Reviewer’s report

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

Version: 4 Date: 26 Jun 2019

Reviewer: Reviewer 2

Reviewer's report:

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.

Differently of what stated, the authors did not apply the same amount of digits after the comma to all different values in Table 1 and 2.

Re: corrected

Figure 1 is not mentioned anywhere in the text.

Re: corrected

In Figure 1 several SD bars are missing (with n=8 is sounds unlikely that SD is equal to 0).

Re: corrected

It's reviewer's opinion that bars with cigarette smoke exposure should be painted differently and maybe grouped together (since statistical comparison is related to C+CS control group).

Re: Done
Whiskey plot is probably a better way to show results. Probably the use of connecting line to better representing the comparison with its own relative control group (ctrl and C+CS) is probably better. Similar changes should be applied to Figure 3.

Re: Box plots with whiskers were plotted according to respect reviewer's request.

Figure 2: panel labels are not present. In the legend, authors refer as (A), (B), etc…but such labelling has not been applied. It is not clear why Fru+CS condition has an additional panel (E) (is the magnitude the same as the other images?)

Re: A, B, etc… labels were added to figure 2.

Re: As described in the text (page 9, paragraph 13), two mice in Fru+Cs subgroup had shown amyloidosis in addition to inflammation that marked as uniform pink sediments located in hepatic sinusoidal wall. The nature of amyloid sediments was confirmed by Congo red staining which became orange. Its image was placed as a separate panel (E) with same magnitude in figure 2. "E: Congo Red" also was added to Figure 2 legend.

The evaluation of a critical event in pathogenesis such as inflammatory probably deserves additional analysis to one cytokine only (TNF alpha) quantification. It looks like smoking condition increases TNF alpha hepatic concentration of approx.25%. Is such increase consistent with the different diets? Please check comparison between smoking and non-smoking diets (ctrl/C+CS; Fru/ Fru+CS is similar to HFD/HFD+CS or WD/WD+Cs)? Similar comparison to Figure 1 is largely suggested.

Re: Done

I hope that the answers to the comments made are clear and meet your requirements for accepting for publication in your high quality and respected journal.

Best regards Seyyed Ali Mard

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes
Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

Yes

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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Please indicate the quality of language in the manuscript:

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