

REVIEWERS' COMMENTS:

Thank you very much for the in-depth review of the paper and for mentioning some very important points which really helped us to improve the manuscript. We have done additional work and re-written part of the manuscript accordingly. We have also carefully gone through the whole manuscript and did language corrections. All modifications are in red text. We hope that the reviewer's will now find the manuscript substantially revised with respect to the points raised by them. Here is the point-by-point response to the reviewers' comments:

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: The paper by Aslam et al. entitled "Umbilical Cord Derived Mesenchymal Stem Cells Preconditioned with Isorhamnetin: Potential Therapy for Burn Wounds" shows how preconditioning hU-MSCs with the flavonoid isorhamnetin improves their ability to promote burn healing in an animal model. Based on histological and gene expression studies, the authors conclude that the treatment improves healing due to the anti-inflammatory and tissue remodelling inducing effect of preconditioned hU-MSCs. The study is interesting and supports the application of MSCs in cell therapy for the treatment of skin wounds.

Remarks:

1- The authors should explain in the introduction why they selected isorhamnetin for study. Furthermore, in the discussion they should attempt to associate the observed effects of MSC preconditioning with this flavonoid.

We have now included the reason of selection of isorhamnetin in the introduction of the revised manuscript (page 5 and 6). Also, in the discussion we have correlated the effect of preconditioning of MSCs with this isorhamnetin (page 27 and 28).

2- Materials and methods. The details of isolation of hU-MSCs from human umbilical cord explants should be explained in more detail: size of the explant, incubation time, etc.

We have now added more information in the Methods section with reference to the isolation of human umbilical cord tissue as suggested by the reviewer (page 6).

3- Materials and methods. hU-MSCs have been characterized by surface markers, but in addition their ability to differentiate into osteoblasts, adipocytes and/or chondrocytes should have been demonstrated.

We have now added the tri-lineage (osteoblasts, adipocytes and chondrocytes) differentiation potential of hU-MSCs in the relevant sections, e.g. Methods, Results, Figure Legends. This is now part of the revised figure 2.

4- Materials and methods. The number of animals used in each treatment group should be included.

In the revised Methods Section, we have now included number of animals used in each treatment group (pages 7, 8 and 10). It is also included in the Figure Legends as “n”.

5- Results. 5 μ M isorhamnetin concentration did not affect MSC viability, but 10 μ M reduced it by approximately 50%. Have the authors tested concentrations lower than 5 μ M?

We have also checked lower concentration (2 μ M) of isorhamnetin. This concentration also did not affect the viability of MSCs. We have now included new figure 3 in the revised manuscript.

6- Results. Figure 3, scratch images are difficult to interpret. The authors should show images of the cultures at lower magnifications.

We have tried to improve figure 3. With improved resolution, the images are now clear. We have earlier published similar images using same magnifications. We hope that the images are now clear and easy to interpret.

7- Results. Figure 4. Why does the wound of the IH+MSCs group appear from day 1 surrounded by hair and the other two groups appear shaved?. Has the same methodology been used for all groups?

The methodology is same for all groups. The appearance of hair near the wounded surface correlates well with the treatment period. We have noticed that due to mislabeling, data interpretation is quite confusing and misleading. Days 4, 7 and 14 in case of burn wound correspond to days immediately after burn infliction, while days 4, 7 and 14 in case of transplantation groups correspond to days after 72 h of burn infliction. We have edited Figure 4 and its legend accordingly.

8- Results. The indication of the statistical significance in figure 3H and especially in figure 6 is very confusing. The text in the figures does not indicate with respect to which treatment the differences are significant. For example, in figure 6F, all the columns are marked with ***.

We have noticed that data interpretation is quite confusing and misleading in some cases. Therefore, we have rewritten part of the text and explained the data in simple and clear manner. We have also redrawn the graphs in both figures 3 and 6. The asterisks that appeared in all bars are due to some error. We have now included both figures in the revised manuscript.

9- Discussion. The histological analysis part is more a repetition of results than a discussion. The authors should correct this.

We have carefully gone through the histology analysis description in the Discussion Section and removed part of the text which is already described in the Results Section (page 25).

10- Discussion. Authors should clarify sentences that create confusion: - They claim that IL-1 β and IL-6 were upregulated at day 7 and 14, but then they write: "Their expression levels were decreased as the wound healing progresses." - In page 23, the authors write: "In contrast, the expression level of Bcl-2 was reduced after 7 days of burn infliction and significantly lower expression was observed as the wound progresses". However, after 14 days, it can be seen in Figure 6 that it only decreases with IH-MSD treatment. The authors should explain what they mean. - In page 23, the authors write: "the expression of Bax was extensively reduced at day 7 and day 14 post burn injury, and Bcl-2 level was elevated as compared to the burn wound control". As shown in figure 6, the expression of Bcl-2 at day 14 in IH+MSDs is lower than in the control. Therefore, the authors should correct the commentary.

We have noticed that data interpretation in case of gene expression analysis is quite confusing and misleading. We also noticed that there is a discrepancy in part of figure 6 and the corresponding text. Therefore, we have now explained the data in simple and clear manner, redrawn the graphs and corrected the errors in both figure and text (pages 25 – 27).

11- It would be interesting that the authors had studied, in addition to gene expression, the protein expression of inflammatory and remodeling cytokines in wound samples, in order to obtain more robust conclusions.

The data is always strong if protein expression analysis is also presented along with the gene expression. In this work, we have analyzed the gene expression of mostly those genes which are involved in the process of inflammation. We have also shown our histology results and our histology data also support the findings of the gene expression analysis. The factors which are involved in the process of remodeling were discussed in discussion and are now highlighted. The inflammatory cells in tissue remodeling were tracked accordingly and shown with arrows in figure 5. It is observed that inflammatory cells were greatly reduced in our treated group as compared to control. We believe that both data are now fully supported and are in line with our conclusion. We hope that the reviewers also find this satisfactory.

Reviewer #2:

Scientific Quality: Grade B (Very good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Minor revision

Specific Comments to Authors: Results carried to Discussion. Define terms (like complete remodeling) Needs some English editing

We have carefully gone through the Discussion Section and removed part of the text which is already described in the Results Section. Terms which need explanation are defined and inappropriate terms are removed. Whole manuscript is revisited for language correction and necessary editing is done.

4 REVISE THE MANUSCRIPT

4.1 Guidelines and Requirements for Manuscript Revision and the Format for Manuscript Revision: Please visit: <https://www.wjgnet.com/bpg/GerInfo/291>.

We have read the guidelines and formatted the manuscript accordingly.

4.2 Preparatory work for revising your manuscript: (1) [Original articles](#); (2) [Review articles](#); and (3) [Case report articles](#).

We have read the guidelines and revised the manuscript accordingly.

4.3 Editorial office's comments: Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

Editorial office's comments: Authors must revise the manuscript according to the Editorial Office's comments and suggestions, which are listed below:

(1) Science editor:

1) Scientific quality: The manuscript describes a basic study of the umbilical cord derived mesenchymal stem cells preconditioned with isorhamnetin. The topic is within the scope of the WJSC. (1) Classification: Grade B and Grade C; (2) Summary of the Peer-Review Report: The study is interesting and supports the application of MSCs in cell therapy for the treatment of skin wounds. However, the language needs some editing. The questions raised by the reviewers should be answered; and (3) Format: There is 1 table and 6 figures. A total of 43 references are cited, including 4 references published in the last 3 years. There are no self-citations.

2). Language evaluation: Classification: Grade B and Grade B.

We have done language editing and answered all queries by the reviewers. We have done modifications according to the reviewer's comments and now hope that the reviewer's will now find the manuscript substantially revised according to their suggestions.

3) Academic norms and rules: The authors provided the Biostatistics Review Certificate, the signed Conflict-of-Interest Disclosure Form and Copyright License Agreement, The ARRIVE Guidelines, the Institutional Animal Care and Use Committee Approval Form or Document, and the Institutional Review Board Approval Form. No academic misconduct was found in the CrossCheck detection and Bing search.

4) Supplementary comments: This is an invited manuscript. The study was supported by Akdeniz University Research Foundation. The topic has not previously been published in the WJCC.

5) Issues raised:

(1) The "Author Contributions" section is missing. Please provide the author contributions;

We have now included the “Author Contribution Section” in the revised manuscript. In this section, contribution of each author is outlined.

(2) The authors did not provide original pictures. Please provide the original figure documents. Please prepare and arrange the figures using PowerPoint to ensure that all graphs or arrows or text portions can be reprocessed by the editor.

All figures are arranged in PowerPoint and submitted for processing by the Editor.

(3) The “Article Highlights” section is missing. Please add the “Article Highlights” section at the end of the main text.

We have now included the “Article Highlights Section” in the revised manuscript at the end of the main text.

6) Re-Review: Required.

7) Recommendation: Conditional acceptance.

(2) **Editorial office director:** I have checked the comments written by the science editor. The study was without financial support. Please don't include any *, #, †, §, ‡, ¥, @....in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters. Statistical significance is expressed as $aP < 0.05$, $bP < 0.01$ ($P > 0.05$ usually does not need to be denoted). If there are other series of P values, $cP < 0.05$ and $dP < 0.01$ are used, and a third series of P values is expressed as $eP < 0.05$ and $fP < 0.01$.

We have revisited description of the statistical data and revised it according to the suggestions of the Editorial Office Director.

(3) **Company editor-in-chief:** I have reviewed the Peer-Review Report, the full text of the manuscript and the relevant ethics documents, all of which have met the basic publishing requirements, and the manuscript is conditionally accepted with major revisions. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report and the Criteria for Manuscript Revision by Authors. Before final acceptance, authors need to correct the issues raised by the editor to meet the publishing requirements.

We have done language editing and answered all queries by the reviewers. We have done modifications according to the reviewer's comments and we now hope that the reviewer's will now find the manuscript substantially revised according to their suggestions.

4.4 Requirements for figures: Please provide the decomposable Figures, whose parts are all movable and editable, organize them into a PowerPoint file, and submit as “58626-Figures.ppt” on the system. The figures should be uploaded to the file destination of “Image File”.

All figures are arranged in PowerPoint. The file is uploaded on the system as “Image File”.

4.5 Requirements for tables: Please provide the decomposable Tables, whose parts are all movable and editable, organize them into a Word file, and submit as “**58626-Tables.docx**” on the system. The tables should be uploaded to the file destination of “Table File”.

Word file of the Table is uploaded on the system as “Table File”.

4.6 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.**).

References are edited accordingly to the suggestion and included in the revised manuscript.

4.7 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.

We have now included the “Article Highlights Section” in the revised manuscript at the end of the main text.

4.8 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.

We have done language editing and corrected the text for any grammar errors.