

Dr. Lian-Sheng Ma
Editor-in-Chief
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Dear Dr Lian-Sheng Ma,

Thank you very much for your positive reply to our manuscript (Manuscript NO.: 77778, Basic Study) and greatly appreciate the reviewers for the constructive comments. We have revised the manuscript according to your guides and the reviewer's comments. Our point-to-point response is described below. We sincerely hope that the manuscript is now acceptable for publication in *World Journal of Gastroenterology*. We thank you for your kind concern with our work. The following steps were performed to revise our manuscript to meet the requirements for acceptance and publication.

Response to Reviewer comments:

Reviewer #1:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Accept (General priority)

Specific Comments to Authors: Please provide breakdown of the materials included in the formula and any long-term sided effects ever reported in conjunction with its use.

Our response:

Thank you for your positive comments.

Jichuan decoction is recommended as a treatment for functional constipation in three clinical practice guidelines of traditional Chinese medicine and one clinical practice guideline of integrated traditional Chinese

and western medicine (Dai, L, L Zhong, and G Ji, *Irritable bowel syndrome and functional constipation management with integrative medicine: A systematic review*. World J Clin Cases, 2019. 7(21): 3486-3504). Three clinical practice guidelines of traditional Chinese medicine and two clinical practice guidelines of integrated traditional Chinese and western medicine use Jichuan decoction as the recommended formula for the treatment of constipation-predominant irritable bowel syndrome (Dai, L, L Zhong, and G Ji, *Irritable bowel syndrome and functional constipation management with integrative medicine: A systematic review*. World J Clin Cases, 2019. 7(21): 3486-3504.). Zhang and coworkers (Shuang-Xi Zhang, Xiang-an Zhang, Yong-kang An. Effect of Jichuanjian on Gastrointestinal Function, Serum Intestinal Neurotransmitters and Intestinal Flora in Elderly with Chronic Functional Constipation. Chinese Journal of Experimental Traditional Medical Formulae, 2018, 24(22): 169-174) used the JCD for chronic functional constipation in 59 senior subjects for one month. The results showed that JCD has an obvious curative effect and high safety.

We reviewed the main active ingredients of six Chinese herbs including *Angelica sinensis* and *Achyranthes bidentata*, in JCD, detected the therapeutic compounds (please check Supplementary Table 5), and constructed an interactive network through network pharmacology. Both network prediction and experiments indicate that AKT is the key factor of JCD for STC treatment, and quercetin derived from *A. bidentata* and *C. deserticola* of JCD was considered the key active ingredient (Table 1). However, long-term effects and side effects have been observed. For instance, Zhang and coworkers (Shuang-Xi Zhang, Xiang-an Zhang, Yong-kang An. Effect of Jichuanjian on Gastrointestinal Function, Serum Intestinal Neurotransmitters and Intestinal Flora in Elderly with Chronic Functional Constipation. The Chinese Journal of Experimental Traditional Medical Formulae, 2018, 24(22): 169-174) used the JCD for chronic functional constipation in 59 senior subjects for one month. The results showed that JCD has an obvious curative effect and high safety. Slight side effects of long-term JCD use were observed in a few subjects, including dry mouth (1/59,

1.7%), dizziness (1/59, 1.7%) and fatigue (1/59, 1.7%); the adverse rate was not statistically higher than that of prucalopride succinate, a positive drug for STC intervention. Taken together, the basic materials included in JCD are beginning to be clear, without any significant long-term adverse effects ever reported in the literature.

Reviewer #2:

Scientific Quality: Grade C (Good)

Language Quality: Grade B (Minor language polishing)

Conclusion: Major revision

Specific Comments to Authors:

1. We conducted an extensive and elaborate study on STC in an animal model to understand the mechanism and changes after TCM treatment.

Our response:

Thank you for your positive comments.

Yes, the model made for STC research is a field of great controversy. Several STC models have been established, including opioid-induced mice. At present, drugs usually applied in STC models are the compounds diphenoxylate, loperamide and morphine. Morphine, as a strictly controlled psychoactive drug, is always unavailable. Both diphenoxylate and loperamide inhibit intestinal motility by stimulating intestinal opioid receptors (Baker et al., 2007), which are characterized by delayed defecation and reduced fecal water content. In addition, atropine was added to diphenoxylate, which also inhibited intestinal motility (Wang et al. 2019). Previous studies have shown that diphenoxylate can induce constipation better than loperamide (Yang et al. 2019). The major mechanism of compound diphenoxylate is the inhibition of local peristalsis. This model stimulates the pathological changes in the colon of STC patients well, with the advantages of simple operation, low cost, and a relatively high achievement ratio. Furthermore, these STC mice were sensitive

to opioids, including diphenoxylate, which is a representative symptom of constipation, and had a short modeling period. Hence, it is one of the most useful models for STC research. However, our work and relevant publications are preliminary studies, and extensive research to understand the anti-constipation mechanisms of TCM is welcome.

2. Authors are advised to mention the chemical composition of six Chinese herbs- *Angelica sinensis*, *Cistanche deserticola*, *Achyranthes bidentata*, *Fructus aurantii*, *Alisma orientalis* and *Cimicifuga heracleifuga* used in JCM. This may help to understand the chemical effects on the COLON and the correlation with ACH / other ACH/other metabolites in STC after JCD treatment.

Our response:

Thank you for your kind guide.

As mentioned above, we probed the active ingredients of JCD (Supplementary material Table 5) that can significantly alleviate the symptoms of STC patients, enhance gastrointestinal function, and regulate the expression levels of intestinal neurotransmitters (Shuang-Xi Zhang, Xiang-an Zhang, Yong-kang An. Effect of Jichuanjian on Gastrointestinal Function , Serum Intestinal Neurotransmitters and Intestinal Flora in Elderly with Chronic Functional Constipation. Chinese Journal of Experimental Traditional Medical Formulae;2018,22). On the other hand, insufficient gastroenteric motility is a crucial factor in STC (Wu, et al., 2021; Jiang, et al., 2017; Chen, et al., 2014). Therefore it is a potential field to understand the chemical effects of enteric neurotransmitters including ACH, in STC subjects after JCD treatment.

3. A paragraph on use of JCD in humans, its long term outcome and side effects from published larger study would be better if included in introduction.

Our response:

Thank you for your suggestions.

JCD is a classical TCM formula set up by Jing-Yue Zhang, an outstanding physician in the Ming Dynasty. JCD has been extensively used for STC and other gastroenteric disorders, and considerable documents have been accumulated. The background and clinical outcomes have been added to the revised manuscript. Nevertheless, less further was included in the revised manuscript only for the case that too little international literature on JCD against constipation are currently available. A meta-analysis focused on STC intervention by TCM is necessary.

4. There was no mention how the STC was confirmed ? inconfirmed? in an animal model after oral administration of diphenoxylate, that all of them developed STC. Objective evidence is necessary.

Our response:

Thank you for your comments.

The identification of STC mode-made is a prerequisite for further experiments. Several key indexes of STC were detected to identify successful STC mice, such as fecal particle count, wet weight, dry weight and water content, intestinal propulsion rate and expression level of colonic ACH. The results showed that all mice orally administered the compound diphenoxylate (10 mg/kg.d) for 14 days were induced as STC models. Such a high molding ratio can be observed in relevant publications. For instance, Zhan et al. (Yu Zhan, Xuegui Tang, Hong Xu, Shiyu Tang. *Maren Pills Improve Constipation via Regulating AQP3 and NF- κ B Signaling Pathway in Slow Transit Constipation In Vitro and In Vivo. Evid Based Complement Alternat Med.* 2020;2020:9837384.) constructed a rat model by using diphenoxylate (10 mg/kg), and all subjects were identified as STC rats. Wang et al. (Wang L, Wang F, Zhang X, Chen Q, Xu J, Li H, Li F, Yang M. *Transdermal Administration of Volatile Oil from Citrus aurantium-Rhizoma Atractylodis Macrocephalae Alleviates Constipation in Rats by Altering Host Metabolome and Intestinal Microbiota Composition. Oxid Med Cell Longev.* 2022;2022:9965334) and

Zhu et al. (Feiye Zhu, Shan Xu, Yongsheng Zhang, Fangming Chen, Jinjun Ji, Guanqun Xie. *Total Glucosides of Paeony Promote Intestinal Motility in Slow Transit Constipation Rats through Amelioration of Interstitial Cells of Cajal*. *PLoS One*. 2016;11(8):e0160398.) used the same chemical and obtained similar results for high molding ratios. Therefore, we believe that diphenoxylate is an effective compound for the construction of STC models in rodents. However, the underlying mechanism remains to be explored.