

Answering the reviewers' comments

Thanks for referees' reviewing our manuscript (**World Journal of Gastroenterology Manuscript NO: 48437**) and giving significant comments and valuable advice on our manuscript. According to the referees' comments, we carefully revised our manuscript. The major changes were marked in red in our revised manuscript. Some unimportant and ambiguous sentences have been revised. Here, some minor revisions are not explained and shown. The revisions in detail are shown below including a point by point response to the reviewers' comments. The English of the manuscript was polished by the professional language editing support recommended by you (Mede Editing Service <http://meditorexpert.com>). All the changes were marked in red in our revised manuscript.

Reviewer #1:

Comment 1: According to the resarch published onto WJG, Volume 15, Issue 19, 21 May 2009, Pages 2414-2417, authors should put as limitation to study the possibility of adverse event as every herbal medication or derivate.

Answer: Thanks for your suggestion. It is true that every herbal medication or derivate may cause some adverse events such as your mentioned report in WJG, Volume 15, Issue 19, 21 May 2009, Pages 2414-2417. However, in this study, we try to evaluate the effect of *Tong Xie Yao Fang* on irritable bowel syndrome (IBS) rats by using metabolomics method, find its potential biomarkers, analyze the metabolic pathways, and explore the pharmacological effects, mechanisms of action and syndrome essence of syndrome model. *Tong Xie Yao Fang* originates from "Danxi Heart method" of the traditional Chinese medicine that is composed of *Atractylodes macrocephala*, *Paeonia lactiflora*, *Chen Pei* and *Fangfeng*. It is a representative prescription for treating liver and spleen deficiency and abdominal pain diarrhea. It shows the effect of tonifying spleen and inhibiting liver and relieving diarrhea, and has unique advantages in the treatment of gastrointestinal dysfunction such as IBS. In addition, the prescription, *Tong Xie Yao Fang*, is safe and effective, and no adverse events have been found in clinic. Therefore, we carried out the study on the therapeutic mechanism of *Tong Xie Yao Fang* on IBS using

rat as model, and did not consider whether herbs or derivatives had other adverse conditions for the time being.

Comment 2: The link with Spleen (26 times mentioned) should be correctly referenced as per -- journal of translational medicine, -Volume 9, Issue 1, 16 August 2011, Article number 136. journal of translational medicine.

Answer: Thanks for your kind advice. Your mentioned literature (journal of translational medicine, -Volume 9, Issue 1, 16 August 2011, Article number 136.) is valuable to investigate Spleen. But this paper pays attention to the change of endogenous metabolites in urine of irritable bowel syndrome (IBS) rat models. The reported method and Liver-spleen axis are not relevant to our study. In Chinese medicine, *Tong Xie Yao Fang* is a representative traditional Chinese prescription for the treatment of the liver and spleen deficiency deficiency, and abdominal pain and diarrhea. In future study, we will consider and refer the mentioned method and obtain the data about Liver-spleen axis etc. to investigate the therapeutic effect of *Tong Xie Yao Fang* on the liver and spleen deficiency deficiency.

Comment 3: The psychological basis and the every day-living stress should be deeply analysed.

Answer: In our study, the effects of psychological stress and emotional changes on IBS in model rats were carried out. Through the open field experiment, dark box test and sugar water consumption experiment in rat behavior, it was concluded that *Tong Xie Yao Fang* could obviously alleviate the depression and anxiety of model rats. These results in detailed will be reported in the future study, because they were not relevant to the topic in our manuscript.

Comment 4: The importance of altered microbiome should be emphasised.

Answer: At present, there are a lot of studies on microflora in disease, especially the changes of intestinal microbiome. In recent years, the study of intestinal microbiome mechanism has attracted more and more attention in the course of IBS. The imbalance of flora can cause / aggravate visceral hypersensitivity, gastrointestinal motility disorder,

intestinal mucous barrier damage and immune mechanism disorder in patients with IBS. Traditional Chinese medicine holds that liver and spleen deficiency is a necessary stage in the development of IBS and plays an important role in the evolution of its pathogenesis. Therefore, the effects of intestinal microbiome on IBS were studied in our group, but the changes of endogenous metabolites in urine were investigated in this manuscript, so the changes of microbiome were not involved in this manuscript.

Comment 5: The link with NAFLD should be pointed out, at large discussed and referenced. here are many typos and repeated words.

Answer: IBS is a common functional bowel disease, and its main symptoms are recurrent abdominal pain, diarrhea, constipation, or alternations between diarrhea and constipation. The main feature is chronic recurrent abdominal pain or abdominal discomfort associated with intestinal dysfunction. It is believed that the pathogenesis of IBS involves the abnormal interaction between brain and intestine, and is a kind of biological-psychological-social disorder. The pathogeny of IBS is complex and various, and its pathophysiological basis is mainly gastrointestinal motility and visceral sensory abnormalities. Nonalcoholic fatty liver disease (NAFLD) refers to acquired metabolic stress liver injury, which is characterized by excessive deposition of intracellular fat caused by other definite liver damage factors except alcohol, which is closely related to insulin resistance and genetic susceptibility. Both IBS and NAFLD are digestive system diseases, but their pathogenic factors, pathogenesis and pathology are different, and the treatment schemes are also different. *Tong Xie Yao Fang* is often used in the treatment of IBS, and rarely involves the treatment of NAFLD. Therefore, the relationship with NAFLD is not pointed out in this manuscript.

The typos and repeated words have corrected in the revised manuscript. Moreover, the English of the manuscript was polished by the professional language editing support recommended by the Journal (Mede Editing Service <http://meditorexpert.com>).

Reviewer #2:

Comment: The methodological design of the study is adequate and evaluates markers of the complex multifactorial etiology of irritable bowel syndrome (IBS), despite the limitations of the experimental studies of this clinical entity. One of the limitations of the studies involves the effects of pharmacological agents in homogenous populations as experimental animals in studies related to functional digestive diseases. I recommend the publication.

Answer: Thanks for your positive comments on our manuscript.