

Manuscript NO: 88866

Dear editor,

Thank you very much for giving us the opportunity to answer, explain, and argue for the questions and concerns raised by reviewers. We have now addressed the reviewer's comments point-by-point and presented them with **Yellow color**, and others changes including respond to the scientis editor in the revised manuscript are marked with **Blue color**.

We deeply appreciate yours and the reviewer's time to go through our manuscript, thoughts, and input, as well as your continued consideration of accepting our manuscript to be published in World Journal of Gastroenterology.

If you have any further questions, please do not hesitate to contact us. Thank you.

On behalf of all co-authors,

Kind regards,

The corresponding author

Ping Zhang

Dec 23th, 2023

Response to Reviewers

Reviewer #1:

Major comments:

- Statistics is not adequately described: What was the group size (i.e. total number of mice in each group)? In the statistics section, the posthoc test used after the ANOVA (to find out which group is significantly different from which group) must be given.

Answer: We appreciate the reviewer's insightful comments.

We have now added the description regarding the group size as “no less than 3 mice per group” (Page 10, marked in Yellow), additionally we have also added the group size in the caption of the figures and tables whenever applicable.

Furthermore, based on the reviewer's suggestion, we have run the posthoc test after ANOVA using the Least Significant Difference (LSD) test and rewrote the Statistical analysis section (Page 10, marked in Yellow), and marked the analysis results in the Figure 7A-F.

- Fig. 1E: What is the reason to write in the result section that the number of goblet cells was reduced after 6d DSS in the KO group? In the HE staining, you see goblet cells as cells with large cytoplasmic vacuoles and your picture shows MORE of these cells compared to the d0 situation. You should perform PAS staining to clarify this point if possible from your material.

Answer: Thank you for the valuable comments.

The image of 6d DSS in the KO group in our manuscript mainly emphasizes on the substantial infiltration of inflammatory cells, disruption of the intestinal epithelial layer, and marked vascular dilation, all indicative of pronounced inflammatory responses. We thank your suggestion that we shall perform PAS staining to clarify reduction of goblet cells, which we will consider for our

future studies, however for the present study it's unable to perform due to unavailable materials.

For this limitation, we have now rewritten the description of the picture, and added a sentence to better explain the potential reasons for the image inclusion, as "abnormal morphology of goblet cells with a large cellular cavity" (Page 11, marked in Yellow)

- Fig. 3: When using the $\Delta\Delta C_t$ method, the expression of the reference gene(s) must be stable under the conditions used (i.e. after the induction of colitis): Was this the case (see The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments; Clin Chem: 611-622, 2009 for testing)? Otherwise all changes might result from changes in the expression of your "house keeping gene" (GAPDH).

Answer: I agree with you very much. DSS is known to inhibit the activities of both polymerase and reverse transcriptase in RT-qPCR. However, it does not alter the integrity and stability of the RNA. In our manuscript, we performed a rapid and efficient method to purify RNA using lithium chloride, and effectively eliminates the inhibitory impact of DSS on qPCR, and it cited on Page 9 (Ref No. 24, marked in Yellow).

[24] Viennois, Emilie et al. "Dextran sodium sulfate inhibits the activities of both polymerase and reverse transcriptase: lithium chloride purification, a rapid and efficient technique to purify RNA." BMC research notes vol. 6 360. 8 Sep. 2013, doi:10.1186/1756-0500-6-360

Furthermore, GAPDH as a housekeeping gene was often used in DSS-induced colitis studies. I have provided references as followed and have added them in revised manuscript (Page 9, Ref No. 25, 26, marked in Yellow).

[25] Yan F, Wang L, Shi Y, et al. Am J Physiol Gastrointest Liver Physiol. 2012;302(5):G504-G514. doi:10.1152/ajpgi.00312.2011

[26] Bauer, Christian et al. "Colitis induced in mice with dextran sulfate sodium

(DSS) is mediated by the NLRP3 inflammasome.” Gut vol. 59,9 (2010): 1192-9.
doi:10.1136/gut.2009.197822

- Fig. 7: The idea that changes in the expression of Nrf2 are involved in the described effects is very interesting. However, the experiment depicted in Fig. 7 is not convincing. Maybe Nrf2 activation by t-BHQ would also improve the situation in the WT mice. So you need the key experiment to study the effect of t-BHQ on the course of the colitis in WT animals! Minor cimments:

Answer: We appreciate the reviewer's valuable suggestion!

Very common that litters of mice crossbred from alk-SMase^{+/-} mice are limited in numbers and sexes, thus the design of multiple groups in an experiment often faces difficult to conduct. Additionally, the previous study has demonstrated that t-BHQ attenuates ferroptosis against 5-fluorouracil-induced intestinal epithelial cell injury and intestinal mucositis via activation of Nrf2, thus with our limited number of the same litter mice, we did not conduct t-BHQ in DSS-induced colitis firstly. However, we fully aware that perform Nrf2 activation by t-BHQ would improve the situation in WT mice, and will keep this in mind for our further studies whenever animal samples are allowable.

The reference was added in the revised manuscript (Page. 17, Ref No. 50, marked in Yellow).

[50] Deng, Shihua et al. “TBHQ attenuates ferroptosis against 5-fluorouracil-induced intestinal epithelial cell injury and intestinal mucositis via activation of Nrf2.” Cellular & molecular biology letters vol. 26,1 48. 18 Nov. 2021, doi:10.1186/s11658-021-00294-5

Without the group you suggested, our study also provides very valuable knowledge inputs as we found that Nrf2 activation could potentially reverse the extent of inflammatory responses in KO mice after DSS.

- p.4, line 9: I assume you mean the microbiom when you speak about "biological barrier". Perhaps the term "microbial barrier" would be more clear.

Answer: In response to the reviewer's valuable feedback, we would like to clarify the concept of a "biological barrier" and suggest that the term "microbial barrier" may provide a more precise understanding (Page 5, marked in Yellow).

- Especially in the method section, many abbreviations are used without definition. All abbreviations must be defined at first use throughout the manuscript.

Answer: In response to the reviewer's comments, we have provided definitions for all abbreviations used in the manuscript upon their initial appearance.

- Western blot section: give the source of all antibodies (not only that of ZO-1)

Answer: The antibodies listed prior to the ZO-1 antibody were all purchased from Abcam, and this information has been added to the revised manuscript (Page 8, marked in Yellow).

- Table 2: Give the gene accession (data base) numbers of all genes for which you give the primers here.

Answer: The gene accession (data base) numbers corresponding to the primers have been added to the table 2, marked in Yellow.

Response to Science editor:

1 Scientific classification: Grade B.

2 Language classification: Grade C.

3 Specific comments:

(1) Please provide the Biostatistics statement.

Answer: Thank you for your reminder. Please see the attached file of Biostatistics statement from Dr. Wenting Cao.

(2) Please provide the Institutional review board statement.

Answer: This study only involved with animals, and according to Hainan Medical University Ethical Committee Regulations that animal studies only need to be approved for Experimental Animal Welfare Ethical Review.

Additionally, at our institution, ethical reviews are conducted based on projects. Therefore, the ethical approval for this study was granted as part of the project approval process, and not individually for the publication of this manuscript.

(3) Please provide the Informed consent statement.

Answer: Again, this study only involves animal not human, therefore no any informed consent statement was obtained.

(4) Please provide the Language certificate. The English-language grammatical presentation needs to be improved to a certain extent. There are many errors in grammar and format, throughout the entire manuscript. Before final acceptance, the authors must provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend:

<https://www.wjgnet.com/bpg/gerinfo/240>.

Answer: Thanks a lot for your recommendation. We have by now received the edited version of manuscript conducted by Springer nature company.

(5) Please provide the Figures cited in the original manuscript in the form of PPT. All text can be edited, including A,B, arrows, etc. With respect to the reference to the Figure, please verify if it is an original image created for the manuscript, if not, please provide the source of the picture and the proof that

the Figure has been authorized by the previous publisher or copyright owner to allow it to be redistributed. All legends are incorrectly formatted and require a general title and explanation for each figure. Such as Figure 1 title. A: ; B: ; C: .

Answer: We have checked our figures according to your requests. We declare all the figures, pictures, and tables within this manuscript are all owned by the authors listed in the author list.

(6) Please obtain permission for the use of picture(s). If an author of a submission is re-using a figure or figures published elsewhere, or that is copyrighted, the author must provide documentation that the previous publisher or copyright holder has given permission for the figure to be re-published, and correctly indicate the reference source and copyrights. For example, "Figure 1 Histopathological examination by hematoxylin-eosin staining (200 ×). A: Control group; B: Model group; C: Pioglitazone hydrochloride group; D: Chinese herbal medicine group. Citation: Yang JM, Sun Y, Wang M, Zhang XL, Zhang SJ, Gao YS, Chen L, Wu MY, Zhou L, Zhou YM, Wang Y, Zheng FJ, Li YH. Regulatory effect of a Chinese herbal medicine formula on non-alcoholic fatty liver disease. World J Gastroenterol 2019; 25(34): 5105-5119. Copyright ©The Author(s) 2019. Published by Baishideng Publishing Group Inc[6]". And please cite the reference source in the references list. If the author fails to properly cite the published or copyrighted picture(s) or table(s) as described above, he/she will be subject to withdrawal of the article from BPG publications and may even be held liable.

Answer: We declare that all the figures, pictures, and tables within this manuscript are all original and owned by the authors listed in the author list.

(7) Please don't include any *, #, †, §, ‡, ¥, @....in your manuscript; Please use superscript numbers for illustration; and for statistical significance, please use superscript letters. Statistical significance is expressed as aP < 0.05, bP < 0.01 (P > 0.05 usually does not need to be denoted). If there are other series of P values, cP < 0.05 and dP < 0.01 are used, and a third series of P values is expressed as eP < 0.05 and fP < 0.01.

Answer: Thanks for your reminders. We have now checked throughout the

manuscript to meet your requested mentioned above.

4 Recommendation: Conditional acceptance.

Response to Company editor-in-chief:

I have reviewed the Peer-Review Report, all of which have met the basic publishing requirements of the World Journal of Gastroenterology, and the manuscript is conditionally accepted. I have sent the manuscript to the author(s) for its revision according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision by Authors.

However, the quality of the English language of the manuscript does not meet the requirements of the journal. Before final acceptance, it is recommended that the author(s) provide the English Language Certificate issued by a professional English language editing company. Please visit the following website for the professional English language editing companies we recommend: <https://www.wjgnet.com/bpg/gerinfo/240>.

When revising the manuscript, it is recommended that the author supplement and improve the highlights of the latest cutting-edge research results, thereby further improving the content of the manuscript.

To this end, authors are advised to apply PubMed, or a new tool, the RCA, of which data source is PubMed. RCA is a unique artificial intelligence system for citation index evaluation of medical science and life science literature. In it, upon obtaining search results from the keywords entered by the author, "Impact Index Per Article" under "Ranked by" should be selected to find the latest highlight articles, which can then be used to further improve an article under preparation/peer-review/revision. Please visit our RCA database for more information at: <https://www.referencecitationanalysis.com/>, or visit PubMed at: <https://pubmed.ncbi.nlm.nih.gov/>.

Answer: Thanks for your suggestions. We have by now revised our manuscript according to your suggestions listed above.