

Dear Editors and Reviewers:

Thank you for your letter and for the reviewers' comments concerning our manuscript entitled "**Total flavone of *Abelmoschus manihot* suppresses epithelial-mesenchymal transition via intervening TGF- $\beta$  signaling in crohn's disease intestinal fibrosis**" (Manuscript NO: 39546). We have studied comments carefully and have made correction which we hope meet with approval. Revised portions are marked in red in the paper. The main corrections in the paper and the responds to the reviewer's comments are as follows:

#### **Reviewer 1**

Some minor changes would improve the manuscript

1) Intro: the authors should include the reference for their previous study TFA and intestinal inflammation.

**Answer:** Thank you for your comments. The previous studies about TFA and intestinal inflammation were published in Chinese.

**Zhang Dan**, Qian Haihua, Yang Bolin, Chen Yugen. The effects of Total flavone of *Abelmoschus manihot* on intestinal fibrosis in Crohn's disease mice induced by TNBS. *Jiangsu Journal of Traditional Chinese Medicine* 2017; 49 (3): 76-79.

**Cheng Yingrui**, Chen yugen, Zhou Jinyong, Qu Dinghao, Zhou Zhengxuan, Yang Bolin, Wang Qiong. Effects of Abelmoschi Corolla Extracts on IBD Treatment in Mice and expression of TNF- $\alpha$  and IFN- $\gamma$ . *Journal of Nanjing University of Traditional Chinese Medicine* 2015; 31 (1): 33-35.

2) The authors should explain the rationale for using rat intestinal epithelial cells instead of human intestinal epithelial cells.

**Answer:** Thank you for your comments. From the previous study we knew that TFA had significantly inhibitory effects on the CD rats, and the Masson staining revealed that collagen fibrils of mucosa and lamina propria of colon were markedly decreased, indicating that TFA had the positive inhibitory effects of CD intestinal fibrosis. Therefore in the present study we chose rat intestinal epithelial cells to carry out the experiments. (**Xu S**, Jiang B, Wang H, Shen C, Chen H, Zeng L. Curcumin Suppresses Intestinal Fibrosis by Inhibition of PPAR $\gamma$ -Mediated Epithelial-Mesenchymal Transition. *Evid Based Complement Alternat Med* 2017; **2017**: 7876064. [PMID: 28203261]; Flier SN, Tanjore H, Kokkotou EG, Sugimoto H, Zeisberg M, Kalluri R. Identification of epithelial to mesenchymal transition as a

novel source of fibroblasts in intestinal fibrosis. *J Biol Chem* 2010; **285** : 20202-20212. [PMID: 20363741])

3) The authors should provide more information on the purity of the TFA preparation.

**Answer:** Thank you for your comments. In the present study, we used rotary evaporation under vacuum at 60 °C to obtain the TFA preparation, and dissolved in DMSO to carry out further studies.

4) The authors should provide more information on the siRNA molecules used (e.g. Dharmacon assay ids) and the antibodies used (monoclonal/polyclonal, species generated etc)

**Answer:** Thank you for your comments. We have revised the manuscript.

#### **Reviewer 2**

This study was based on a publication mentioned in the introduction and in the discussion, without a bibliographic reference. This reference is essential for the evaluation and judgment of this publication.

**Answer:** Thank you for your comments. The previous studies about TFA and intestinal inflammation were published in Chinese.

**Zhang Dan**, Qian Haihua, Yang Bolin, Chen Yugen. The effects of Total flavone of *Abelmoschus manihot* on intestinal fibrosis in Crohn's disease mice induced by TNBS. *Jiangsu Journal of Traditional Chinese Medicine* 2017; **49**: 76-79.

**Cheng Yingrui**, Chen yugen, Zhou Jinyong, Qu Dinghao, Zhou Zhengxuan, Yang Bolin, Wang Qiong. Effects of *Abelmoschi* Corolla Extracts on IBD Treatment in Mice and expression of TNF- $\alpha$  and IFN- $\gamma$ . *Journal of Nanjing University of Traditional Chinese Medicine* 2015; **31**: 33-35.

#### **Reviewer 3**

Overall a well written study investigating not only the potential mechanisms behind the development of fibrosis in CD, but how steps in this fibrosis pathway (in the experimental rat intestinal epithelial cell line) may be potentially modified. While the article itself will be of greatest interest to those with a background in basic laboratory research, the topic and well organized presentation is accessible enough for the more “clinically” oriented reader.

Minor comments: Title: Rather than saying “intervening with TGF- $\beta$ ...” should read “interfering with

TGF- $\beta$  ..." or "modifying TGF- $\beta$  signaling..." Abstract: The abstract does not address any of the study "methods". Some mention should be added Introduction: In the first paragraph would consider adding a reference or two when addressing surgical rates and CD. While the rates may be decreasing, other population studies have shown higher percentages than the single reference cited. Paragraph beginning line 57. Would add a sentence or two referencing the background/traditional uses of *Abelmoschus manihot*. Why, how did it become a candidate for investigation? Results: Section 3.1, sentence beginning line 171 appears to belong in the methods section rather than the results section Section 3.4 Line 208 "The activation of phosphorylated..." belongs in the introduction or discussion section. It is not a result. Section 3.5 Line 225 "In addition to the Smad2/3 signaling...." Also belongs in the introduction. It is not a result. Section 3.5 Line 227 "To explore the involvement...." Belongs in the methods section Discussion: The entire first paragraph really belongs in the Introduction, or should follow the second paragraph which begins on line 263

**Answer:** Thank you for your comments. We have revised the manuscript.

Your sincerely

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