

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "Hydrogen-rich water protects against inflammatory bowel disease by inhibition of endoplasmic reticulum stress and promotion of heme oxygenase-1 in mice" (Manuscript NO.: 30657). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the reviewer's comments are as following:

Responds to the reviewer's comments:

Reviewer #1:

1. Response to comment: (The main focus of this study is to investigate the possible beneficial effect of hydrogen-rich water on inflammatory bowel disease on DSS mice model. The subject of the review is very interesting and topical. The manuscript is very good structured. The experiments were well designed and the methods used are appropriate. However, there are some details that need to be improved and corrected before publication. When using animals in research it is important to be aware that there are many factors that can affect the results. In light of responsible conduct of animal experimentation and validity and reproducibility of the results there is demand to include all necessary data into manuscript-more information can be found on the website - link <http://iclas.org/committees/ethics-and-animal-welfare-committee> <http://iclas.org/wp-content/uploads/2012/07/ICLAS-Ethical-Guidelines-Editor.pdf>. It can be seen that authors already include most of the necessary information but they forgot to mention a few important data such as: - **How many mice did you use – the number, - Diet (code of the diet and manufacturer) - Exact name of the strain of mice – C57BL/6 is not enough (see Nomenclature <http://www.informatics.jax.org/mgihome/nomen/strains.shtml>)** When using DSS solution it is important that it is prepared on daily basis – however, authors did not provide information whether they prepared solutions daily or once a week.

**Please include this data.)**

Response: It is really true as reviewer suggested that we should provide as many details as possible about the mice experiment. The detailed responses are: 1) Six mice per group are used in the present study; 2) The diet for the mice are purchased from Jiangsu Xietong Pharmaceutical Bio-technology Co., Ltd. The code of the diet is NO.120161128007. 3) the exact name of the strain of mice is C57BL/6J. 4) The DSS solution was prepared on daily basis for use. All these revision were marked red in the revised version.

2. Response to comment: (Section 2.4. Euthanasia Authors wrote - "blood samples were collected from the eyeballs" - this can not be true – it could be from periorbital plexus – please correct)

Response: We have made correction according to the Reviewer's comments. "blood samples were collected from the eyeballs" was revised by "blood samples were collected from periorbital plexus" which was marked red in the revision.

3. Response to comment: (Authors provide three different scorings, which is difficult to follow. I would suggest presenting scoring system in the table.)

Response: It is really true as Reviewer suggested that three different scorings are difficult to follow. We have presented the scoring system in three tables. However, in our opinion, these three tables are oversize for the article which may affect the overall layout. We hope these three tables can be put in the supplementary materials.

4. Response to comment: (Please explain how you calculate % of shortening of colon length – what did you use as a base line?)

Response: According to the previous study, the base line of the colon length and weight is calculated from the mice in control group.

5. Response to comment: (Section 2.7. – measurement of hepatic oxidative stress – Hepatic?? Please correct.)

Response: We are really sorry for this mistake. “hepatic oxidative stress” has been revised by “colonic oxidative stress” in the revision which is marked red.

6. Response to comment: (Section 2.9. – in animal experimentation usually standard error of mean (SEM) instead of standard deviation (SD) is used (due to small number of animal per group—below 30).)

Response: Considering the Reviewer’s suggestion, Standard error of mean (SEM) has been calculated and used in the revised version. Standard deviation (SD) is replaced by SEM according to the Reviewer’s comments.

7. Response to comment: (It is known that different parts of colon (distal, middle, proximal) can be differently affected by DSS. Thus, it is very important that the sampling is done correctly. Please include in the manuscript how did you perform the sampling for the histology, oxidative stress and western blot. There are some typing error that need to be corrected.)

Response: It is really true as reviewer suggested that different parts of colon (distal, middle, proximal) can be differently affected by DSS. In the present study, we selected the distal part of colon as the object of study. Moreover, we have added the detailed performance description of histology, oxidative stress and western blot in the revision. Finally, we have asked American journal experts for help to improve the English.

Special thanks to you for your good comments.

Reviewer #2:

1. Response to comment: (Congratulations is a very well designed work with very interesting results I just wanted to ask: Was there any examination or histological study of the puncture site? It would have been interesting to know if there is any reaction in that place.)

Response: The primary and classic pathological change in DSS-induced is the distal part of colon. So the distal colon is the research emphasis in the present study.

Histological study of the puncture site can be launched in the next step which may have interesting association with the disease.

Special thanks to you for your good comments.

We tried our best to improve the manuscript and made some changes in the manuscript. These changes will not influence the content and framework of the paper. And here we did not list the changes but marked in red in revised paper.

We appreciate for Editors/Reviewers' warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your comments and suggestions.

Chang Liu