

## ANSWERING REVIEWERS



June 9, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 11028-review.doc).

**Title:** Effects of the Danshao Huaxian capsule on *Gremlin* and *bone morphogenetic protein -7* in hepatic fibrosis rats

**Author:** Xue-Ke Zhao, Ming-Liang Cheng, Rong-Min Wu, Yu-Mei Yao, Mao Mu, Juan-Juan Zhu, Bao-Fang Zhang, Ming-Yu Zhou

**Name of Journal:** *World Journal of Gastroenterology*

**ESPS Manuscript NO:** 11028

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

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

Reviewer No.2314: This is an interesting study. My suggestions: 1) it is important to explain why the two doses (8 and 16 times the clinical doses) were selected: what is the translational value of the study? 2) add a statement on ethical approval; 3) please add a scale bar on microscopic images; 4) the English style needs careful revision to improve clarity.

(1) A: Danshao Huaxian Capsule has been approved to be marketing by SFDA in China. In clinics, the total daily dose is 4.5 g, and it should be taken in 3 times. The dose for human body was calculated to be 0.0643 g/kg or so (for 70 kg human body). The dose of intragastric administration for rats was 0.5 g/kg, 7.78 times that of the human body; 1.0 g/kg (15.56 times that of the human body). That is to say, the doses close to 8 times or 16 times the doses of the human body. The two doses were chosen according to previous reference<sup>[1]</sup>.

[1]Geng XX, Yang Q, Xie RJ, Luo XH, Li CX, Cheng ML. Effects of Dan-Shao-Hua-Xian on the expression of collagen type I and III in rats with hepatic fibrosis. *Hepatobiliary Pancreat Dis Int* 2004; 3: 558-563.

(2) A: “Statement on ethical approval” has been supplemented.

(3) A: The amplification of the micrograph has been marked. The positively expressed cells have been marked by arrow. A group of immunohistochemical figures (Fig 2A, B, C, and D; Fig 3A) have been re-supplemented.

(4) A: English writing has been modified by a corporation for professional medical translation recommended by WJG.

(5) The effect of the Danshao Huaxian Capsule (DHC) on the expression of TGF-beta1 in rat liver tissue has been studied in our previous experiment. DHC was found to be capable of down-regulating the expression of TGF-beta1 in rat liver tissue, which has been published in academic journals included by Medline<sup>[1]</sup>. Therefore, the effect of DHC on the mRNA and protein expression of TGF-beta1 in rat liver tissue has not been repeated in this experiment. We only detected the level of TGF-beta1 in the serum and the liver homogenate. I am very sorry that we mistook the concentrations of HA and TGF-beta1 in the serum for the levels in the liver homogenate in our manuscript. We have corrected the mistake and added the levels of HA and TGF-beta1 in the liver homogenate in Tab 2.

[1] Zhang Wensheng, Cheng Mingliang, Lu Mengying. Effect of Chinese Herbal Compound on cytokines of liver fibrotic rats. *Chinese Journal of Hepatology*, 2003, 11 (5): 285-287.

(6) The revisions have been marked **by red** in the manuscript.

Reviewer No.1810523: In this manuscript, Zhao and colleagues reported protective effect of Chinese herb medicine DHC on the pathogenesis of liver fibrosis using a CCl4 induced liver fibrosis rat model. The authors concluded that the DHC effect is likely through its inhibition of TGF-beta and gremlin expressions, and its upregulation of BMP-7. Although the role and mechanism of gremlin and BMP-7 in liver fibrosis have been reported by several groups, the therapeutic potential of DHC in liver fibrosis is of interesting. Unfortunately, the manuscript is poorly written and difficult to understand, which raise many concerns of the reviewer. 1. The authors must provide statement that the experiments involve animals are approved by the institutional animal care and use committee. 2. The authors should have consulted a scientific writer/editor and got help on manuscript writing in English and data presentation in the Results section. 3. The manuscript should have been numbered on each page. 4. Inhibition of either gremlin or TGF-beta would result in amelioration of liver fibrosis. Hence, the major effect of DHC is likely through downregulation of TGF-beta; while the decreased gremlin could be secondary, given that gremlin could be regulated by TGF-beta signaling. In vitro experiment using isolated primary cells should be performed to address this question. 5. TGF-beta measurement in the liver tissue should be normalized by the tissue weight, not by the concentration of the solution. 6. What is the "Positive protein expression rate" in Tables 4 & 5, and how is it rated? 7. I am not convinced that the images in Figures 2 & 4 are all taken using 400x magnifying power. In addition, the location of the positive staining and whether it correlates with fibrosis in these figures should be indicated.

(1) A: "Statement on ethical approval" has been supplemented.

(2) A: English writing has been modified by a corporation for professional medical translation recommended by

WJG.

(3) A: Page numbers have been listed at the lower right corner in every page.

(4) A: In vitro experiment using isolated primary cells are ongoing.

(5) A: The effect of the Danshao Huaxian Capsule (DHC) on the expression of TGF-beta1 in rate liver tissue has been studied in our previous experiment. DHC was found to be capable of down-regulating the expression of TGF-beta1 in rate liver tissue, which has been published in academic journals included by Medline<sup>[1]</sup>. Therefore, the effect of DHC on the mRNA and protein expression of TGF-beta1 in rate liver tissue has not been repeated in this experiment. We only detected the level of TGF-beta1 in the serum and the liver homogenate. I am very sorry that we mistook the concentrations of HA and TGF-beta1 in the serum for the levels in the liver homogenate in our manuscript. We have corrected the mistake and added the levels of HA and TGF-beta1 in the liver homogenate in Tab 2.

[1] Zhang Wensheng, Cheng Mingliang, Lu Mengying. Effect of Chinese Herbal Compound on cytokines of liver fibrotic rats. *Chinese Journal of Hepatology*, 2003, 11 (5): 285-287.

(6) A: How the positive protein expression rate was calculated has been supplemented in this paper.

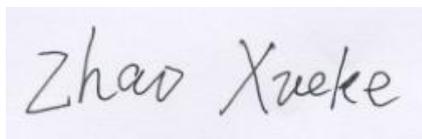
(7) A: A group of immunohistochemical figures (Fig 2A, B, C, and D; Fig 4A) have been re-supplemented. The amplification of the micrograph has been marked. The positively expressed cells have been marked by arrow.

(8) The revisions have been marked **by red** in the manuscript.

3 References and typesetting were corrected

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

Sincerely yours,

A rectangular box containing a handwritten signature in black ink that reads "Zhao Xueke".

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