Author’s response to reviews

Title: A New Method to Induce Nonalcoholic Steatohepatitis (NASH) in Mice

Authors:
Feryal Savari (feryal.savary@yahoo.com)
Seyyed Ali Mard (alimard77@gmail.com)
Mohammad Badavi (badavim@yahoo.com)
Anahita Rezaei (rezaie20a@yahoo.com)
Mohammad Kazem Gharib-Naseri (mgharibnaseri@yahoo.com)

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Author’s response to reviews:

Dear Editor-in-Chief

BMC Gastroenterology

Thank you for giving us a chance to revise our manuscript entitled: "A New Method to Induce Nonalcoholic Steatohepatitis (NASH) in Mice ". We answered and revised our manuscript according to editor and reviewer’s comments. For your convenience all changes made in the revised manuscript are highlighted (in green) and reviewer comments together with the corresponding responses are presented below.

Reviewers’ Comments to Author:

Reviewer: 1

- About the cigarette smoke model used in this study, I recommend a better explanation about the employed dose of 4 cigarettes/day, once it can be consider a low smoking load to develop chronic diseases.

Previous studies have used 2 cigarette per day for 8 weeks to investigate its damaging effect on the live in rats. We also used this dose in our pilot study, but did not find acceptable hepatic changes (damages). Notably, difference susceptibility of rats and mice has been established.
However, despite another report using dose of 6 cigarette/day for 4 and 8 weeks to induce hepatic injury in mice, we succeeded to induce liver damage employing dose of 4 cigarette/day as more reasonable approach of chronic disease model induction.

1- Tobacco Smoke-Induced Hepatic Injury with Steatosis, Inflammation, and Impairments in Insulin and Insulin-Like Growth Factor Signaling


- Some minor corrections can be done.

On pdf page 11, line 11: use "serum level" instead of "Serum level".

On page 12, line 17: use "inflammation" instead of "Inflammation". Add a dot after "(central vein)".

Please, consider review the term "pronounced" on page 16, line 15.

On figure legends, please mention the congo red stain on figure 2 legend and also include the magnification of the images.

According to the respected reviewer comments, needed corrections were done.

Reviewer: 2

- Firstly, it is not clear what the authors aimed to prove or investigate. The generation of an "ideal disease model" for NASH disease appears to be one of the aims (as stated at page 3), but the animals exposed to different diets are not new and their relevance is questionable.

We attempted to induce more applicable model of NAFLD progression (NASH), especially in terms of duration of the model induction, by its ability to mimic the etiology and its histopathologic changes of human disease.

Based on previous studies, fructose and also HFD feeding have led to steatosis. moreover, considering the exacerbating effects of cigarette smoking on NAFLD progression, we hypothesized possibility of NASH model induction using combination of these intervention which has not been done so far.

Accordingly, we designed a comparative study on combined pattern of interventions have nowadays converted to habits of modernized life style.
We believe that our used method successfully developed NASH model in a strain of animals (NMRI) firstly used because previous studies introduced C57BL/6 mice as a susceptible strain to develop metabolic and histopathological features of NAFLD. (related references presented below)


According to the respected reviewer comment, the aim of this study defined in a part of introduction (End) and (first) discussion.

- The parameters measured and analyzed are not sufficient and poorly informative. additional analyses are encouraged with more specific and punctual measurements based on different diets/smoking effects.

That’s a quiet suggestion. I did perform the following relevant experiments to show the induction of NASH according to the respected reviewer comment.

Additional parameters measured and analyzed (fasting serum glucose and insulin, HOMA-IR index and GGT activity) added to respective part in result and discussion sections of the revised manuscript.

Insulin resistance (IR) is one of the main metabolic disturbances linked to NAFLD/NASH.

Pathogenesis of NASH has been explained according to multiple hypothesis and insulin resistance as a pivotal cause (major contributor), is considered to be involved in the progression of simple steatosis to NASH.

Insulin resistance could be used to distinguish NASH from simple steatosis especially when has accompanied with elevated serum ALT.

Beside, a positive correlation between insulin resistance and GGT level has been established. Accordingly, GGT could be used to independently determination of IR to NAFLD association.


- Finally, the authors should focus their Conclusion on data obtained during the study, limiting comments and discussion on previous work. Negative results, as "fructose consumption was not able to change significantly the studied biochemical parameters" (as stated at page 13), may be of importance, but only if the analyzed parameters are of relevance and specifically targeted.

According to the respected reviewer comment, the results and discussion sections were summarized as far as possible.

- English language editing is recommended.

English was edited by an expert.