Author's response to reviews

Title: Chlorpromazine-induced Perturbations of Bile Acids and Free Fatty Acids in Cholestatic Liver Injury Prevented by the Chinese Herbal Compound Yin-Chen-Hao-Tang

Authors:

Qiaoling Yang (cherish0814@126.com)
Fan Yang (fanyangforever@gmail.com)
Xiaowen Tang (shs912vv@126.com)
Lili Ding (nail8219@126.com)
Ying Xu (skyxu_1983@163.com)
Yinhua Xiong (xiongyhfriend@hotmail.com)
Zhengtao Wang (wangzht@hotmail.com)
Li Yang (yangli7951@hotmail.com)

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Author's response to reviews: see over
Dear Editors and Reviewers:

Thank you very much for the reviewers’ comments concerning our entitled manuscript “Chlorpromazine-induced Perturbations of Bile Acids and Free Fatty Acids in Cholestatic Liver Injury Prevented by the Chinese Herbal Compound Yin-Chen-Hao-Tang” (Manuscript No. : 1057055714147219). We have done additional analysis as suggested. Our revised manuscript has submitted according to the editors and reviewers' comments. Revised parts are marked in red in new version.

We sincerely hope that the revised manuscript meets the standard of BMC Complementary and Alternative Medicine.

Thank you and regards,

Li Yang, Prof., Ph.D.
Institute of Chinese Materia Medica, Shanghai University of Traditional Chinese Medicine.
1200 Cailun Road, Shanghai 201210, China
Tel: 86-21-51322506
Fax: 86-21-51322519
E-mail: yangli7951@hotmail.com
Response to Reviewer A (kewei Li)

1) The major weakness was the unreasonable grouping. WHY group 1 received intragastrical treatment of peanut oil while group 3 received YCHT in water and it was not mentioned what and how group 2 received. Would the authors comment this?
Answer: We are truly sorry for the mistakes and we have cleared the misunderstandings in revised manuscript (page 4, line 30).

2) If the authors want to get the more convincing outcomes, the more groups should be needed such as the groups of different YCHT dose and different administration time
Answer: We appreciate your suggestions. In our preliminary study, we did dose-responsive experiments, and turned out that YCHT showed better protective effect on chlorpromazine-induced liver injury at 8g/kg. Besides, published papers showed the protective effect of YCHT ranges from 2g/kg to 12g/kg in absence of toxicity. Therefore, the dose of YCHT at 8g/kg was preferred in current study.

3) It was difficult to decide the dose of chinese herbal quantitatively. Would the authors tell how to calculate the dose?
Answer: Crude drug materials of Rhubarb, Gardenia and Artemisia capillaries were prepared as mentioned in Method and Materials section. Then the solution was freeze-dried into powder and the extract yield is 24.6% (The extract yield = (the weight of the freeze-dried powder/ the weight of the crude drug)*100%). Accurately weight the powder and dissolve into distilled water for 0.8g/ml.

4) The authors should tell us the animal model was successfully made in the first part of results.
Answer: We measured the ALT, AST and ALB as liver injury parameters and TBIL as cholestasis parameter after CPZ administration. The significant increase of that as well as H&E staining changes indicate liver damage along with mild cholestasis occurred, which has shown in revised manuscript (page 7, line 28, Figure 2, Figure 3).

5) The authors should show the effects of YCHT on the histological changes quantitatively or semi-quantitatively.
Answer: Thanks for the suggestion. We did the histological analysis in a semi-quantitative manner, which has shown in Figure 3D.
6) Liver tests are not complete. Bilirubin should be measured.

Answer: We measured the TBIL in all groups, please refer to Figure 2.

Response to Reviewer B (Jian Luo)

1) The profile of the bile acids reported here doesn’t seem to be or at least not a complete profile of rodent serum bile acids. Different forms of muricholic acids are major components of rodent serum bile acids. They are missing from the profile. (Major Compulsory Revisions)

Answer: We detected α-, β- and ω-muricholic acids in our study by LC-MS as suggested (Figure 5).

2) Cholesterol is the source of bile acid synthesis. It is known that cholestatic liver injury may disturb the bile acid synthesis and as such cholesterol metabolism. Hypertriglyceridemia has been observed in cholestasis and could be resulted from a defect of plasma triglyceride catabolism due to liver injury. The authors seem to link the chlorpromazine-induced lipids (FFA) change to “lipotoxicity” in the liver and as a result, change of FFA is part of the liver injurymechanism. There are no data to support this. (Major Compulsory Revisions)

Answer: The change of FFA is due to the liver injury and as a result of triglyceride or even overall lipid homeostasis imbalance. In our study, we intended to exert the hepatoprotective effect of YCHT against CPZ based on correcting the BA and FFA profiles. The underlying mechanism of how liver injury was attenuated by YCHT through BA and FFA correcting warrants further investigation.

3) The dosage of YCHT used seems to be very high (8 g/kg) as compared others reported in the literature (e.g., Lee et. al., J Ethnopharmacol, 2007). (Major Compulsory Revisions)

Answer: In that reported literature, YCHT was exposure for 27 days at dose of 500mg/kg (Lee et. al., Journal of Ethnopharmacol, 2007.) in order to avoid toxicity for long term use. In addition, the other published literature showed protective effect of YCHT at dose range from 2g/kg to 12 g/kg (Liu Lian ,et al, World Science and Technology/ Modernization of Traditional Chinese Medicine and Materia Medica, 2007). Moreover, based on our previous study, the dose of YCHT at 8g/kg for 9 days treatments was effective and non-toxic. In conclusion, we preferred this dose in current study.