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:
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Jundishapur Journal of Natural Pharmaceutical Products
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Peer Review Report for "The Effect of Hydro-Alcoholic Extract of *Nigella sativa* on *Bmp7* and *Bmp8b* Expression in Rats Fed with a High-Fat Diet"

Author(s): Akram Yaghoobi , Keihan Ghatreh Samani, Effat Farrokhi

Review Timeline:

Submit Date: 11 Mar 2018
Revised Date: 31 Oct 2018
Accepted Date: 6 Jan 2019

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)

25 Apr 2018

Dear author:
Thank you for choosing JJNPP for publishing your manuscript!

The manuscript is the result of an experimental animal study which contains new findings; however there are some scientific , designing, writing, and presentation defects which need a complete revision. Please do consider the followings comments:

1. The first sentence of abstract is not a good background for this study. It can be more relevant with the main topic of the study. It can be deleted or replaced by another sentence.
2. Introduction: The plant Nig.. should be explained in more details: Where does it grow; Where is it used, In which medicines is it present…..
3. SOD must be written complete for the first time.
4. Methods: a paragraph is repeated twice.
5. The plant and its extraction must be explained in more details. Where was it collected or purchased? How was it confirmed? Which part of the plant was used????? Which form of the extract was used? wet or dry?
6. GPT and GOT or SGOT and SGPT must being used with same format as in the text. However, AST and ALT are the new and more scientific abbreviation for these enzymes.

7. Measurement of these factor has not done by just one kit.
8. Please spicify the details of HPLC apparatus: column, eluent, flow rate, detector, machine Brand …….
9. How did you prepare live homogenate?
10. More details should be presented about Catalase, PON , and Spectrophotometer. Point out the basis of methods shortly.
11. What was the method for analysis of the ReT PCR?
12. SDS is not gel, it should be SDSPAGE.
13. What was the method for protein assay?
14. SPSS is a software. What were the statistical Tests?
15. Figure 1 or figure 1, use the same format.
16. Discussion: The author several times points out to the first and second group which are not completely clear. This should be clarified.
17. As you measured CAT and PON in liver, it should be mentioned liver cat..
18. Tables 1 & 2 : None of the factors have a measurement unit. All factors must have it.
19. What is CHO??? Glc is a more common abbreviation for glucose.
20. Two set of experiments were done in this study, one is preventive and the other is treatment; but the authors did not show or explain or conclude which one is better or more effective or can be recommended?
21. Table 2: the data for FRAP and ALP are not correct.
22. The format of some references are not correct i.e 19 and 26.
23. There are some grammatical mistakes, the manuscript needs an English writing revision.
24. In the attached file, some defects or mistakes are highlighted and some comments are presented as well.

Best wishes!

REFEREE: Reviewer | **Revision** (0) 18 May 2018

Dear Editor –in-Chief;
The article entitled “The effect of hydro-alcoholic extract of Nigella sativa on Bmp7 and Bmp8b expression in rats fed with high-fat diet” is an interesting study and well designed. However the article needs major revision.
Dear author;
Please do consider the below mentioned comments!
1- The text should be revised for English errors.
2- In abstract, first, please don’t use abbreviation in the abstract, second, this abbreviation is a kit or method so this is no abbreviation for “total antioxidant capacity”.
3- In the manuscript and also abstract use the full name of the group for example “control”, instead of one or second or third group.
4- I think that in introduction section the authors should present more evidence regarding to the herbal drug that used in the study.
5- In material and methods; Who approved your herbal drug? Did you consult with any herbalist? Please insert the herbarium code number! In the set A of the study, the time of

treatment is not mentioned.

6- The code and date of ethical approval should be mentioned in the main text

7- In page 4, line 10, the paragraph which started with "after one week", is a repeated paragraph, and should be deleted.

8- Which method was used for preparation of Hydroalcoholic extract of the Nigella sativa? Please describe in detail.

9- In which solvent, the Nigella sativa extract was dissolved? How much of the extract was dissolved to obtain a final concentration?

10- Why chloroform was used for anesthesia? Please report a reference for this case. Wasn't it better to use Ketamine/Xylazine? The method for collection of blood samples is not determine.

11- Catalase unit of measurement and PON1 unit of activity should be report both in the text and tables.

12- The source of primers used in the study should be mentioned.

13- How have you measured and checked the purity of extracted RNA? Please describe in detail.

14- Mention the ingredients of reaction mixture in real time PCR. Please determine the concentration of c.DNA, as well as the primers. PCR product size should be determined in the body of manuscript and table.

15- How did you verify the specificity of amplified target genes? Please describe it in the manuscript. How much adipose tissue (gr) was used for lysis in western blot? You mentioned (300 µg) protein was used for western blot loading. This is a very high amount for loading. Please describe the reason for doing this. Usually, 30-50 µg should be used.

16- Statistical analysis is very poor and incomplete. You should describe how did you check the normal distribution of data. Which kind of test was used? And what was the reason for sing the test. How did you present the results? And if the distribution of all data was normal. If not, you should use nonparametric test for analysis. There is no report not in the text nor in the footnotes of tables about the way of presenting the data. Only in western blot you reported that the data expressed as mean ± SEM. Why did you use SEM? I Suggest using SD. The very wide error bar in figures 1 and 2 showed that data distribution is not normal. Please describe this issue in detail.

17- All tables format should be corrected. In table 1, in column 2, insert PCR product length. In table 2 and 3, first, the title should be corrected; Second, the title should be written above the tables and column 1 must be deleted. Each group must be described as: control = normal diet; fat= high fat diet…

18- How did you calculate the relative gene expression in the mRNA level? Please describe completely in the text.

19- In page 7, line 9, "and visualized by TMBD" is not correct. Please correct it.

20- In figures 1 and 2, the mean value of samples for control must be adjusted to 1.0 and the all sample values must be expressed relative to this adjusted mean value in fold change determination of target gene expression; But the author compared the results with the second group.

21- Which gel documentation system was used for blot visualization? By which software optical densities of bands were analyzed? Please clarify these.

22- In page 7, line 10, the sentences, "western blot analysis…….is not correct". Correct it.

23- Please consider, each abbreviation (SGOT, SGPT, MDA) should be written in full name

when used for the first time throughout the text.

24- In page 8, gene expression section, line 2 the sentence "no changes……" doesn't have any verb.

25- Final conclusion should be reported after discussion.

26- The discussion is poor and redundant or a reiterating of results, and needs to be corrected. A more deep discussion should be provided about these interesting data. Your results should be compared with the results of other studies.

With best wishes!

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 information and reviewer comments. The suggestions are helpful for us to refine this paper.

We have revised our manuscript according to the reviewers' comments.
All changes were highlighted in green in the revised manuscript.

Reviewer 1:

1. The first sentence of abstract is not a good background for this study. It can be more relevant with the main topic of the study. It can be deleted or replaced by another sentence.

R: The first sentence was deleted.

2. Introduction: The used plant Nig.. should be explained in more details, where dose it grow, in which country it is used, …… in which medicine it is present…..

R: These information were added to the introduction.

3. SOD must be written complete in first time.

R: SOD was written complete.

4. Method : a paragraph is repeated twice.

R: The mentioned paragraph was deleted.

5. The plant and its extraction must be explained in more details. Where was collected or purchased the plant? How was it confirmed? What part of the plant was used?????wet or dry?

R: Requested items were added as "Preparing the plant extract" on Page 3-4.

6. GPT and GOT or SGOT and SGPT they must being used with same format in the text. However, AST and ALT are the new and more scientific abbreviation for these enzymes.

R: All of GOT or SGOT was changed to AST. All of GPT or SGPT was changed to ALT.

7. Measurement of these factor is not done by just a kit.

R: This sentence was corrected.

8. What was the details of HPLC : column, eluent? Flow rate, detector? Machine Brand ? …….

R: Description for these data was added on Page 5-6.

9. How did you prepare liver homogenate?

R: Preparation of liver homogenate was explained on 6 page.

10. About Catalase and PON1 more details should be presented, Spectrophotometer? Point out the basis of methods shortly.

R: These details were added, although reference was mentioned for every method.

11. What was the method for analysis of the ReT PCR?

R: The method for RT-PCR analysis was added.

12. SDS is not gel, it should be SDSPAGE.

R: This sentence was corrected.

13. What was the method for protein assay?

R: The method for protein assay was added.

14. Data analysis" SPSS is a software what was the statistical Tests?

R: The used statistical tests were described in page 8.

15. Figure 1 or figure 1 , use the same format.

R: All of them were writhen the same format.

16. Discussion: In discussion the author several times point out to first and second group that are not clear what groups they mean? It should be clarified.

R: All of the groups were clarified in the whole text and were highlighted.

17. Since apparently they measured CAT and PON in liver , it should be mentioned liver cat..

R: The "liver" word was added.

18. Tables 1 &2 : none the factors have unit. All factors must have unit,

R: The units were added in tables.

19. What is CHO??? Glc is a more common abbreviation for glucose.

R: The abbreviations were corrected.

20. Two set of experiment were done in this study, one is preventive and the other is treatment, but the authors did not show or explain or conclude which one is better or more effective or can be recommended?

R: The Comparison of two sets was explained in the discussion section.

21. Table 2: the data for FRAP and ALP are not correct.

R: The data for FRAP was corrected. About ALP, the data was correct.

22. The format of some references are not correct i.e 19 and 26.

R: Endnote software *was* used for references managing. The format of references are correct.

23. There are some grammatical mistakes, the manuscript needs an English writing revision.

R: The text was revised and grammatical mistakes were corrected.

24. In the attached file some defects or mistakes are highlighted and some comments are presented as well.

R: The attached file was not sent to us.

Reviewer 2:

1. The text should be revised for English errors

R: The text was revised and grammatical mistakes were corrected.

2. In abstract, firs , please don't use abbreviation in the abstract (FRAP), second this abbreviation is a kit or method so this is no abbreviation for " total antioxidant capacity"

The " FRAP" was deleted in abstract.

3. In abstract and thought the manuscript instead of one or second or third group should be use full name of the related group for example " control" , et ..

R: All of the groups were clarified in the whole text and were highlighted.

4. I think that in introduction section the authors should present more evidence regarding to the herbal drug that used in the study.

R: These information were added to the introduction.

5. In material and methods, who approved your herbal drug? Did you have any herbalist? Please insert the herbarium code number? And in the set A of the study time of treatment is not explored?

R: Requested items were added as "Preparing the plant extract" on Page 3-4. The time of treatment was mentioned in 5 page.

6. The code and date of ethical approval should be mention in the main text

R: The code and date of ethical approval were added.

7. In page 4, line 10, the paragraph which stared with after one week, is a repeated paragraph, and must be delete?

R: The repeated paragraph was deleted.

8. Please describe in detail which method was used for preparation of Hydro alcoholic Extract of the Nigella sativa?

R: Requested items were added as "Preparing the plant extract" on Page 3-4.

9. For gavage, the Nigella sativa extract was dissolved in which solvent? And, how much of the extract was dissolved to obtain a final concentration (200 mg/kg)?

R: Nigella sativa extract was dissolved in distilled water (It was mentioned in the manuscript). We dissolved 40 mg dried powder of extract in 1ml distilled water for every rat.

10. What was used chloroform for anesthetization of animal? Please report a reference in this point? It is better use Ketamine/Xylazine? The method for collection of blood samples was not determined?

R: The method for collection of blood samples was described.
Ketamine/Xylazine is used as an injection and may interfere with the measurement of compounds such as MDA.

11. The unit of catalase and PON1 activity measurement should be report both in the text and tables?
R: The units were added in tables.

12. The source of primers used in the study should be determined in the manuscript, provided from article or authors deigned?
R: It has been mentioned in the manuscript.

13. How you measured and checked the purity of extracted RNA? Please describe in detail
R: It was added in the manuscript.

14. For real time PCR please determine the reaction mixture consisting of which materials, also, concentration of c.DNA, as well as concentration of primers should be describe in the manuscript, and PCR product size should be determine in the manuscript and table.
R: The data of reaction mixture were added in the manuscript. PCR product size was mentioned in the manuscript table.

15. How you verified the specificity of amplified target genes? Please describe in the manuscript? For western blot how many adipose tissue (gr) was used for lysis? Also, you mentioned (300 µg,) protein was used for western blot loading? This is very high for loading? Please describe the reason? usually, 30-50 µg should be use

R: These items were described in the manuscript and highlighted. in the manuscript. The amount of protein for the western blot was 30µg and it was edited in the manuscript.

16. Statistical analysis is very poor and incomplete, and should be completed; you must describe how checked the normal distribution of data, which kind of test was used? And the reason? How presented the results? And, whether, all data distribution was normal? If not, you must use nonparametric test for analysis. There is no report not in the text nor in the footnote of table about how presented the data? Only in western blot reported data expressed as mean ± SEM? Why SEM? Suggest use SD? The very wide error bar in figures 1 and 2 showed that data distribution is not normal? Please describe this point?

R: We used nonparametric test for analysis. The used statistical tests were described in page 8.

17. All tables format should be correct? In table 1, in column 2, insert PCR product length, in table 2 and 3, first, the title should be correct? Second, the title should be reported in the top of tables? And column 1 must be deleting? And each group must be describe as: control = normal diet; fat= high fat diet …

R: All tables format were corrected.

18. How you calculated the relative gene expression in the mRNA level? Please describe completely in the text?

R: The calculation of relative gene expression was added to the manuscript.

19. In page 7, line 9, the sentence, and visualized by TMBD, this sentence is not correct? Please correct it

R: This sentence was corrected.

20. In figures 1 and 2, for fold change determination of target gene expression, the mean value of samples for control must be adjusted to a value of 1.0 and the all sample values must be express relative to this adjusted mean value. But the author compared the results relative to the second group?

R: For fold change determination of target genes, all sample values were expressed relative to control mean value. But we compared the results of second group relative to the control group and other groups to second group.

21. For blot visualization which gel documentation system was used? And optical densities of bands were analyzed by which software? Please clarify these points?

R: We visualized blot by POD substrate in dark room. It was mentioned in the manuscript.

22. In page 7, line 10, the sentences, western blot analysis…….is not correct? and should be correct

R: This sentence was corrected.

23. Please consider, each abbreviation when used in the first time throughout the text, should be clarify in the full name? Such as SGOT, SGPT, MDA.

H: The abbreviations were corrected.

24. In page 8, gene expression section, line2, sentence, no changes……doesn't have any verb?

R: This sentence was corrected.

25. Final conclusion should be report after discussion?

R: The conclusion was added.

26. The last comment is about the discussion which is poor and redundant or reiterated of results, and need to be correct. A more deep discussion about these interesting data could be provided. And your results should be comparing with the results reported in the literature?

R: The discussion was revised.

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) 22 Jun 2018

Dear author;

The manuscript is corrected based on the reviewers' comments. It considerably is improved now; however, it still contains several defects and needs more revision.

1- In part "aims": it should be mentioned that gene expression is carried out on white adipose tissue.
2- Abbreviation for gram is "g."
3- The detection of blots is not explained properly. What was the conjugated enzyme? Is substrate TMB or POD? Both of them are mentioned.
4- Lines 18 & 19: Western blot detect at "protein" level not "mRNA" .
5- Line 17 : Liver pon1 in serum???!!!
6- Line 26: The measurement unit that is mentioned for enzymes (AST…..) activity is not correct.
7- When the activity of an enzyme is measured in tissue homogenate, the activity of enzyme must be expressed as unit per mg of protein or at least in gram or mg of tissue.
8- The manuscript still needs a linguistic revision.
9- Two new sentences that are added in conclusion are not clear and are grammatically incorrect.
10- The used font for figures legend must be similar to the other parts.

With best wishes!

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

We have revised our manuscript according to the reviewers' comments.
All changes were highlighted in green in the revised manuscript.

Reviewer 1:

1. In aims: it should be mentioned that gene expression is carried out on white adipose tissue.

R: This sentence was corrected.

2. 2. Abbreviation for gram is "g".

R: This word was corrected.

3. The detection of blots is not explained properly. What was the conjugated enzyme? Substrate is TMB or POD? Both of them are mentioned.

R: This sentence was corrected.

4. Pages 18 & 19:

Western blot detect at protein level not mRNA.

R: These sentences were corrected.

5. Page 17:

Liver pon1 in serum!!!

R: This sentence was corrected. PON1 was measured in serum as a marker of liver.

6. Page 26: The unit that is mentioned for enzymes (AST…..) activity is not correct.

R: AST and ALT were measured in serum and the unit for these enzymes is IU/dl.

7. When the activity of an enzyme is measured in tissue homogenate, the activity of enzyme must be expressed as unit per mg of protein or at least in gram or mg of tissue.

R: The unit for CAT was corrected (IU/mg protein) in tables.

8. The manuscript still needs a linguistic revision.

R: The manuscript was revised again.

9. Two new sentences that are added in conclusion are not clear and are grammaticaly incorrect.

R: These sentences were corrected.

10. Just as an example: incorrect sentence

R: There is no such sentence in the text.

11. The used font for figures legend must be similar to the other parts.

R: The font was corrected.

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) 20 Oct 2018

Dear author;
The manuscript is improved now due to the corrections and modifications that are made by authors; however still needs some corrections.

1- As I stated in my previous comments the measurement unit for AST and ALT activity is not correct; I recommend authors to have a look in similar papers, it should be IU/L .
2- There are still some grammatical mistakes which need revision. The most of sentences in "Result" are written in "passive voice" while they should be in "active voice".
Just as an example :
Abstract: MDA was increased.
Results: Total antioxidant capacity was increased..... and many other sentences.
3- "serums" is not correct.
4- page 8: : This sentence "the second, third and forth groups..... " needs revision.
5- The font of legends for Fig 1 and 2 are not corrected, they should be similar to Tables.

Kind regards!

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

We have revised our manuscript according to the reviewers' comments.
All changes were highlighted in green in the revised manuscript.

Reviewer 1:
The manuscript is improved now due to the corrections and modifications that are made by authors; however still needs some correction
1. As I stated in the previous comments the unit for AST and ALT is not correct; I recommend the authors to have a look in similar papers, it should be IU/L.

R: The units in table were corrected.

2. There are still some grammatical mistakes that needs a revision. The most of sentences in "Result" are written in "passive voice" while they must be in "Active voice" .
Just as an example:
Abstract: MDA was increased
Results: Total antioxidant capacity was increased..... and many other sentences.
" serums" is not correct.

R: These sentences were corrected.

page 8: the second. third and forth groups..... this sentence needs revision.

R: This sentence was corrected.

The font of legends for Fig 1 and 2 are not corrected, they should be similar to Tables and

R: The font for figures legend is similar to the other parts.

OPEN PEER REVIEW

Revision (3)

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

REFEREE: Reviewer | **Revision** (3) 16 Dec 2018

Dear author;
According to the correction in this version, I recommend it for publication.
Good Luck!