

## ANSWERING REVIEWERS



August 29, 2014

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 12306-review.doc).

**Title:** Mechanism Study of Gypenosides on Type 2 Diabetes and Non-alcoholic Fatty Liver Disease in Rat

**Author:** Qin He, Jin-ke Li, Fang Li, Ru-gui Li, Guo-qing Zhan, Gang Li, Wei-xing Du, Hua-bing Tan

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

**ESPS Manuscript NO:** 12306

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 1:

This is an interesting paper which may improve our knowledge in the fields. The subject matter is suitable for the intended audience and it fits the journal scope. Article is mostly clearly written, but Title is suggestive of the article's content. Article is appropriately organized and the headings are indicative of content, I suggest to accept this paper in the present form.

Thanks.

### Reviewer 2:

This manuscript presents a series of well thought out experiments related to effects of "gypenosides" on rat model of type 2 diabetes mellitus combined non-alcoholic fatty liver disease (T2DM-NAFLD). However, the data shown in this manuscript is insufficient and failed to clarify the mechanism. This study contains many dubious biological response, problems and confused statements.

(1) It is well known that single high dose STZ injection (>60 mg/kg BW) results in massive pancreatic beta cell destruction, more characteristic of T1DM, whereas intermediate dosages of STZ injections (between 40 and 55 mg/kg BW) cause only partial impairment to insulin secretory mechanisms seen in T2DM. These models are usually characterized by fasting or non-fasting hyperglycemia, lowered serum insulin levels with hyperlipidemia. However, the levels of serum insulin in HFSD-induced T2DM-NAFLD rats was much higher than that of controls in Table 2.

In this model, we used HFSD combined STZ to induce T2DM-NAFLD model, not single STZ induce, and this method showed a significantly effect in T2DM-NAFLD model. In addition, lowered serum insulin levels with hyperlipidemia is one phenomenon in T2DM, that is although the levels of serum insulin is high, but it did not work.

(3) In Materials and methods section, the body weight of rats was 220 to 250 g before the experiments. In figure 1, the body weight was about 180 g at week 1. The experimental protocol is very dubious.

Sorry, this is our typo, and now we have modified it.

(4)How long did the gypenosides intervention?

Six weeks.

(5)Treatment durations should be consistent all of the experiments. Six or fourteen weeks? P9, it seems like the figure legends were copied in the two paragraphs. The transcriptional activity of peroxisome proliferator activated receptor  $\gamma$  should be determined.

The drug treatment groups were raised on HFSD and injected with STZ for the first 4 weeks, and the second 4 weeks continued fed the same HFSD, but not inject STZ. And the next 6 weeks, use GPs to treat and also continue fed. So GPs treatment durations is 6 weeks, and in the manuscript, we have modified the inaccurate description.

### **Reviewer 3:**

The work has potential interest.

(1)The overall english is poor and need to be corrected.

We have revised the whole language, which we have ask Dr Takshi Ikejima to help us to modify the whole language. Dr Takshi Ikejima is a pharmacology professor in our department. I think the present language would be accept.

(2)The group division (I-V) needs to be clarified earlier because it is confusing. A table should help to understand the different conditions. Also, a scheme of the treatment and times would help greatly.

We have modified and described this in the manuscript.

(3)The abbreviations need to be explained not only in the abstract but also the first time they appear in the text and also in the legends to figures.

We have modified in the whole manuscript.

(4) As it is known and the authors explained in the discussion, TNF-alpha is mainly produced by monocytes macrophages. The difference observed herein could be due to diminish macrophages in the liver. It would be useful to measure by qPCR and immunohistochemistry if the macrophages F4/80+ are altered in the model group and what is the effect of GP on macrophage number and/or F4/80 expression.

Thanks for your good advice. In our study, we just use immunohistochemistry to detect the effect of TNF-alpha, and we did not detect the macrophage number and F4/80 expression, so, I could not give the explanation in the effect of GP on macrophage number and/or F4/80 expression. We will carried out this study in our next step plan to make clear this issue. Thanks.

(5)Are the doses administered here comparable to those used for the pharmacological studies mention?

Yes

(6)Is there any toxic effect of GP that might be considered at high doses?

In our study, we did not find the toxic effect at high doses.

(7)In the figure 1, it is not clear the difference between the groups. A graph showing the final body weight would be more useful. Also, "Liver Index" should be defined in the legend and also, in the text. In the figure 2, a higher magnification (200 x) should be included.

We have defined Liver Index in the text and in the legend. And we have provided the higher resolution figure.

(8) The authors stated that HFSD induced lipid deposition. Did the model group show inflammation or ballooning of the hepatocytes? Did the GP treatment affect these two features?

Yes, the model group show inflammation or ballooning of the hepatocytes and the GPs treatment showed lesser these features, especially the high dose of GPs.

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

Sincerely yours,

A handwritten signature in black ink that reads "Huabing Tan". The signature is written in a cursive, slightly slanted style.

Huabing Tan

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