OPEN PEER REVIEW

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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.

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Peer Review Report for "An Efficient Model of Non-alcoholic Fatty Liver Disease (NAFLD) Versus Current Experimental Models: Effects of Fructose, Fat, and Carbon Tetrachloride on NAFLD"

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Review Timeline:

Submit Date:	8 Jul 2021
Revised Date:	28 Sep 2021
Accepted Date:	14 Oct 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)

30 Jul 2021

Dear editor,

1. Write the abbreviation of all words where they are mentioned for the first time within the manuscript (abstract & text body). For instance, the abbreviation of NAFLD, ALT, AST, VLDL, and GGT should be written where it has been mentioned for the first time either in the abstract or manuscript body.
2. In the abstract/result section, it would be well if you could write some of main findings as quantitative or mean ±SD within the abstract. Furthermore, write the p-value of all significant differences and comparisons within the results of abstract. For instance, you wrote “After 6 weeks, the ALT, AST, GGT levels increased significantly in CI”, but you didn’t mention the p-value of this significant correlation or difference. Please revise this type of mistakes within the abstract and results of manuscript body.
3. The introduction section is still immature. Write about the problems, the novelty of your study, and your study goals within the introduction. Why you selected carbon tetrachloride

to induce NAFLD?
4. Don't start sentences with numbers, so remove the numbers at the beginning of all sentences. For example, write "One hundred and fifty-five patients" or "twenty five percent" instead of "155 patients." And 25%, respectively. Correct this type of errors within the manuscript.
5. In methods section, "Pastor Institute" is incorrect. Please change it to Pasteur Institute of Iran (Tehran, Iran).
6. How did you select CCL4 dose? Did you consider its cytotoxicity effect in advance?
7. There are several grammatical errors within the manuscript which need to be revised in advance. Please consider the academic writing of the manuscript again and revise it accordingly. Furthermore, you can also check the word format of the manuscript with right click and revise some of grammatical errors.
8. All abbreviations in tables or figures should be explained in related legends.
9. Write the name of all applied apparatuses, chemicals, kits, solutions you applied for biochemical and the other examinations. You should also mention the name of all companies, cities and countries in parenthesis.
10. How did you collect blood samples? For example from the abdominal aorta?
11. Show abnormalities in microscopic images using arrows. Moreover, according the diagnostic criteria of NAFLD, the provided HE is not clear, and no ruler.
12. It was better if you explain figures and tables a bit more in each legend.

Regards

Mohammad Heiat: Associate Editor | **Revision** (**0**) 17 Aug 2021

Dear EIC,

Manuscript needs major revision.

Thanks

Seyed Moayed Alavian: EIC | **Revision** (**0**) 17 Aug 2021

Dear Authors,

Thanks for your submission. Please follow the peer-review comments and submit a revision.

Regards,
EIC

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.

Reviewer 1

1. *Write the abbreviation of all words where they are mentioned for the first time within the manuscript (abstract & text body). For instance, the abbreviation of NAFLD, ALT, AST, VLDL, and GGT should be written where it has been mentioned for the first time either in the abstract or manuscript body.*

It has done.

2. In the abstract/result section, it would be well if you could write some of main findings as quantitative or mean ±SD within the abstract. Furthermore, write the p-value of all significant differences and comparisons within the results of abstract. For instance, you wrote "After 6 weeks, the ALT, AST, GGT levels increased significantly in CI", but you didn't mention the p-value of this significant correlation or difference. Please revise this type of mistakes within the abstract and results of manuscript body.

It has done.

3. The introduction section is still immature. Write about the problems, the novelty of your study, and your study goals within the introduction. Why you selected carbon tetrachloride to induce NAFLD?

It has improved.

Studies indicated that CCl4 in combination with a high-fat diet causes chronic oxidative stress, which leads to inflammation and apoptosis. As fibrosis progresses in hepatocytes, fatty liver leads to steatosis. As a result, CCl4 induces NAFLD through this mechanism.

Guodong Zhang et al. (2020) examined CCl4 to induce NAFLD/NASH in mice. In their study, it was proved that the use of CCl4 accelerates and intensifies the

induction of NAFLD/NASH, and it was mentioned as the best animal model. For this purpose, based on their study, CCl4 was used to increase the vulnerability of hepatocytes and induce NAFLD.

References: Guodong Zhang, Xiaoli Wang, Tzu-Yang Chung, Weiwei Ye, Lauren Hodge, Likun Zhang, Keefe Chng, Yong-Fu Xiao & Yixin Jim Wang. Carbon tetrachloride (CCl4) accelerated development of non-alcoholic fatty liver disease (NAFLD)/steatohepatitis (NASH) in MS-NASH mice fed western diet supplemented with fructose (WDF) BMC Gastroenterology volume 20, Article number: 339 (2020).

Another study by Norihiro Kubota et al. (2013) found that consuming a high-fat diet with CCl4 administration leads to chronic oxidative stress, which leads to inflammation and apoptosis. As a result, as fibrosis progresses in hepatocytes, the fatty liver progresses to steatohepatitis.

References: Norihiro Kubota, Shoichi Kado, Mitsuyoshi Kano, Norie Masuoka, Yuriko Nagata, Toshihide Kobayashi, Kouji Miyazaki, Fumiyasu Ishikawa. A high-fat diet and multiple administration of carbon tetrachloride induces liver injury and pathological features associated with non-alcoholic steatohepatitis in mice. Clin Exp Pharmacol Physiol. 2013 Jul;40(7):422-30. doi: 10.1111/1440-1681.12102).

4. Don't start sentences with numbers, so remove the numbers at the beginning of all sentences. For example, write "One hundred and fifty-five patients" or "twenty-five percent" instead of "155 patients." And 25%, respectively. Correct this type of errors within the manuscript.

It has done.

5. In methods section, "Pastor Institute" is incorrect. Please change it to Pasteur Institute of Iran (Tehran, Iran).

It has done.

6. How did you select CCL4 dose? Did you consider its cytotoxicity effect in advance?

According to the Guodong Zhang et al. (2020) study, which examined the effect of CCl4 injection on NAFLD induction, the basis of our study on CCl4 dose was based on the results of their study. Also, since we did not achieve definite results in the groups during the pilot study, we modified the prescribed dose of CCl4 in this study as a c1 group that it show NAFLD.

7. There are several grammatical errors within the manuscript which need to be revised in advance. Please consider the academic writing of the manuscript again and revise it accordingly. Furthermore, you can also check the word format of the manuscript with right click and revise some of grammatical errors.

The manuscript has been revised and checked by ARTICLEX (Kowsar publisher).

8. All abbreviations in tables or figures should be explained in related legends.

It has done.

9. Write the name of all applied apparatuses, chemicals, kits, solutions you applied for biochemical and the other examinations. You should also mention the name of all companies, cities and countries in parenthesis.

It has done.

10. How did you collect blood samples? For example, from the abdominal aorta?

According to the ethical principles of laboratory animals, sampling was performed on rat tails in the 2nd and 4th weeks, and at the end of the intervention (at 6th week, before anesthesia and dissection), blood samples were taken from abdominal aorta.

11. Show abnormalities in microscopic images using arrows. Moreover,

according the diagnostic criteria of NAFLD, the provided HE is not clear, and no ruler.

It has done and the pictures replaced.

12. It was better if you explain figures and tables a bit more in each legend.

It has done.

Reviewer 2

1. *The abstract should present and introduce the best model of NAFLD in bold.*

It has done.

2. *“phases” steps.*

It has done.

3. *FCI or FC1??*

FC1 is correct and it is improved in all manuscript.

4. *A large part of method has been dedicated to group introduction. method should be expanded more about whole procedure. This is a methodological manuscript.*

It has improved.

5. *“CI” CI group*

It is corrected.

6. *“C2” CII. The unified writing should be observed in all parts of the manuscript.*

C2 is correct and it is unified in all parts of manuscript.

7. *"NAFLD Activity privilege" Do you mean NAFLD Activity Score (NAS)?*

Yes, it is improved.

8. *"efficient" Not a good word*

It is improved.

9. *"NAFLD, High-fat diet, Fructose, CCl4, Animal model" It should be in an alphabetic order.*

It has done.

10. *The background should be more focused on the issue you are following.*

It has done.

11. *space between population and (. all through the manuscript.*

It has done.

12. *The title doesn't reflect this goal "Therefore, the present study sought to develop a reproducible rat NAFLD/ NASH model, comparing different forms of intervention".*

The title is modified to "An efficient model of non-alcoholic fatty liver disease (NAFLD) versus current experimental models: Effects of fructose, fat, and carbon tetrachloride on NAFLD"

13. *"male" all throughout the test needs to be revised by a native speaker.*

It has done.

14. *"ad libitum"?*

This word is used to mention free access to water and food.

15. *"The ordinary diet will have 4.30 kcal per gram including 3.87% fat (soy oil), 17.46% casein protein, 68.7% carbohydrates, 8.97% minerals, and 1%vitamins." More suitable structure is needed here.*

The standard diet for the animals contained 4.30 kcal per gram with 3.87% fat (soy oil), 17.46% casein protein, 68.7% carbohydrates, 8.97% minerals, and 1% vitamins.

16. *"(NIH Publication No. 86 – 23, revised 1985" INCOMPLETE PARENTHESES.*

It has done.

17. *"C1" nomination should be unified al through the manuscript.*

It has done.

18. *"Triglycerides(TG)" space*

It has done.

19. *"Data were expressed as mean ± SD. The statistical significance of differences between groups was specified by the Kruskal-Wallis H test and the Mann-Whitney U test was used to compare the two groups. P-values < 0.05 were considered significant." Grammatical error.*

Data are expressed as median (interquartile range). Significant differences between the groups were determined by Kruskal-Wallis H test. Also, Mann-Whitney U test was used to compare the groups. P-values less than 0.05 were considered statistically significant.

20. *This is the Ethics Committee Code for the following title: "Evaluation of effect coadministration of atorvastatin with flaxseed flour and flaxseed oil*

on serum levels of lipid profile liver enzymes trans fatty acids and liver tissue in male Rats with fatty liver".

The Ethics Committee Code is corrected.

21. "food intake" Capital letter Everywhere.

We sent the manuscript to ARTICLEX (Kowsar publisher) for Native and writing edition.

22. "shows" the academic writing tense should be used.

It has checked by ARTICLEX (Kowsar publisher) for Native and academic writing edition.

23. "Weight and Food intake" section should be re-write in a better phrase. As it was mentioned above, all parts of the manuscript should be revised grammatically and literary.

It has rewrited by ARTICLEX (Kowsar publisher) for grammar, Native and academic writing edition.

24. "Figure 3" the details of measurements should be presented in a table.

It has done.

25. "CHO"

It is changed to TC (total cholesterol)

26. "Figure 4" the details of measurements should be presented in a table.

It has done.

27. "meaningfully"

It is changed to significantly.

28. Was it possible to determine the fatty liver grading?

We added data such as NAFLD Activity Score (NAS) in Table 2 that include hepatocyte ballooning, Lobular inflammation and Steatosis to determine NAFLD grading.

29. How can a reader make sure that this model can act as a fit NAFLD model in his studies?

Based on changes in serum levels of biochemical parameters (such as increased ALT and AST, which are serum markers of liver damage) as well as changes observed in H&E staining of liver tissue, in the intervention groups compared to the healthy control, induction of NAFLD can be proved. Also, since the gold standard method in the diagnosis and evaluation of NAFLD is its pathology, therefore, by measuring hepatocyte indices such as hepatocyte ballooning, Lobular inflammation, and steatosis and calculating the level of NAS, induction of NAFLD can be ensured. The best induction model has a higher level of steatosis and NAS. The results of our study showed that CCl4 injection alone (C1) or in combination with fructose (FC1) induces NAFLD and liver steatosis, although of the two, CCl4 injection alone is the most effective model in inducing NAFLD model.

30. use the complete form (H&E).

It has done.

31. Altogether, this article should present and introduce the best model in bold to be used by other researchers.

CCl4 injection alone (C1) is the most effective model in inducing NAFLD model that explained in abstract and discution.

32. "Discussion" the literature of this part should be revised and categorized subtly.

It has done.

33. "references 15,16" both of them are for 2017 some updates may exist.

We added updated article (2020). Jensen VS et al. claimed that one of the reasons for the non-reproducibility and insignificance of comparisons in preclinical studies of NAFLD can be possible changes in sampling and non-standard sample sites.

*34. what do you mean. what are the differences between * and # and+?*

*The data in figures insert into the table and the symbols of * and ** were shown for significance.*

35. the figs have not made in a high quality way.

We draw and put them again.

36. The lesions should be pointed by arrow.

It has done.

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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) 6 Oct 2021

Dear editor,

This manuscript has been revised accordingly. It can be accepted for publication.

Thanks

Mohammad Heiat: Associate Editor | **Revision** (1) 14 Oct 2021

Dear EIC,

The manuscript is accepted.

Thanks

Seyed Moayed Alavian: EIC | **Revision** (1) 14 Oct 2021

Dear Authors,

I am delighted to inform that your manuscript is accepted for publication in Hepatitis Monthly journal.

Regards,
EIC