

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 5309-review.doc).

Title: Effectiveness of a hydroxynaphthoquinone fraction from *Arnebia euchroma* on experimental colitis

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Name of Journal: *World Journal of Gastroenterology*

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The manuscript has been improved according to the suggestions of reviewers: (My revisions are highlighted in yellow in the paper)

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

(1) The index of injury provided at best only qualitative information. From the histology figures it appears that the degree of protection is modest (at least according to my understanding). A more quantifiable index or exact parameter of injury is required, functional assays, such as gut permeability should be also tested.

Re: Regarding the histology figures, it is apparent that the colon of TNBS-treated rat was completely destructed, yet HM obviously reduced the extent and area of destruction. The index of injury is a common indicator for measurement of colonic injury. In this study, reduction of MPO activity also reflects the anti-inflammation of HM. Thus, the present data is able to confirm HM's beneficial effect. Of course, we will consider the review's suggestion in our future investigation with conditions allowing.

(2) Please obtain independent statistical review of the manuscript as the difference between groups in figure 5 may not be significant in many statistical tests. Did you check the distribution of your data? My guess is that you should use non-parametric tests, ANOVA instead of the Student's t test. Moreover, p-values should be given as exact value, the expression "obviously higher" as mentioned in the results is unknown to me.

Re: Thank you for the comment. We have revised these sections. Please refer to the statistical analysis and results sections on page 9.

(3) Figure 6 A and B: please show TNBS and mesalazine data with relevant statistics. These are missing. Figure 7: please show TNBS and mesalazine data with relevant statistics.

Re: We are sorry for it. Colonic samples from mesalazine-treated rats were not measured in this experiment, since we just wanted to explore HM's mechanism of action initially.

(4) The conclusion (HM is a potentially valuable candidate for IBD treatment) goes well beyond the data in this study.

Re: I am sorry for my statement. I have revised it.

(5) Mesalazine (apparently unformulated) was used as a positive control drug in then study. Typically mesalazine is specially formulated to allow delivery to the colon. Please explain how mesalazine worked as an anti-colitis drug in this study (i.e., topically or systemically). Add these comments to the Introduction and /or Discussion, and also provide appropriate literature support.

Re: Thank you for the comment. I have supplemented related content. Please refer to the discussion section in paragraph in 1 on page 13. Meanwhile, I found that the actual dosage of mesalazine was 100 mg/kg in this study, but not 200 mg/kg. The dose of 200 mg/kg was used in another experiment. I am sorry for my carelessness, and I have corrected it.

(6) Why was no data shown for mesalazine in figures 6 and 7? These data would have been useful for delineating and contrasting the MOA of mesalazine and HM extract, and should be included in the paper.

Re: We are sorry for it. Colonic samples from mesalazine-treated rats were not evaluated in these two experiments, since we just wanted to explore HM's mechanism of action initially.

(7) Are there any macroscopic pictures related to the data shown in figure 2? If so, these pictures could be included in the paper.

Re: Thank you for the suggestion. We have supplemented macroscopic pictures. Please refer to Figure 2 on page 20.

(8) HM extract apparently has anti-TNF-alpha effects. However, there is no specific mention in the paper, as to how these effects might be occurring. It would be easy/important to look at the NF-kappa B signaling pathway proteins (p65 and/or I-kappa B), by immunohistochemistry

and/or western blot. Please consider doing these types of evaluations for the revised manuscript.

Re: Thank you for the comment and suggestion. Regarding HM's anti-TNF-alpha effect, we have supplemented related content. For NF-kappa B signaling pathway, I am sorry to say that now we have not enough colonic sample, and the paraffin blocks can not be used due to improper preservation for a long time. We will consider doing these in another model of experimental colitis.

(9) Some statements in the paper are too strong and unwarranted. See the comment in the core tip section on superiority of natural products for IBD. This remark is not based in scientific fact in 2013, and should be amended.

Re: I am sorry for the statement. I have re-written it.

3 References and typesetting were corrected

Thank you again for publishing our manuscript in the *World Journal of Gastroenterology*.

Sincerely yours,

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