Format for ANSWERING REVIEWERS

August 7, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3956-review.doc).

Title: Curcumin Protects Against Acetaminophen-Induced Apoptotic in Hepatic Injury

Author: Gang Li, Jun-Bao Chen, Chao Wang, Zhi Xu, Hao Nie, Xiao-Yan Qin, Xiao-Mei Chen, Quan Gong
Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 3956

The manuscript has been improved according to the suggestions of reviewers:
1 Format has been updated: Yes

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

Reviewer 1

1. One report (ref. 8) has described that "curcumin protects rats against acetaminophen-induced hepatorenal damage……". Authors should analyze the main differences and improvements of their study with ref. 8.

Response: In reference 8, the authors discussed the effects of curcumin on liver and kidney toxicity induced by APAP. In our present study, we mainly investigated the anti-apoptotic effect of curcumin on APAP caused hepatic injury.

2. Could authors explain the reason for the administration of both drugs by using peritoneal injection to mice rather than oral administration?

Response: Previous studies have administrated APAP and curcumin via intraperitoneal injection, so we adopted the method in our study to detect the efficacy of curcumin on APAP-induced hepatic injury.

Reference

Zhong F, Chen H, Jin Y, Guo S, Wang W, Chen N. Analysis of the gene expression profile of curcumin-treated kidney on endotoxin-induced renal inflammation. Inflammation. 2013; 36: 80-93

Liang YL, Zhang ZH, Liu XJ, Liu XQ, Tao L, Zhang YF, Wang H, Zhang C, Chen X, Xu DX. Melatonin protects against apoptosis-inducing factor (AIF)-dependent cell death during acetaminophen-induced acute liver failure. PLoS One. 2012; 7: e51911

3. On the second paragraph of phage 5, authors should explain using normal saline as the buffer of tissue homogenization. Because normal saline lacks protease inhibitors, I worry proteins may be degraded in homogenate and influence the results.

Response: The preparation of liver homogenate was processed in ice boxes in our study, after preparation of liver homogenate, we measured the content of SOD and MDA immediately. So, we think the measured values are credible.

4. Could authors provide more detail procedure about TUNEL assay and the measurement of SOD and MDA.

Response: We have provided more detail procedure about TUNEL assay and the measurement of SOD and MDA (Page 4, line29- 33, Page 5, line1, line8-11, the green font).

5. On the second paragraph of page 7, the first sentence is too long to read.

Response: We have revised the sentence in our manuscript and now it is easy to read (Page 6,
Line20-22, the green font).

6. On the second paragraph of page 7, "…., pretreatment of mice with APAP induced a significant increase in the activity of SOD", I think the "APAP" would be "curcumin".

Response: Thanks a lot. We have corrected the "APAP" into "curcumin" (Page 6, line24, the green font).

7. In Fig. 4B, reverse transcription PCR is not a suitable quantitative method for gene expression. Authors may provide Q-PCR or protein expression results (such as western blot or immunohistochemistry) to analyze the expression of Bax and Bcl-2.

Response: Thanks for your advice. Previous studies also used the RT-PCR to detect the gene expression. However, we believe that combined RT-PCR and Q-PCR (western blot or immunohistochemistry) should be more persuasive. In our later research, we would adopt your advice.

Reference

Pereira MM, Raposo NR, Brayner R, Teixeira EM, Oliveira V, Quintão CC, Camargo LS, Mattoso LH, Brandão HM. Cytotoxicity and expression of genes involved in the cellular stress response and apoptosis in mammalian fibroblast exposed to cotton cellulose nanofibers.Nanotechnology. 2013; 24: 075103

Yao K, Yang Y, Hu R, Miao M, Liao AJ, Yang W, Liu ZG. Influence of TIEG1 on apoptosis of HL-60 cells and expression of Bcl-2/Bax. Zhongguo Shi Yan Xue Ye Xue Za Zhi.2013; 21: 587-90

8. Could authors discuss the possible reasons that curcumin could enhance Bcl-2 expression and suppress Bax in hepatocytes, since the same drug could suppress Bcl-2 and enhance Bax in some cancer cells?

Response: The pathogenesis of APAP-induced hepatic injury and cancer are different, so the change of Bcl-2 and Bax also different. Others studies can prove it.

Reference

Kumari A, Kakkar P. Lupeol prevents acetaminophen-induced in vivo hepatotoxicity by altering the Bax/Bcl-2 and oxidative stress-mediated mitochondrial signaling cascade. Life Sci. 2012; 90: 561-70

Sharma S, Singh RL, Kakkar P. Modulation of Bax/Bcl-2 and caspases by probiotics during acetaminophen induced apoptosis in primary hepatocytes. Food Chem Toxicol. 2011; 49: 770-9

Reviewer 2

Major 1. The figures are not very consistent. They have to show the same treatment time of CMN before and after APAP treatment and also dose-dependent (10 or 20 mg/kg) effects of CMN on APAP induced-hepatic toxicity in all figures.

Response: Thanks a lot. From the results of ALT, we found that pretreatment with

CMN (20 mg/kg body weight) 2 h before APAP injection show the best liver protection effect. So, we mainly discussed the CMN (20 mg/kg body weight) 2 h before APAP injection to investigate its effect on MDA, SOD and apoptosis.

Major 2. Curcumin is known to have antioxidant activity via regulating mitochondrial system. I wonder CMN could regulate caspase activity induced by APAP in this model.

Response: CMN could down-regulate caspase activity induced by APAP. Previous study has proved it.

Reference

Bulku E, Stohs SJ, Cicero L, Brooks T, Halley H, Ray SD. Curcumin exposure modulates multiple pro-apoptotic and anti-apoptotic signaling pathways to antagonize acetaminophen-induced toxicity. Curr Neurovasc Res.2012; 9: 58-71

Minor 1. The reason they choose the concentrations (10 or 20 mg/kg) of CMN have to discuss. Since the CMN could have role of antoapoptotic and proaptototic depending on concentration.

Response: In this reference of "Vizzutti F, Provenzano A, Galastri S, Milani S, Delogu W, Novo E, Caligiuri A, Zamara E, Arena U, Laffi G, Parola M, Pinzani M, Marra F. Curcumin limits the fibrogenic evolution of experimental steatohepatitis. Lab Invest.2010; 90: 104-15: male C57BL/6 mice weighing between 20 and 25□g and received curcumin intraperitoneally 25□μg per mouse", and this equal to 10 mg/kg curcumin.

In the reference of "Shen SQ, Zhang Y, Xiang JJ, Xiong CL. Protective effect of curcumin against liver warm ischemia/reperfusion injury in rat model is associated with regulation of heat shock protein and antioxidant enzymes. World J Gastroenterol.2007; 13: 1953-61: 50 mg/kg curcumin was injected through a branch of superior mesenteric vein",so we chose 20 mg/kg curcumin.

Minor 2. In Fig. 1B. and 4A, numbering is missed and x10 and x40 looks misprint.

Response: Thanks a lot. We have revised the numbering and mistakes in Fig .1B and Fig. 4A.

3 References and typesetting were corrected: Yes

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

Sincerely yours,

Quan Gong PhD
Department of Immunology, Medical School, Yangtze University, 434023, Jingzhou, China
e-mail: gongquan1998@163.com
Tel/fax: +86 0716 8062733