Dear Editors

World Journal of Gastroenterology

Ref.: Revision of the manuscript MS: Manuscript NO-39217

Dear Lian-Sheng Ma,

On behalf of all my colleagues, I would like to thank you and the reviewers for critiquing the manuscript. We found that the comments from the reviewers are constructive and helpful, and have addressed the questions and carefully revised our manuscript accordingly. The reviewer and editor's suggestions have been adopted and we have sent our manuscript to LetPub (http://www.letpub.com.cn/) for improving the clarity and readability of our writing. We anticipate that the revised manuscript entitled " Moxibustion treatment modulates the gut microbiota and immune function in a dextran sulphate sodium -induced colitis rat model" to be considered for publication in "World Journal of Gastroenterology". Below are the point-by-point responses.

Reviewer #1: Microbial dysbiosis is an important factor in the pathogenesis of inflammatory bowel disease including ulcerative colitis (UC). Xiao-Mei Wang et al., show that moxibustion treatment for 7 days significantly restored the colonic mucosa and reduced submucosal inflammatory cell infiltration in rats eliciting UC, caused by intake of dextran sulphate sodium from drinking water. The salutary effects of moxibustion on colitis were mediated via concomitant restoration of healthy gut

microbiota and altered expression of cytokines associated with pro- and anti-inflammatory states. Specifically, moxibustion therapy led to reduced alpha diversity of the microbiome, associated with altered ascorbate, aldarate and amino acid metabolism. The authors concluded that alleviation of UC by moxibustion was mediated via its ability to alter the gut microbiome and intestinal mucosal immunity. Although the findings reported in this manuscript support the overall conclusion, the authors need to address a number of concerns as outlined below:

1. The Abstract and Core Tip sections must be carefully revised to indicate that the salutary effect of moxibustion treatment was restricted to 7-day regimen and that 14-day treatment fared far worse. Also, the authors should more SUCCINCTLY state their cytokine expression data in both Abstract and Core Tip sections. The Abstract should convey the central message of this work without burdening the readers with the details of the PCR and ELISA data, and statistics.

Response:

Thanks for the advice. We have made some appropriate changes accordingly in the abstract and core tip sections of the manuscript and marked in red.

2. The authors need to outline the experimental methodology more clearly. For example, with regard to the model of UC developed with 7 days of 4% DSS administered in drinking water, authors need to explain in Materials and Methods why it was necessary to keep giving rats 1% DSS in their drinking water? Was the quality and quantity of UC different in the absence of continued presence of DSS in water?

Response:

Thanks for the question, after the success of the model building, UC, UC-7, UC-W and UC-14 rats continued to receive a low concentration of DSS (1%) to maintain state of inflammation because this UC model rats had a self-healing tendency, such as relieved congestion, oedema and ulcers, according to our previous studies.

3. How did the authors determine histopathological scores (shown in Fig. 3). The colonic tissue sections, stained with H&E shown in Fig. 2 should be labelled (with arrows or other markers) to indicate key features that reveal differences in the healthy tissue versus abnormal tissue architecture seen in UC with or without treatment.

Response:

Thanks for the question. The detailed information of histopathological scores has been added in the manuscript (Table 1) and marked in red. We have added arrows to indicate the differences in the healthy tissue versus abnormal tissue architecture seen in UC with or without treatment accordingly in the images.

4. Figure Legends need to be more clearly described so the reader can understand the intended explanation of the data contained in the Figures. As an example, it would be helpful for the reader if arrows or some other markers indicated the change pointed out by the authors (infiltration of mononuclear cells or disorganized glands) in Figure 2.

Response:

Thanks for the advice. We have added arrows to indicate the differences in the healthy tissue versus abnormal tissue architecture seen in UC with or without treatment accordingly in the images.

5. The authors should make judicious use of abbreviations throughout the manuscript, without unnecessary repetition. For example, OC in the Core Tip and other sections of the manuscript.

Response:

Thanks for the comments. We have made the appropriate changes about abbreviations accordingly in the manuscript.

6. The Method of collection of blood plasma is unclear as written “Blood plasma were collected by abdominal aortic.” Please clarify this.

Response:

Thanks for your comments. Blood samples were collected by abdominal aortic, after 1 hour’s standing, the samples were centrifuged at 3000 rpm for 10 minutes at 4 ℃ to separate the plasma. The obtained plasma was stored at -80 ℃ until analysis. We have added the content of this part in the manuscript.

7. In the Discussion, authors write that “This finding suggests that short-term (7 day) but not long term (14 day) moxibustion treatment may significantly affect the gut microbiome.” What is the possible explanation of this suggestion and what type of

experimental strategies will be able to discern the mechanism of this difference? Do the quality/quantity of the evolving microbiome and its metabolic consequences (between days 7 and 14) in the GI tract support this suggestion? Please speculate why longer duration of treatment is not helpful and put this observation in a proper context of previously published observations.

Response:

Thanks for the question. In our study, we found that UC-7 had a higher alpha diversity than UC-14, suggests that short-term (7 day) but not long term (14 day) moxibustion treatment may significantly affect the gut microbiome. The short-term moxibustion of UC group can increase the alpha diversity of the bacteria, and the long-term moxibustion cannot further increase the diversity of the bacteria, which may be related to the state of the disease. In addition, the relative abundance of probiotics such as lactobacillus in UC-7 group was higher than that of UC-14.

Reviewer #2: The authors investigated the gut microbiome profiling and the expression of inflammatory cytokines in serum and colon mucosa of healthy rats and DSS induced UC rats with or without moxibustion treatment and reported that reduced diversity, gut microbial dysbiosis, increased inflammatory cytokines and decreased anti-inflammatory cytokines in DSS induced US rats and showed that these effects could be alleviated by moxibustion treatment. Based on these findings, they conclude that moxibustion exerts its therapeutic effect by modulating the microbiome and intestinal mucosal immunity. The paper is well-written and has interesting

findings. Some points should be revised.

1. page 3, line 11-12: This sentence did not show what samples the authors used for cytokine analysis in this study. “in colon mucosa and serum” should be added after “the expression of inflammatory cytokines”. “, respectively” should be added after “by PCR and ELISA”.

Response:

Thanks for your comments. We have added the content of this part accordingly in the manuscript. The detailed information has been added to the abstract of the manuscript and marked in red.

2. references: Page numbers should give full-spelling. For example, the reference No.1:”688-93” should be changed to “688-693”.

Response:

Thanks for your comments. We have made the changes accordingly in the “References” section.

Reviewer #3: This paper has impact to elucidate the mechanisms how moxibustion effect for UC. It is novel to find that moxibustion improved gut microbiome and cytokine expression. However, there are several points for revision as follows.

1, There is discrepancy between microbiome diversity and inflammatory cytokine expression (Ex, Fig5d and Fig 8 or 9). The author described that gut microbial

variation may affect mucosal immunity, but it cannot be concluded.

Response:

Thanks for your comments. In our study, we found that the expression levels of inflammatory cytokines, such as IL-6, IL-12, IL-17, IL-23, IFN-γ, TNF-α, TNFR1 and TNFR2, were increased and that anti-inflammatory cytokine IL-10 and TGF-β expression was decreased in UC rats compared with HC rats. Studies have shown that Lactobacillus and Bifidobacterium can down-regulate inflammatory cytokines[1] and stimulate anti-inflammatory cytokines, such as IL-10[2], so we speculated that gut microbial variation may affect mucosal immunity through up-regulating inflammatory cytokines and down-regulating anti-inflammatory cytokines. However, the specific relationship between gut microbiome and mucosal immunity needs further investigation.

References

1. Llopis M, Antolin M, Carol M, Borruel N, Casellas F, Martinez C, Esp ń-Basany E, Guarner F, Malagelada JR. Lactobacillus casei downregulates commensals' inflammatory signals in Crohn's disease mucosa. Inflamm Bowel Dis. 2009;15:275-283. PMID: 18839424 DOI: 10.1002/ibd.20736

2.Sokol H, Pigneur B, Watterlot L, Lakhdari O, Berm údez-Humar án LG, Gratadoux JJ, Blugeon S, Bridonneau C, Furet JP, Corthier G, Grangette C, Vasquez N, Pochart P, Trugnan G, Thomas G, Blotti ère HM, Dor é J, Marteau P, Seksik P, Langella P. Faecalibacterium prausnitzii is an anti-inflammatory commensal bacterium identified by gut microbiota analysis of Crohn disease patients. Proc Natl Acad Sci U S A.

2008;105:16731-16736. PMID: 18936492 PMCID: PMC2575488 DOI: 10.1073/pnas.0804812105

2, The “A”,”B”,”C”,…in Fig 4 and 5 needs to be changed to “HC”, “UC”, “UC7”.. like other figures.

Response:

Thanks for your advice. We have made the changes accordingly in the figures and figure legends.

We have carefully addressed all the questions above. Thank you for providing the comments to improve our manuscript. If you have any other questions, please do not hesitate to contact us.

Thank you and best regards.

Xiaomei Wang

Shanghai Research Institute of Acupuncture-moxibustion and Meridian, Shanghai University of Traditional Chinese Medicine, 650 South Wanping Road, Xuhui District, Shanghai 200030, China