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.

What is "Open Peer Review" process?

An "Open Peer Review" process is making the details of all review process (including reviewers, associate editors, and EICs comments) as "Public" as it is agreed by EIC, Authors, and reviewers.

Advantages of "Open Peer Review" process

- More transparency, constructiveness and tactful comments of the peer review process: leads to an increase in the quality of reviews
- More motivations for all involved roles in the review process
- 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:
https://sites.kowsarpub.com/kowsar/knowledgebase/category/tree.html#opr.html

Jentashapir Journal of Cellular and Molecular Biology
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Peer Review Report for "Cytotoxic Effects of Hydro-Alcoholic Extract of the Leaf of *Elaeagnus angustifolia* in Hepatocellular Carcinoma Cell Line (HepG2)"

Author(s): Manijeh Ghanghareh, Maryam Zare

Review Timeline:

Submit Date:	22 Aug 2020
Revised Date:	22 Sep 2020
Accepted Date:	22 Sep 2020

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)

23 Aug 2020

Dear AE,
This study evaluates the anticancer effect of hydro-alcoholic extract of the leaf of Elaeagnus
angustifolia HepG2 cells. Study well designed, however, there are some issues, need to be noted by the authors:
1. The authors are encouraged to use an anticancer drug as a positive control. It would be beneficial for comparison and conclusion.
2. More details about the IC50 value should be presented. Cell viability formula for IC50 should be added to the method section.
3. The unit of IC50 should describe as µg/ml.
4. Evaluation of HepG2 morphological changes, at IC50 concentration of extract, also need to be provided to confirm cell shrinkage.
5. Control group must be defined clearly for readers in the method section.
6. What concentration of the extract was used for HepG2 treatment in RNA extraction protocol? It should be mentioned.
7. The authors are missing details about error bars representing SD or SE.

8. The interpretation of the discussion section needs refining.
9. The manuscript should carefully check and editing the English language. There also are some typographical errors.
Thanks.

Dian Dayer: Reviewer | **Revision** (0) 26 Aug 2020

Dear AE,
The manuscript includes useful content. But it needs major revision. Too many mistakes in structure, scientific writing, and English writing were noted.
Please find the attached file.
Thanks.

REFEREE: Reviewer | **Revision** (0) 26 Aug 2020

Dear AE,
Comments for the authors:
As the study aimed to evaluate the Cytotoxic effects of hydro-alcoholic extract of the leaf of Elaeagnus angustifolia in a hepatocellular carcinoma cell line (HepG2), one of the major concerns of the study was its methods.
1- The lack of a section as "cell culture": after the part of "preparation of extract", you should add a section as "cell culture" to explain your cell culture method in detail.
2- To prove apoptosis, we should assess at least 3 apoptotic markers, and evaluation of bax and bcl2 alone, are not enough for apoptosis assessment. Moreover, Real time-PCR is not an acceptable test alone and needs to confirm with western blotting or flow cytometry.
3- Totally, there's much room for the improvement of manuscript writing. Please, pay more attention to writing.
Thanks.

Seyyed Ali Mard: Associate Editor | **Revision** (0) 26 Aug 2020

Dear EIC,
Minor revision is recommended by reviewers.
Thanks.

Seyyed Ali Mard: EIC | **Revision** (0) 26 Aug 2020

Dear Author,
Minor revision is recommended.
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 in Chief

Thanks a lot for considering and reviewing the manuscript "Cytotoxic effects of hydro-alcoholic extract of the leaf of *Elaeagnus angustifolia* in hepatocellular carcinoma cell line (HepG2)". The comments of reviewers were replied and the manuscript was edited according to the precious comments of respectable reviewers.

Reviewers' Comments:

Reviewer 1:

Dear Editor
This study evaluates anticancer effect of hydro-alcoholic extract of the leaf of Elaeagnus anustifolia HepG2 cells. Study well designed, however there are some issues, need to be noted:

1. The authors are encouraged to use an anticancer drug as a positive control. It would be beneficial for comparison and conclusion.
Response: As the respectable reviewers mentioned, applying the positive control was exactly useful for comparing the outcome results. Cisplatin anticancer drug was used as positive control. The related explanation was added in revised manuscript (yellow highlighted in methods, section 2.3 to 2.8, and results; section 3.1 to 3.6 pages 3, 4, 5)

2. More details about IC50 value should be presented. Cell viability formula for IC50 should be added to method section.
Response: The explanation about IC50 was added to method section according to the comment of respectable reviewer (blue highlighted, section 2.3. MTT Assay, page 3).

3. The unit of IC50 should describe as µg/ml.
Response: The unit of IC50 was edited according to the comment of respectable reviewer (blue highlighted in page 3 and 5)

4. Evaluation of HepG2 morphological changes, at IC50 concentration of extract, also need to be provided to confirm cell shrinkage.
Response: According to the comment of respectable reviewer, the image of morphological changes was added to the revised manuscript (figure 6) (grey highlighted in page 5).

5. Control group must be defined clearly for readers in method section.
Response: According to the comment of respectable reviewer, definition about the control groups was added to method section (yellow highlighted in methods, section 2.3 to 2.8, pages 3 and 4).

6. What concentration of extract was used for HepG2 treatment in RNA extraction protocol? It should be mentioned.

Response: As mentioned in the results section, RNA was extracted from all treated groups (50, 100, 200, 400 and 600 µg/ml of extract). However, according to the comment of respectable reviewer, extra definition was added to method section (blue highlighted in page 4).

7. The authors are missing details about error bars representing SD or SE.

Response: The error bar represents the SD and according to the comment of respectable reviewer, the definition was added to method section (green highlighted, section 2.8, page 4).

8. The interpretation of the discussion section needs refining.
Response: the discussion section was edited and rewrote. Some new explanation was added to discussion in revised manuscript (red color in page 6).

9. The manuscript should carefully check and editing the English language. There also are some typographical errors.
Response: According to the comment of respectable reviewer, the manuscript was completely checked and corrected (all line sin blue)

Reviewer 2

Dear Author
The manuscript includes useful content. But it needs major revision. Too many mistakes in structure, scientific writing and English writing were noted
Response: According to the comment of respectable reviewer, the manuscript was completely checked and corrected (all blue lines).

Reviewer 3:

Reviewer Comments:
As the study aimed to evaluate the Cytotoxic effects of hydro-alcoholic extract of the leaf of Elaeagnus angustifolia in hepatocellular carcinoma cell line (HepG2), one of the major concerns of the study was its methods.

1- The lack of a section as "cell culture": after the part of "preparation of extract", you should add a section as "cell culture" to explain about your cell culture method in details.
Response: According to the comment of respectable reviewer, Cell Culture section was added in methods in revised manuscript (green highlighted in page 3).

2- To prove apoptosis, we should assess at least 3 apoptotic markers, and evaluation of bax and bcl2 alone, are not enough for apoptosis assessment. Moreover, Real time-PCR is not an acceptable test alone, and needs to confirm with western blotting or flow cytometry.
Response: As the respectable reviewer mentioned, western blotting and flow cytometry analysis could clearly represent the apoptosis. But there was no funding for this research. So, it was impossible for us to perform these tests because of the budget limitation.

3- Totally, there's much room for the improvement of manuscript writing. Please, pay more attention to writing.
Response: According to the comment of respectable reviewer, the writing of manuscript was completely checked and edited (all blue lines).

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) 9 Sep 2020

Dear AE,
Comments for the authors:
The study of Cytotoxic effects of hydro-alcoholic extract of the leaf of Elaeagnus angustifolia in a hepatocellular carcinoma cell line (HepG2), was reviewed by reviewers, and still, the major concern remains is writing.
1- Please, check the manuscript writing completely. There are a lot of grammar mistakes.
2- Put the figures sequentially, according to their numbers.
3- Pay attention to figure legends and revise their writing.
3- Organize all sections. Justify the text body in all sections.
Thanks.

REFEREE: Associate Editor | **Revision** (1) 10 Sep 2020

Dear EIC,
A Minor revision is commended by reviewers.
Thanks.

Seyyed Ali Mard: EIC | **Revision** (1) 10 Sep 2020

Dear Authors,
Minor revision is recommended
Thanks.

OPEN PEER REVIEW

Revision (2)

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 in Chief,

Thanks again for considering and reviewing the manuscript entitled "Cytotoxic effects of hydro-alcoholic extract of the leaf of Elaeagnus angustifolia in hepatocellular carcinoma cell line (HepG2)". The comments of reviewers were replied and the manuscript was revised according to the precious comments. Below, there is a point by point response to the reviewers' comments.

Kind Regards,

Maryam Zare
Assistant Professor, Ph.D in Molecular Genetics
Department of Biology, Faculty of Sciences, Payame Noor University, Tehran, Iran P.O.BOX: 14155-6343

Reviewer 1:
Reviewer Comments:
The study of Cytotoxic effects of hydro-alcoholic extract of the leaf of Elaeagnus angustifolia in hepatocellular carcinoma cell line (HepG2), was reviewed by reviewers and still, the major concern remains is writing.

1- Please, check the manuscript writing completely. There are a lot of grammar mistakes.
Response: The manuscript writing has been edited completely in two steps: it was editted by a native English language colleague, and also edited by the qualified expert company of English editing services. All edited parts were written with red color in revised manuscript.

2- Put the figures sequentially, according to their numbers.
Response: The figures were put sequentially.

3- Pay attention to figure legends and revise their writing.
Response: The figures legends were revised.

3- Organize all sections. Justify the text body in all sections.
Response: The manuscript was checked. All lines were justified.

OPEN PEER REVIEW

Revision (2)

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

REFEREE: Reviewer | **Revision** (2) 22 Sep 2020

Dear AE,
The authors have shown a lot of effort to improve the manuscript and this should be well appreciated. It is clear that the authors have addressed all reviewer's comments carefully. As a result, it seems that the current form can be accepted for publication.
Thanks.

Seyyed Ali Mard: Associate Editor | **Revision** (2) 22 Sep 2020

Dear EIC
Based on the authors' answers to the reviewers' comments, the reviewer(s) have recommended publication.
Kind regards.

Seyyed Ali Mard: EIC | **Revision** (2) 22 Sep 2020

Dear Authors,
I am pleasure to inform you that your manuscript is provisionally accepted right now. Please mention the Ethical approval of your paper in the part of your manuscript just before references to get scientific acceptance. Do not hesitate to contact with journals staff Mr. Zamani if you had any inquiries.
Best Regards
Dr. Mard

OPEN PEER REVIEW

Revision (3)

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 in Chief,

Thanks again for considering the manuscript entitled "Cytotoxic effects of hydro-alcoholic extract of the leaf of Elaeagnus angustifolia in hepatocellular carcinoma cell line (HepG2)". The Ethical Approval was added in footnote section, and Materials and Methods section in the manuscript (highlighted with yellow).

Kind Regards,

Maryam Zare
Assistant Professor, Ph.D in Molecular Genetics
Department of Biology, Faculty of Sciences, Payame Noor University, Tehran, Iran P.O.BOX: 14155-6343

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Revision (3)

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

Seyyed Ali Mard: EIC | **Revision** (3) 22 Sep 2020

Dear Author,
The document is reviewed carefully by the reviewers. According to their comments and your revisions as requested, this manuscript is accepted.
Thanks.