

Reviewer #1:

**Scientific Quality:** Grade B (Very good)

**Language Quality:** Grade B (Minor language polishing)

**Conclusion:** Major revision

**Specific Comments to Authors:** General comments: This manuscript "Spinal anesthesia alleviated DSS-induced colitis by modulating gut microbiota" revealed that spinal anesthesia alleviates DSS-induced colitis in mice by affecting the microbiota. Spinal anesthesia reduced increased noradrenaline levels in DSS-induced mice, which may be one of the mechanisms underlining the effect on gut microbiota. This is a new method to reduce colitis and impressive me. The experiment is well designed and the presented data support the conclusion. However, some specific details and mismatch or between figure and text should be revised to improve the quality of the manuscript. Major Revision.

Questions:

1: Why is the quantitative statistical result of Fig. 7B inconsistent with the information shown in Fig. 7A?

Thanks for pointing out the error. We made a mistake for uploading pictures. And we have changed the right picture.

2: Is there no more specific picture of saline+DSS group in HE staining? From the picture of HE staining, we can only see intestinal villi fracture in the saline+DSS group, but there are no other clear inflammatory indicators. Please provide more specific pictures.

Thanks for the suggestions. Yes, the picture of HE staining only show the fracture changes. And the inflammatory indicators have been expressed with inflammation index (Fig 2. F) and serum interleukins (Fig 3.A.B.C).

3: Can you provide the Venn diagram of OTUs in microbial analysis?

Yes, we have Venn diagram and we attached it in the re-uploading manuscript.

4: It is suggested to refer to the article "Dietary Taxifolin enhances the intestinal barrier and regulates intestinal flora to prevent sodium dextran sulfate induced colitis through NF-KB signaling". Spinal anesthesia is a novel approach to alleviate DSS-induced colitis by modulating gut microbiota, however, some discussion of its comparison with other ways should be added, such as

bioactive peptides and taxifolin: "Dietary Bioactive Peptide Alanyl-glutamine Attenuates Dextran Sodium Sulfate-induced Colitis by Modulating Gut Microbiota. Oxidative Medicine and Cellular Longevity", "Dietary Taxifolin Protects Against Dextran Sulfate Sodium-induced Colitis via NF- $\kappa$ B Signaling, Enhancing Intestinal Barrier and Modulating Gut Microbiota. Frontiers in Immunology", "The Potential of Food Protein-derived Bioactive Peptides against Chronic Intestinal Inflammation. Mediators of Inflammation".

Thanks for the suggestion, it is really important to compare our spinal anesthesia way with other ways to against chronic intestinal inflammation. And we have added some discussion as your advised, we marked it with red line in the last paragraph.

5: Line 32-35: Rewrite this sentence. Line 35: Change "reducing" to "reduce". Line 46: Change "inflammation" to "inflammatory". Line 98: Change "mg/kg" to "mg/kg BW". Line 130: Delete "blank". Line 119: Change "microvillus" to "Microvillus". Line 131: Change "was" to "were". Line 139: Change "andthen" to "and then". Line 252: Delete ")". Line 257: Change ";" to ":". Line 265: Change "NY) ." to "NY)". Line 275: Change "stool consistency" to "stool consistency reduce". Line 289: Change "were" to "was". Line 294: Change "the the expression" to "the expression". Line 294: Change "TNF- $\alpha$ in" to "TNF- $\alpha$  in".

Really thanks for pointing out so many spelling and grammar errors. We have corrected all these by ourselves and also, we sent the manuscript to be polished by the recommended English language editing companies.

5: Line 294: Rewrite all "induced by DSS" in this manuscript.

Yes, we have rewritten all "induced by DSS" to "DSS-induced mice" in this manuscript.

6: Line 331-334: Supplement the figure of PCoA.

Thanks, we have made a supplement of the PCoA figure.

7: Line 332-334: Supplement the description about the figure.

Yes, the description of the figure has been added.

8: Line 349-350: Rewrite this sentence.

Thanks for the suggestion, we rewrite the sentence as following: The DSS mice and lidocaine+DSS mice were cohousing for two weeks.

9: Line 367: Please add some references to prove "Several studies".

Thanks for the suggestion, we have added some references to prove "Several studies" in the revised manuscript.

10: Line 370: Change "Figure 1A" to "Figure 7A". Line 374: Change "underline" to "underlining". Line 392: Change "increase" to "increases". Line 414: Change "is" to "are". Line 422: Change "main catecholamine" to "the main catecholamines". Line 140: Write the full name of "BCA". Line 143: Supplement "NC" manufacturer information. Line 144: Delete "a degree of". Line 434: Change "have" to "has". Line 435: Delete "by". Line 439: Change " $\beta$ -Actin" to "Occludin". Line 441: Change "was" to "were". Line 449: Change "underline" to "underlining".

Really thanks for pointing out so many spelling and grammar errors. We have corrected all mistakes in the revised manuscript.

10: Line 583: The pictures in "Figure Legends" are seriously inconsistent with the description in the manuscript,

So sorry, we haven't check carefully, and now we make all the mistakes and keep the legends inconsistent with the descriptions in the revised manuscript.

11: PCoA pictures are missing, and the meaning of pictures 5A and 6A is unclear.

Yes, we have added PCoA picture in revised manuscript, and we rewrote the description of picture 5A and 6A.

12: Please check and modify carefully. Line 628: Change "(DAI) (F)" to "(DAI), (F)". Line 638-639: Supplement the figure of PCoA. Line 643: Change "(DAI) (F) colon length" to "(DAI) (F), colon length".

Thanks, we have added the figure of PCoA and made the writing mistakes in the revised manuscript.

13: Line 653-654: The quantitative statistical results of Figure 7B are inconsistent with the information shown in Figure 7A. Please provide the data (including peak area) used for quantification.

Thanks for pointing out the error. We made a mistake for uploading pictures. And we have changed the right picture.

Reviewer #2:

**Scientific Quality:** Grade C (Good)

**Language Quality:** Grade C (A great deal of language polishing)

**Conclusion:** Major revision

**Specific Comments to Authors:** In this manuscript, the authors investigated the potential effect of spinal anesthesia on inflammatory bowel disease (IBD) by modulating gut microbiota, suggesting a new treatment option for IBD. Overall, the design and performance are good. However, there are some issues with the result presentation and discussion.

1: Rephrase this sentence 'The composition of the fecal microbiota was determined by analysis of ~30,000 paired reads per mouse of the ~250-bp v4 region of the 16S rRNA gene by Illumina MiSeq sequencing'. In addition, in method V3-V4 region was measured, how do you can analyze the V4 region?

Thanks for the advice. We rephrased the sentence as the following: "The composition of the fecal microbiota was determined by analysis of ~30,000 paired reads per mouse of the ~250-bp V3-V4 region of the 16S rRNA gene by Illumina MiSeq sequencing. "

2: Grammar errors: Line 80: co-caged individual > co-caged individuals; Line 86: after approved from > after approval from; microvillus in colon were > was (or microvilli); Line 158: different group > different groups; Line 195: incubated for in the dark, remove for; Line 277: H&E straining > H&E staining; Line 289: the inflammatory response were> was; Line 344: no significantly difference > significant; Line 378: epithelia barrier > epithelial barrier; Line 389: As we known> As we have known; Line 392: which increase gastrointestinal > which increases gastrointestinal; Line 412: relive disease > relieve disease; Line 434: Increasing sympathetic tone have > has; Line 435: development intestinal inflammation > development of intestinal inflammation; etc.

Really thanks for pointing out so many spelling and grammar errors. We have corrected all mistakes in the revised manuscript.

3: Figure 1B and 1C, day 0 to day 14, maybe not count day 15, as well as Figure 5G or 5H. In addition, how to calculate DAI.

Thanks for your advice, we have done the Fig 1B and 1C as you suggested. DAI (disease activity index): a clinical index widely used for Crohn's disease, based on subjective symptoms (body weight loss, colon shortening, rectal bleeding,

and stool consistency reduction). The marks of abscissa of (Figure 1B and 1C, Figure 5G or 5H, Figure 6G or 6H) have been altered as reviewer's advice.

4: Figure 2D to 2J order is now correct, context vs figure. Similarly, checking the order of Figure 3B and 3D.

Thanks, as your suggestion, we have checked the figure order and we made changes in the Fig legend3.

5: The western blotting figure needs semi-quantification, then describes the results.

Thanks for the suggestion, we have attached the semi-quantification figure in the revised manuscript, but as the arrangement of the big Figure 3, we only put one picture of West blot result. And we added the results description as your suggestion.

6: Section 3.6, the result description was not correct. Verrucomicrobia does not decrease in data of Figure 6, induced by FMT.

Thanks, Verrucomicrobia decreased in the data of Figure 5 induced by FMT, not in Figure 6.

7: Several studies have reported the beneficial effects of local or systemic lidocaine in experimental colitis, Topical treatment with Lidocaine in Patients with Ulcerative Proctitis/Colitis. Discussion should be added to explore the novelty of current study.

Thanks for the advice. We added discussion of novelty of current study in the last paragraph.

8: Minors: The phylum name of bacteria is not italic.

Thanks for the suggestion, we made all the bacteria name of italic to normal typeface.

9: Written errors: Line 103: was cut ^and > was cut and; Line 110: 3), , remove one ,; Line 139: andthen blocked > and then blocked; Line 201: and ^the: and the; Line 265: Armonk, NY) ^.; Line 294: the the expression of IL> remove one of the; Line 439: ^Moreover; Number and unit: ml or mL, 1part, 1: 5,000 or 1:5,000

Thanks for pointing out so many spelling and grammar errors. We have corrected all mistakes in the revised manuscript.

Re-reviewer:

The authors revised the comments according to reviewer's suggestion. Good job.

Thanks for your comments.

**1) Science editor:**

The authors have performed a mouse model of DSS induced colitis and used spinal anaesthesia to determine changes caused in intestinal permeability, inflammation and microbiota. They found that spinal anaesthesia improved the intestinal barrier, reduced inflammation and changed the microbiome and these microbiome changes could reduce colitis in other DSS mice. The possible mechanism was proposed to be through changes in sympathetic activation with spinal anaesthesia. It is an interesting and novel study. There are issues with the presentation of results and discussion that have been detailed by the reviewers that need to be addressed.

Language Quality: Grade B (Minor language polishing)

Scientific Quality: Grade C (Good)

**(2) Company editor-in-chief:**

I have reviewed the Peer-Review Report, the full text of the manuscript, and the relevant ethics documents, 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. Before final acceptance, uniform presentation should be used for figures showing the same or similar contents; for example, "Figure 1 Pathological changes of atrophic gastritis after treatment. A: ...; B: ...; C: ...; D: ...; E: ...; F: ...; G: ...". Please provide decomposable Figures (in which all components are movable and editable), organize them into a single PowerPoint file. Before final acceptance, the author(s) 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>.

Thanks so much, we have strictly revised the manuscript according to the Peer-Review Report, Editorial Office's comments and the Criteria for Manuscript Revision. And we unify the figures with the main contents as suggestion.

We have got an English Language Certification from the editing company that editor recommended.