OPEN PEER REVIEW

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Advantages of "Open Peer Review" process

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https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Gene, Cell and Tissue
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Peer Review Report for "Transcriptional Levels of Autophagy and UPR Markers in the Brain and Liver of Pregnant Rats"

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Review Timeline:

Submit Date: 1 Dec 2020
Revised Date: 29 Dec 2020
Accepted Date: 5 Jan 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 Dec 2020

Dear AE,
The authors investigated the mRNA levels of authophagy-related genes and UPR markers in tissues of pregnant rats. There are some comments for the authors:
1- Why did the authors categorized the animals in 3 groups and not 4 groups? It would be more conclusive if there were also 6 rats receiving resveratrol alone.
2- The authors need to mention the Ethical code for the study.
3- The Real-Time PCR protocol and selected melting temperatures should be described briefly.
4- The name of all genes should be Italicized.
5- Please mention references for selected doses of Eth and RSV.
6- Please add vertical axis titles for all the figures.
7- Please mention the limitations of the study.
8- What are the application size of PCR products? Please re-check the primer sequences. For Actin and Beclin1, no target templates were found in NCBI-primer-blast database.
Thanks.

REFEREE: Reviewer | **Revision** (0) 13 Dec 2020

Dear AE,
Comments for the authors:
1- Full name of some abbreviation should be written in the first mention.
2- Which method was used for RNA extraction?
3- It is better to write the official gene names in the capital and italic format.
4- Please recheck the sequence of the Beclin-1 and
GRP78 primers.
5- The references of used primers should be mentioned in the text.
Thanks.

REFEREE: Reviewer | **Revision** (0) 16 Dec 2020

Dear AE,
The study is reviewed carefully. According to comments, this manuscript needs still revisions.
Thanks

Saeedeh Salimi: Associate Editor | **Revision** (0) 18 Dec 2020

Dear EIC,
The manuscript needs major revision according to the comments of reviewers
best regards

Zahra Heidari: EIC | **Revision** (0) 19 Dec 2020

Dear Authors,
Based on the reviewers' and AE comments, this manuscript needs major revisions and a precise point by point reply to the comments.
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,

Gene, Cell and Tissue

We are highly pleased to have an opportunity to revise and resubmit our manuscript entitled “Transcriptional levels of autophagy and UPR markers in the brain and liver of pregnant rats” in Gene, Cell and Tissue.

We greatly appreciate you, the editor and the reviewers for taking time to provide constructive comments which helped improve this manuscript. We have carefully considered your comments and insightful queries rose by reviewer and tried to provide a detailed response to each comment.

Here you can find our responses to reviewer's comments. We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have.

Responses to the reviewer's comments

Reviewer #1:

Comment 1: Full name of some abbreviation should be written in the first mention.

Response1: We have revised the whole manuscript carefully and written the full name of abbreviation in the first mention.

Comment 2: Which method was used for RNA extraction?

Response 2: RNA extraction was carried out using Trizol reagent (Invitrogen, CA, USA) which was added into the revised manuscript under qRT-PCR subheading.

Comment 3: It is better to write the official gene names in the capital and italic format.

Response 3: Based on your useful comment, all of the official gene names have been changed to capital and italic format in the revised manuscript.

Comment 4: Please recheck the sequence of the Beclin-1 and GRP78 primers.

Response 4: Thank you for your valuable point. The sequence of primers was rechecked and wrong nucleotides due to typographical error were corrected.

Comment 5: The references of used primers should be mentioned in the text.

Response 5: The ACTIN, BECLIN-1 and GRP78 primers were designed in our lab using AlleleID software.

Reviewer #3:

Comment 1: Why did the authors categorized the animals in 3 groups and not 4 groups? It would be more conclusive if there were also 6 rats receiving resveratrol alone.

Response1: Thank you very much for your suggestion. Indeed, resveratrol is insoluble in water and normal saline and the mentioned amount of the ethanol is the minimum concentration of ethanol that resveratrol can be solved in.

Comment 2: The authors need to mention the Ethical code for the study.

Response 2: Thank you for precise consideration. This study has been approved by the Vice-chancellor for Research Affairs with grant number 91-6140 that is added to the material and method part.

Comment 3: The Real-Time PCR protocol and selected melting temperatures should be described briefly.

Response 3: Real time qRT-PCR was performed with a total of 40 cycles for amplification.

We evaluated single-peak melting curves as specific qRT-PCR reactions (figure 1). Electrophoresis of real-time PCR products on agarose gel was also carried out to confirm the accuracy of molecular weight of amplicons. Please see the materials and methods part.

Comment 4: Name of all genes should be Italicized.

Response 4: Thank you for your useful comment, all of the official gene names have been changed to italic format in the revised manuscript.

Comment 5: Please mention references for selected doses of Eth and RSV.

Response 5: The dose for Eth and RSV was selected based on the observation from our previous studies (25, 26), that added to the material and method part.

Comment 6: Please add vertical axis titles for all the figures.

Response 6: All figures have the title for the vertical axis in panel A, we just removed it from panel B as they are in the same layout to be easier for readers to compare the changes in their expression.

Comment 7: Please mention the limitations of the study.

Response 7: Thank you for your suggestion. We provided a paragraph regarding the limitations of the study in the end of the discussion of the revised manuscript. Please see the highlighted part.

Comment 8: What are the amplicon size of PCR products? Please re-check the primer sequences. For Actin and Beclin1, no target templates were found in NCBI-primer-blast database.

Response 8: Thank you for your valuable point. The products size of qRT-PCR for *ACTIN*, *BECLIN1*, and *GRP78* are 138,119, and 181 nucleotides respectively, which were added to the table of primers sequence in the new manuscript.

We also rechecked the sequence of primers that wrong nucleotides of *BECLIN1* and *GRP78* primers due to typographical error were corrected. However, the NCBI primer blast results have shown that the primers of ACTIN had been written correctly (figure 2).

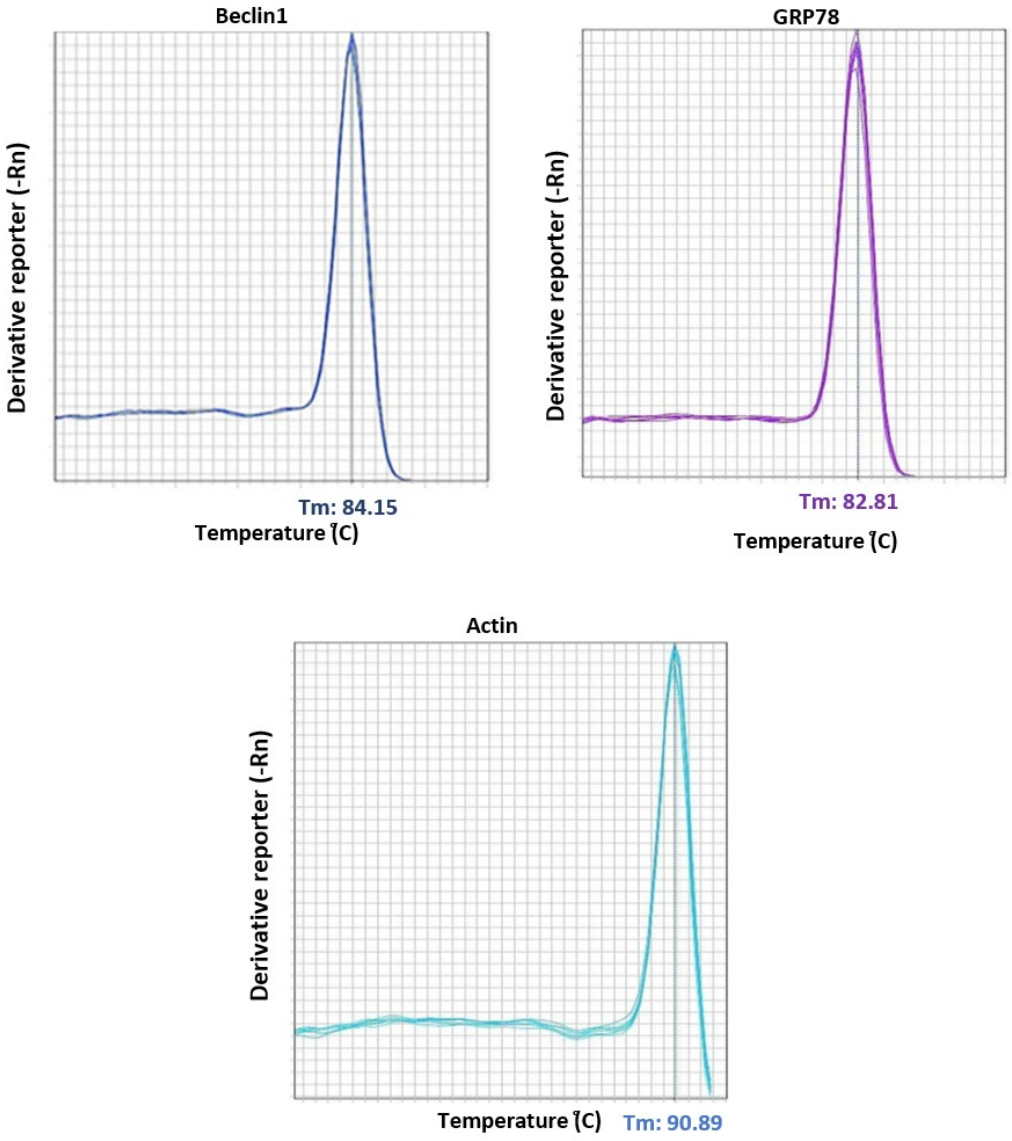

Figure 1. melting curves of BECLIN, GRP78, and ACTIN.

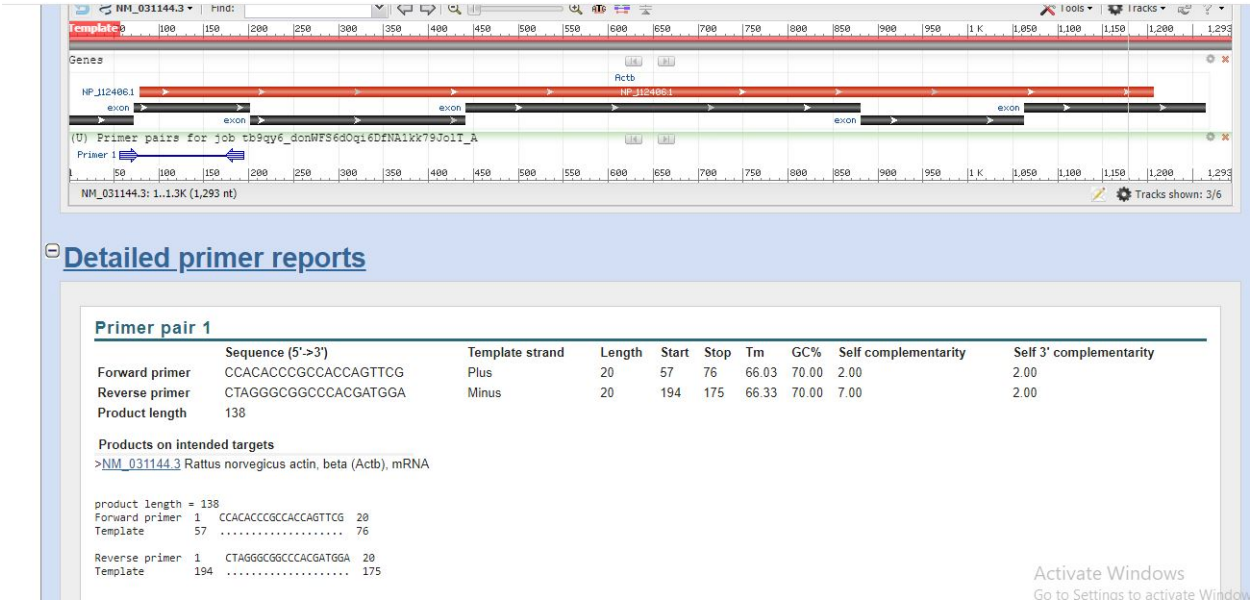

Figure 2. NCBI primer blast results of ACTIN primers.

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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) 30 Dec 2020

Dear AE,
The article is well-revised and all my concerns were addressed.
Thanks.

REFEREE: Associate Editor | **Revision** (1) 5 Jan 2021

Dear EIC,
The manuscript is suitable for publication now.
Best

Zahra Heidari: EIC | **Revision** (1) 5 Jan 2021

Dear Authors,
Based on the reviewer and AE comments revisions were done well. Thus this manuscript can be accepted for publication in GCT.
Thanks.