July 10, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 11541-review.doc).

**Title:** Novel insights into the mechanisms whereby isoflavones protect against fatty liver disease

**Author:** Long-Xin Qiu, Tong Chen

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

**ESPS Manuscript NO:** 11541

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

(1) Reviewer 1

The manuscript aims to review the literature about the mechanisms whereby isoflavones protect against fatty liver disease. The authors conducted an extensive and thorough review of the subject allowing the reader a good update. Suggestions:

1- The pathophysiology of nonalcoholic fatty liver disease should be updated. Other important points have emerged as microbiota, genetics, etc;

We agree with the comment of the reviewer and the pathophysiology of nonalcoholic fatty liver disease has been updated, including the candidate factors, the hypothesis of "two hits", and the hypothesis of "multiple parallel hits". Some sentences are added in the first paragraph of the section of CAUSES OF ALD AND NAFLD (Page 4, line 11-29), and references 17,18,19,21 have been also added.

2 - The literature is limited to animal models, so we need more studies in order to affirm that it may represent a future treatment for fatty liver disease in humans.

We agree with the comment of the reviewer. Data on humans about the anti-FLD effect of isoflavones are scarce. We thus revised the last paragraph of CONCLUSION section (Page 12, line 4-7).

(2) Reviewer 2

It is a review study, the subject is current and interesting. However, I have some considerations: In the title of the article, I suggest adding the information about the group to which limited this review: rodents. As the title of Table 1; There is no methodological description of how the review was conducted.

We thank the reviewer for the helpful comment. The word count for the title of minireview for "*World Journal of Gastroenterology*" should be less than 12, so we can only add the information about the group in the title of Table 1.

(3) Reviewer 3

Authors have summarized the possible pathophysiological mechanisms that are mediated by inhibition of aldose reductase and are responsible from beneficial effects of isoflavones in fatty liver disease. The paper is a basic research with little clinical implication.

We agree with the comment of the reviewer. Data on humans about the anti-FLD effect of isoflavones are scarce. We thus revised the last paragraph of CONCLUSION section (Page 12, line 4-7).

(4) Reviewer 4

Authors suggest that isoflavones should be employed as protective agents for liver steatosis and steatohepatitis. The bakground and the experimental data reported are suggestive and in keeping with the hypothesis. However, some major revisions are necessary. 1-Introduction: the pathogenesis of ALD

and NAFLD is different, even if some biochemical pathways are common. I think that the two aspects should be differently explained.

We have differently explained the two aspects of ALD and NAFLD even though the two aspects were described in the same paragraph.

2- The observation that AR is induced in human livers obtained from patients with different chronic or acute liver damage does'nt indicate that AR may play an important role in the development of liver injuries. In other terms, in all the text, the relationships among different mechanisms not necessary document a cause-effect

We have extensively discussed the role of AR in the development of FLD in Page5-6. Genetic ablation of the AR gene resolved high-glucose-diet-induced hepatic steatosis in mice. Inhibition of AR ameliorated hepatic steatosis in db/db diabetic mice, and lentivirus-mediated knockdown of AR gene alleviated MCD-diet-induced NASH in db/db mice. These studies can confirm the involvement of AR in the development of FLD.

3-also in the discussion there is a confusion about the effects of isoflavones in ALD and NAFLD. Also in this case, the documented actions of each isoflavone should be reported for ALD or NAFLD in different paragraph

We thank the reviewer for the helpful comment. We think that we report the action of isoflavones for ALD or NAFLD in the order of soy isoflavones, kudzu isoflavones, and then red clover isoflavones. It may help the reader to get the information about the anti-FLD action of different isoflavones.

4- Conclusions: authors are unable, on the basisd of reported data, to conclude that " the supplement of isoflavones may be useful in preventing ALD and NAFLD/NASH". .... and provide a new therapeutic strategy for FLD patients ". Data on humans are very poor, without informations about the bioavailability, in vivo, of each of the substances reported, and in absence of well performed trials

We agree with the comment of the reviewer. Data on humans about the anti-FLD effect of isoflavones are scarce. We thus revised the last paragraph of CONCLUSION section (Page 12, line 4-7).

(5) Reviewer 5

1- Introduction: Please provide the statistics about the increase of FLD.

We have done as suggested. One sentence is added in the first paragraph of INTRODUCTION section (Page 3, line 8-10). Reference 1 has also been added.

2- Introduction: Please provide the specific reference(s) about the pharmacological agents for the prevention of FLD and side effects.

We have done what was suggested.

3- Please clarify the meaning of the toxic inflammatory cytokine.

Proinflammatory cytokines are toxic to the hepatocytes.

4- Please cite the recent references about the "two hits" hypothesis. Also, as I know, there is an alternative "two hits" hypothesis in more recent publication(s).

We agree with the comment of the reviewer. Reference 20, 21 were cited for the report of “two hits” hypothesis, and we describe the hypothesis of “multiple parallel hits” either. Some sentences are added in the first paragraph of the section of CAUSES OF ALD AND NAFLD (Page 4, line 11-29), and references 18 has been also added.

5- Please double-check the reference numbers 27 and 28 and correct them if necessary.

We have done what was suggested.

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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