Author's response to reviews

Title: Attenuation of early liver fibrosis by herbal compound "Diwu Yanggan" through modulating the balance between epithelial-to-mesenchymal transition and mesenchymal-to-epithelial transition

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Author's response to reviews: see over
Dear editor:

Thank you very much for reviewing the manuscript MS. 8325922581333335 by X. Shen et al.

Please find enclosed a revised version of the manuscript with improvements according to the reviews’ suggestions. Specifically, we reply to their concerns as follows:

**Referee 1 (Rui-Qiong Ran):**

1. Conclusion is well written but needs to be a bit shorter by eliminating unnecessary sentences. My concern is that the conclusion is a bit too long and you'll lose the reader.  
**Answer:** We have modified the “Conclusions” section in the revised manuscript.

2. The last paragraph of background needs to be revised. You should add the main results to this paragraph. In addition, “in order to decipher the clinical efficacy and make better application of DWYG” needs to be rewritten.  
**Answer:** We have modified the last paragraph of background according to the referee’s suggestion in the revised manuscript.

3. I rewrote or corrected the following sentences or words (see blue color)  
Page3, line 71: severe morbidity and significant mortality; considerable morbidity and mortality  
Page3, line 77: Please delete “as even advance fibrosis is reversible”  
Page3, line 88: be paramount for normal embryonic development; be paramount importance for normal embryonic development  
Page4, line 91: Various in vitro and in vivo studies; In vitro and in vivo studies  
Page4, line 103-104: Through obviously decreasing the serum levels of ALT and AST; as evidenced by the results of an obvious decrease in serum ALT and AST levels.  
Page4, line 112: it has been shown to provide good clinical application; provide clear therapeutic benefit  
Page4 line 115: Based on above; Based on the above mentioned information  
Page5 line 133: recovered ethanol by reduced pressure; removed ethanol by reduced pressure  
Page6 line 158: Serum AST/ALT determination; Determination of serum ALT and AST activities  
Page8 line 216: treatment with DWYG; DWYG treatment  
Page8 line 221: inhibited the increases of serum ALT and AST; inhibited the increase in serum levels of AST and ALT.  
Page8 line 234-235: was drastically decreased to 4.41±1.02% # was drastically decreased from 8.10±0.92% to 4.41±1.02% #  
Page8 line 237-239: indicating that DWYG had an inhibition effect #indicating that DWYG has an inhibitory effect.  
Page8, line 239: induced a disastrous elevation of hydroxyproline at 6 weeks; induced a considerable elevation in the level of hydroxyproline at 6 weeks.  
Page9 line 243: inhibition effect; inhibitory effect  
Page9 line 260: induction; treatment  
Page9 line 268: accompanied by repression of the protein; accompanied by diminished protein  
Page10 line 282: stimulating a mean 1.5-fold increase in transcript expression levels; stimulating
1.5-fold increase in transcript expression levels

Page 10 line 284: a mean 3-fold decrease; 3-fold decrease

Page 10 line 286: expression ratio TGF-#1/BMP-7; expression ratio of TGF-#1/BMP-7

Page 10 line 291-292: liver fibrosis in CCl4-induced rats; CCl4-induced liver fibrosis in rats

Page 10 line 297: has been implicated in EMT induction #has been implicated in the regulation of EMT

Page 11 line 305-306: compared to CCl4-induced model group; compared to that of rats treated with CCl4 alone

Page 11 line 309: the robust mRNA and protein expressions of Smo exhibited in untreated CCl4-induced fibrotic rats were considerably diminished; robust increases of Smo mRNA and protein expression in untreated CCl4-induced fibrotic rats were considerably diminished.

Page 11 line 311: correspondingly weakened; was correspondingly diminished

Page 11 line 321-322: chronic CCl4-injury rat liver: CCl4-induced chronic liver injury

Page 11 line 323: provoked the reverse of EMT to MET; could result in reversal of EMT to MET

Page 11 line 324-325: up-regulation expression of E-cadherin and down-regulation expression of Vimentin #up-regulation of E-cadherin expression and down-regulation of Vimentin expression

Page 11 line 326: anti-fibrotic efficacy#anti-fibrotic mechanism

Page 12 line 333: have been ascendant due to its effects of; are in the ascendant due to their effects on

Page 13 line 356-366: As shown in our study; Our study showed that chronic exposure

Page 13 line 368: liver fibrogenesis by CCl4 induction; CCl4-induced liver fibrogenesis

Page 13 line 369: were paralleled to the elevation; were paralleled by the elevation

Page 17 line 356: contributed to alleviate the degree of; contributed to alleviating the degree of

Page 17 line 358: Anti-#-actin blotting was used to control for equal protein loading; Anti-#-actin blotting was used as control for equal protein loading.

Answer: We have modified the above sentences according to the Referee’s suggestions.

Referee 2 (Wei-Fen Xie):

Major Compulsory Revisions:

Usually, the rat liver fibrosis should be induced by injection of CCl4 for at least 8 weeks. The authors only treated rat for 6 weeks. In Fig 2A and Fig 2B, they showed that CCl4 treatment induced broad necrosis of hepatocyte, but only weak fibrosis. In addition, these figures also indicated that DWYG did not significantly decrease the level of necrosis. This observation is controversial with the data of plasma ALT and AST (Fig. 2E). So the animal experiment should be repeated to achieve more convincible data.

Answer: We are sorry for selecting the wrong H&E-staining image of DWYG-treated group in Fig 2A and this image is actually for CCl4-induced model group in H&E staining. As for Fig 2B, there are really difference on the degree of steatosis, necrosis and fibrosis in liver tissues between CCl4-induced model group and DWYG-treated group, and we might have no select representative area so that no obvious difference was seen. So in the revised manuscript we reselected the representative histological images (see revised Fig. 2A and 2B) and modified the inaccurate claims in the Results section. As for choosing 6 weeks for the duration of CCl4 administration in the
animal experiment, we thought that in this study DWYG was mainly as a preventive medicine for CCl₄-induced liver fibrosis in rats and its inhibitory effect on hepatic fibrogenesis in rats during the initial phases was able to fully exhibit in the CCl₄ induction of 6 weeks. Moreover, we also emphasized the concept of “early liver fibrosis” in the title. In addition, in the experiment we also observed that the histological grade of hepatic fibrosis in model groups was mainly between S2 and S3, which were consistent with the reports of Li et al. [¹] and Wang et al. [²].

Reference:

Minor Essential Revisions:
The figures should annotate as the order they mentioned in the text. Especially for figure 2.
Answer: We have modified the above section according to the Referee’s suggestion. In addition, we have modified the mistakes of spelling and grammar in the revised manuscript.

We appreciate the helpful suggestions of the reviewers, and please feel free to contact us if there are further questions or queries. Meanwhile, we also hope that the revised manuscript is now suitable for publication in BMC Complementary and Alternative Medicine.

With kind regards,

Hanmin Li