OPEN PEER REVIEW

Open Peer Review (OPR)

As a new feature for progressing towards transparency, we decided to open a new window for all of our editors as well as authors titled "**Open Peer Review**". We hope by this new facility, our reviewers will be more motivated and authors will be more satisfied with the review process. We believe that publishing our peer review reports could make a transparent and clear environment for all our efforts within a journal, but not all reviewers tend to publish their comments.

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Advantages of "Open Peer Review" process

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- Authors' satisfactions from the review process: Increases honesty between authors and reviewers
- Education of both authors and new students
- Prevents reviewers from following their individual agendas and leading to the detection of reviewers' conflicts of interests

You can find out more at:
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Peer Review Report for "Acrylamide Induced Oxidative Cellular Senescence in Embryonic Fibroblast Cell Line (NIH 3T3): A Protection by Carvacrol"

Author(s): Mehdi Evazalipour, Pouya Safarzadeh Kozani, Pooria Safarzadeh Kozani, Sahar Shabani, Bahar Rezaei Soufi, Ehsan Zamani

Review Timeline:

Submit Date:	13 Sep 2020
Revised Date:	1 Feb 2021
Accepted Date:	16 Feb 2021

Revision (0)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (0)

7 Jan 2021

Dear AE,
Comments for the authors:
1- The references need to be updated and use more recent references.
2- The References need to be corrected (11, 14, 16, 18, 31, 33).
3- Detailed further the histological analysis and add the scale bar to understand the magnification ratio with the real dimension.
4- Revise carefully and reorganize the discussion in order to better value the good results obtained.
5- The English must be revised throughout the paper, full of mistakes and poorly formed sentences.
6- In note of text and figs, the "p" should be italic. The same changes to others.
7- Given the same effects of doses of 50 and 100, explain why you chose dose 100 as the effective dose.

8- Glutathione peroxidase plays an important role in maintaining GSH / GSSG homeostasis in stressful circumstances, and it would have been interesting to have evaluated any changes in the GSH / GSSG ratio.
9- Carvacrol and acrylamide production, lot number and should be added.
10- Carvacrol vehicle should be mentioned
Thanks.

Mohammad Javad Khodayar: Reviewer | **Revision** (0) 18 Jan 2021

Dear AE,
Here are my comments for the authors regarding this manuscript as follow;
1- According to mentioned study on cell cycle arrest, why this study was not performed on cancer cells. Please mention this in the discussion.
2- Explain more about the cell line used in the research.
3- What is the basis for choosing carvacrol concentrations and why these concentrations? Please refer to the relevant reference.
4- Explain why the concentration of 100 µM is effective in preventing cytotoxicity but 150 µM is not.
5- Concentrations in X-axis should be mentioned as µM.
6- Specify the carvacrol concentration in Figures 2 to 5.
7- Refer to the selection of the most effective carvacrol concentration in the manuscript.
8- How do you explain that carvacrol has not been effective in preventing cytotoxicity in figure 1 while in Figures 2 to 5 it has been shown to be effective in preventing H2O2 toxicity?
Thanks.

REFEREE: Associate Editor | **Revision** (0) 19 Jan 2021

Dear EIC,
This article still needs major revisions.
Thanks.

REFEREE: EIC | **Revision** (0) 21 Jan 2021

Dear Authors,
The manuscript needs to revise extensively and resubmit for further consideration. Please revise the manuscript accordingly; include your revised manuscript, a point-to-point answer to the comments provided, and highlighted them.
The following point should be considered as well.
- The introduction section is not organized well. Revised it; consider scientific writing standards; prevent from adding so many definitions. Check coherent and cohesion thoroughly. For example, the first and second paragraphs are focusing on the same subject.
- I am afraid if the purity of chemicals is missing, it seems necessary.
- “H2O2” not “H2O2” check the whole document to prevent the same typos.
- Starts the first letter of Figures with capital F!
- Most of the discussion section is the introduction; in this part, you should discuss your results and compare your findings with others. Revised according to writing standards and

use clear English.

- In the conclusion section, the sentences are repeated several times in the manuscript.

write a more transparent and concise conclusion

Thanks.

OPEN PEER REVIEW

Revision (1)

Reply to Reviewers

Ideally, the reviewing process can significantly improve the submitted manuscripts by allowing the authors to take into account the advice of reviewers. Author(s) must reply to all reviewers' comments in a separate Word file, point by point. A "**Reply to Reviewers**" document is submitted along with revised manuscript during submission of revised files, summarizing the changes that the authors made in response to the reviewers' comments. The responses to reviewers' comments specifies how the authors addressed each comment the reviewers made.

You can read the authors' responses to the reviewers' comments in the next page.

Dear Editor,

Thank you for your letter and constructive comments of respected reviewers concerning our manuscript entitled "**Acrylamide induced oxidative cellular senescence in embryonic fibroblast cell line (NIH 3T3): a protection by Carvacrol**". We have studied all comments carefully and made correction which we hope meet with your approval. We answered all questions or comments in details in the following texts which the changes are **shown with yellow band** in manuscript file**.**

Reviewer1:

1- According to mentioned study on cell cycle arrest, why this study was not performed on cancer cells. Please mention this in the discussion.

Answer: senescence-associated beta-galactosidase activity and cellular senescence have been correlated in several cell type. SA-βgal activity is undetectable in immortal cells, except when they are genetically or chemically manipulated to undergo senescence. Then we used fibroblast cells for valuation of cellular senescence. thank you for valuable comment of respected reviewer. We add about this mention in discussion section.

2- Explain more about the cell line used in the research.

Answer: as requested by respected reviewer, we explain about cell line. (please see the material and methods/ Cell culturing and treatment section).

3- What is the basis for choosing carvacrol concentrations and why these concentrations? Please refer to the relevant reference.

Answer: cells treated with concentration of carvacrol based on pretests and similar studies. We add relevant references as requested by respected reviewer.

4- Explain why the concentration of 100 µM is effective in preventing cytotoxicity but 150 µM is not.

Answer: thank you for valuable comment of respected reviewer. Concentration is a main parameter for toxicity or protective effects of compounds. Furthermore, antioxidant can act as pro-oxidant at high concentrations.(1)

Reference: 1. Sotler R, Poljšak B, Dahmane R, Jukić T, Pavan Jukić D, Rotim C, et al. PROOXIDANT ACTIVITIES OF ANTIOXIDANTS AND THEIR IMPACT ON HEALTH. Acta clinica Croatica. 2019;58(4):726-36. doi:10.20471/acc.2019.58.04.20. [PubMed:32595258].

5- Concentrations in X axis should be mentioned as µM.

Answer: we revised the figures and their legends.

6- Specify the carvacrol concentration in Figures 2 to 5.

Answer: thank you for valuable attention of respected reviewer, we corrected figure legends.

7- Refer to the selection of the most effective carvacrol concentration in the manuscript.

Answer: selection of the most effective carvacrol concentration was based our finding in MTT test and our pretests. Also, same previous studies were considered. We mention them in “Cell culturing and treatment” and “ Results of cell viability assay” sections.

8- How do you explain that carvacrol has not been effective in preventing cytotoxicity in figure 1 while in Figures 2 to 5 it has been shown to be effective in preventing of H2O2 toxicity?

Answer: thank you for valuable attention of respected reviewer. We honestly reported our observations. However, figure 1 refers to cell death and other figures are pointing to beta galactosidase enzyme activity. Cell senescence is a sub-lethal procedure and beta galactosidase activity assays could be contributed to sub-lethal events in cells that were evaluated in figure 2 to 5. Our data showed carvacrol can inhibit cell senescence more than cell death. Maybe other mechanisms have role in cell death, too.

Reviewer2:

1- The references need to be updated and use more recent references.

Answer: as requested by respected reviewer, we add some new references.

2- The References need to be corrected (11, 14, 16, 18, 31, 33).

Answer: in most journal of Hindawi publisher, year of publication is equal to issue and/or volume number. However, we revised them and added article ID.

3- Detailed further the histological analysis and add the scale bar to understand the magnification ratio with the real dimension.

Answer: we add scale bar and more information about figure 2. Please see the figure.

4- Revise carefully and reorganize the discussion in order to better value the good results obtained.

Answer: thank you for valuable comment of respected reviewer. We revised it.

5- The English must be revised throughout the paper, full of mistakes and poorly formed sentences.

Answer: thank you for valuable comment of respected reviewer. We revised the mnuscript.

6- In note of text and figs, the "p" should be italic. The same changes to others.

Answer: we change “p” to italic form.

7- Given the same effects of doses of 50 and 100, explain why you chose dose 100 as the effective dose.

Answer: selection of the most effective carvacrol concentration was based on our finding in MTT test and our pretests. Also, same previous studies were considered. Furthermore, we prefered the highest effective concentration based on our pre-tests and same studies.

8- Glutathione peroxidase plays an important role in maintaining GSH / GSSG homeostasis in stressful circumstances, and it would have been interesting to have evaluated any changes in the GSH / GSSG ratio.

Answer: thank you for valuable comment of respected reviewer. Unfortunately, because of some limitation of study's budget, we had to choose between tests. On the other hand, first count of cells in each well were same and finally the protein content of all samples were standardized by Bradford assay before measurement of GSH. Therefore, GSH content can indicate GSH/GSSG ratio, although measuring of this ratio is more valuable.

9- Carvacrol and acrylamide production, lot number and should be added.

Answer: as requested by respected reviewer, we add product their numbers.

10- Carvacrol vehicle should be mentioned

Answer: carvacrole was dissolved in PBS with 2% DMSO. (please see "material and methods/ Cell culturing and treatment" sections)

EIC Decision:

- The introduction section is not organized well. Revised it; consider scientific writing standards; prevent from adding so many definitions. Check coherent and cohesion thoroughly. For example, the first and second paragraphs are focusing on the same subject.

Answer: as requested by respected EIC, we revised it.

- I am afraid if the purity of chemicals is missing, it seems necessary.

Answer: thank you for valuable comment of EIC, we add purity and product number of main chemicals.

- "H2O2" not "H2O2" check the whole document to prevent the same typos.

Answer: we corrected it.

- Starts the first letter of Figures with capital F!

Answer: we corrected it.

- Most of the discussion section is the introduction; in this part, you should discuss your results and compare your findings with others. Revised according to writing standards and use clear English.

Answer: as requested by respected EIC, we revised it and *compare our findings with others*.

- In the conclusion section, the sentences are repeated several times in the manuscript. write a more transparent and concise conclusion

Answer: as requested by respected EIC, we revised it.

OPEN PEER REVIEW

Revision (1)

Here, you can see the **Reviewers**, **Associate Editors** and **EICs'** comments from the beginning to the end of the revision process.

REFEREE: Reviewer | **Revision** (1) 15 Feb 2021

Dear AE,
All the comments are addressed in the revised manuscript; I appreciate it. but consider the following point in the final draft.
According to the MTT result, the dose of 50 and 100 had the same effect and the lower dose should have been selected as the effective dose.
Thanks.

REFEREE: Associate Editor | **Revision** (1) 15 Feb 2021

Dear EIC,
The authors have made the required changes to render the paper accepted for publication.
Thanks.

REFEREE: EIC | **Revision** (1) 16 Feb 2021

Dear Authors,
The referee of your manuscript is now completed. I am glad to inform you that reviewers support the publication of your manuscript; please consider the final reviewer's correction in the proof of the document.
We thank you to considering our journal for publication of your research results and hope to receive more of your valuable manuscripts in the near future.
Kind regards,