Format for ANSWERING REVIEWERS

January 18, 2015

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 14896-review.doc).

Title: Glycyrrhizic acid inhibits apoptosis and fibrosis in CCl4-induced rat liver injury
Author: Bo Liang, Xiao-Ling Guo, Jing Jin, Yong-Chun Ma, Zheng-Quan Feng
Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 14896
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 00003472

(1) CCl_4 is well known to induce necrosis as well as apoptosis. The authors should add comments regarding the effect of necrosis in Discussion.

Thanks for your advice. The comment "Necrosis as well as apoptosis was involved in the process of liver fibrosis[1]. In our previous study we found that hepatocyte apoptosis could induce liver fibrosis."have been added in Disscussion

(2) Please add the data of serum transaminases in this model.

Thanks for your advice. In our previous study, we found that GA significantly reduced serum activity of ALT (from 526.7 ± 57.2 to 342± 44.8, $p< 0.05$) and AST (from 640 ± 33.7 to 462.8± 30.6, $p< 0.05$) after 8 weeks of treatment[2].

(3) The authors mentioned CCl_4 was injected subcutaneously in CCl_4 group whereas CCl_4 was intraperitoneally injected in GA group. The authors should treat mice by same methods. In addition, a vehicle of CCl_4 is generally used oil but not water. Did authors mix CCl_4 and GA, and administrated simultaneously.

Thanks for your advice. In the GA group, rats were also treated with a 40% solution of CCl_4 via hypodermic injection at a dose of 3 mL per kilogram plus 0.2% GA solution in water by means of the intraperitoneal injection of 3 mL per rat, and three times a week beginning at the first week.

(4) It is interesting whether glycyrrhizic acid directly inhibit an activation of stellate cells. How about the in vitro experiments using hepatic stellate cells?

Thanks for your advice. We will use glycyrrhizic acid to intervene the hepatic stellate cells in our further study soon.

(5) Figure 6 is poor quality. In my eyes, GA rather promoted liver fibrosis. Demonstrable photos are required to strength authors' conclusion.

Thanks for your advice. We have added more persuasive photos in the text. Steatosis and ballooning of hepatocytes are the earliest, most frequent, and most striking pathological changes observed in CCl_4-induced liver injury[3], and we found this pathological change using H and E staining. From the study, a strange phenomenon was found that most rat livers in the CCl_4-treated group appeared to have pseudo lobules were less ballooning of hepatocytes than what were not in 8 weeks.

Reviewer 00187937

(1) In the present study, since number of patients in each group is less than 30, parametric assumptions have not been met. Therefore, for comparisons, authors should use Kruskall Wallis Variance analysis for three groups and Mann Whitney U test for two groups.

Thanks for your advice. Statistical methods in the study were according to the recommendation of

Sun, etal[4]. For the data was normal distribution and homogeneity of variance, it could be used the one-way classification ANOVA . For the data was non-normal distribution or variance of data, it could be carried out using one-way classification variance analysis after the variable transformation or use Kruskall Wallis. In addition, the one-way classification variance analysis of the recommendation of Huang, etal[5], it seems no special requirements for number of cases in each group.

Reviewer 01943107

(1) The addition of in vitro data with silencing of specific apoptotic pathways and fibrogenic genes could be relevant to explain the actual molecular mechanism affected by GA.

Thanks for your advice. We will silence α-SMA and other related genes to validate the molecular mechanism of GA on hepatic stellate cells in our further study.

(2) Furthermore, the quality of WB is poor and quantitative data of TUNEL are lacking.

Thanks for your inspiring advices. And we will further improve our results in the future.

3 References and typesetting were corrected

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

Sincerely yours,

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1 Gwak GY, Moon TG, Lee DH, Yoo BC. Glycyrrhizin attenuates HMGB1-induced hepatocyte apoptosis by inhibiting the p38-dependent mitochondrial pathway. World J Gastroenterol 2012; 18: 679-684 [PMID: 22363140 DOI: 10.3748/wjg.v18.i7.679]

2 Guo XL, Liang B, Wang XW, Fan FG, Jin J, Lan R, Yang JH, Wang XC, Jin L, Cao Q: Glycyrrhizic acid attenuates CCl(4)-induced hepatocyte apoptosis in rats via a p53-mediated pathway. World J Gastroenterol 2013;19:3781-3791[PMID: 23840116 DOI: 10.3748/wjg.v19.i24.3781]

3 Merino N, Gonz áez R, Gonz áez A, Remirez D. Histopathological evaluation on the effect of red propolis on liver damage induced by CCl_4 in rats. Arch Med Res 1996; 27: 285-289 [PMID: 8854383]

4 Sun ZQ, Xu YY. Medical Statistics, 3nd ed, Beijing,People's Medical Publishing House,2010.8,55-59.

5 Huang PX, Song HL, Li GC. TCM Statistical Practice Guidance and the Application of SPSS15.0, Beijing, Science Press,2009,89-101.