P3) cells were used for subsequent experiments. We have specified in the text of manuscript.

3) The identification and characterization method of isolated mesenchymal stem cells must be discussed in detail. Also, it could better to add the flow cytometry diagrams in the “figure 2”. You can use and refer the following paper which explained elaborately and completely the “flow cytometric analysis and multi-lineage differentiation method for characterization of mesenchymal stem cells” in “Identification of Immunophenotype” and “Multilineage differentiation” sections: | Immunophenotypic characterization, multi-lineage differentiation and aging of zebrafish heart and liver tissue-derived mesenchymal stem cells as a novel approach in stem cell-based therapy. Tissue and Cell. 2019 Apr 1;57: 15-21.

Responses: Thank you for your detailed comments. We have added the flow cytometry diagrams in “Figure 2” and revised the corresponding part by referring to your recommended reference.

4) Also, the Annexin/V assessment method must be explained in detail. You can use and refer the following paper which explained and analyzed elaborately the “Annexin-V/PI”. | Cytokines secreted from bone marrow derived mesenchymal stem cells promote apoptosis and change cell cycle distribution of K562 cell line as clinical agent in cell transplantation. PloS one. 2019;14(4).

Responses: Thank you for your valuable comments. We have revised the corresponding part by referring to your recommended reference.

5) It could be better to calculate the Bax/Bcl2 ratio in the figure 4. This calculation was previously reported by Fathi et al. (2019) as appears bellow: \ Cytokines secreted from bone marrow derived mesenchymal stem cells promote apoptosis and change cell cycle distribution of K562 cell line as clinical agent in cell transplantation. PloS one. 2019;14(4).

Responses: Thank you for your detailed suggestion. We have revised the corresponding part by referring to your recommended reference.

6) Which software was used for primer designing? Please involved in the text of the manuscript.

Responses: Thank you for your detailed suggestion, the corresponding description as “Primer 5.0 (Premier Biosoft, Palo Alto, CA, USA) software was used to design the primer sequences” was added in the manuscript.

7) The number of ethical code must be added in the section of “methods section “.

Responses: Thank you for your valuable comments. The ethical code (All procedures were approved by the Research Ethics Committee of Clinical Medical College of Yangzhou University (No. SBY2019-023)) was added in the section of “methods section” now.

Reviewer #3:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The manuscript by Nan et al, “6-Gingerol protects nucleus pulposus derived mesenchymal stem cells from oxidative injury by activating autophagy” reports a protective effect exerted by 6-Gingerol against hydrogen peroxide-induced injury on nucleus pulposus derived mesenchymal stem cells (NPMSC) showed. The experiments are well designed and described. The results are satisfactorily exposed and discussed. The literature is properly cited.

Minor suggestions: Line 14: “Expressions of extracellular matrix (ECM)”, “Expression” should be deleted; Line 98: “complete medium for MSCs”, please specify; Line 108,265: “HLA-DR” should be explained and mentioned in the results (line 238) Line 130: bafilomycin A1 (BAF) should be explained at its first appearance; Line 138: “(5 × 10⁵ cells/well)”, please check; Line 150: “2’7’-dichlorofluorescein diacetate”, please add “(DCFH-DA)” Line 196: “Capasee” should be “Caspase” Line 258: bar calibrations are poorly visible; please check “800µm” in Fig. 2D: Line 274, 303: “cell viability induced by hydrogen peroxide”, in this form the expression might be misleading, please rewrite. Line 351, 407, 447: please check magnification “100X” and “200X”; Line 405: “(D-F)” should be “(D-G)”; Line 448: “(G)” should be “(G-I)”; Line 472: Panels A and B should be mentioned in the legend; I believe that a further language revision is suitable. Namely, some expressions should be checked: Line 99: “Next, resuspended the cells in complete medium for MSCs and cultured at 37°C with 5% CO₂. Refreshed the culture medium every 3 days. Each primary culture were digested...”; Line 128: “To further with 10 mM 3-MA (3-Methyladenine, MedChem, China, catalog no. HY-19312) prior to exposure to hydrogen peroxide for 2 h”; Line 146: “The analyzed the ratio of green to red fluorescence by flow cytometry”; Line 320: “In additionally, the....”;

Responses: Thank you for your detailed suggestions. Those comments are all valuable and very helpful for and improving our researches and revising our paper. We have studied the comments carefully and made necessary correction in the revised paper. The corrections in the paper were marked in red in the revised manuscript.

Reviewer #4:

Scientific Quality: Grade C (Good)

Language Quality: Grade A (Priority publishing)

Conclusion: Minor revision

Specific Comments to Authors: This study evaluated the effect of 6-GIN on ROS-induced apoptosis in NPMSC. The molecular mechanisms in which 6-GIN increases Bcl-2 expression may be discussed more in detail.

Responses: Thank you for your valuable advice, we discussed the molecular mechanisms of 6-GIN increases Bcl-2 expression in detail as much as possible in line 714 to 725.

Reviewer #5:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors: The authors evaluation of autophagy is very weak. The autophagy evaluation should be confirmed using TEM, ICC for LC3 puncta, and measuring autophagy flux. The localization of Bcl2 family should be provided.

Responses: Thank you for your valuable advice, which is of great significance for us to improve the quality of our paper and guide further research. We have tried our best to perfect the experiment according to your requirements.

After consulting relevant experts and referring to related published high-quality study (Spermidine promotes nucleus pulposus autophagy as a protective mechanism against apoptosis and ameliorates disc degeneration. J Cell Mol Med, 2018, 22(6):3086-3096) in this field, transmission electron microscopy (TEM) detection considered as the gold standard for autophagy detection was added to better evaluation of autophagy.

However, this study is a preliminary study on the effect of 6-GIN on NPMSCs by regulating autophagy. Research on the localization of Bcl2 family and detailed mechanisms will be taken in the further study.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper.

We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you.

Once again, thank you very much for your comments and suggestions.

Yours sincerely, Liang Zhang Corresponding author: Name: Liang Zhang E-mail:

zhangliang6320@ sina. com