Author’s response to reviews

Title: Berberine moderates glucose metabolism through the GnRH-GLP-1 and MAPK pathways in the intestine

Authors:

  Qian Zhang (rubiacordifolia@sohu.com)
  Xinhua Xiao (xiaoxinhua@medmail.com.cn)
  Ming Li (liming_pumch@sohu.com)
  Wenhui Li (Liwenhui_pumch@sohu.com)
  Miao Yu (yumiao_pumch@sohu.com)
  Huabing Zhang (zhanghuabing_pum@sohu.com)
  Zhixin Wang (wangzhixinpumch@sohu.com)
  Hongding Xiang (xianghongdingpu@sohu.com)

Version: 2 Date: 19 March 2014

Author’s response to reviews: see over
Dear editor:

First, we would like to thank you for your efforts and the helpful suggestions. The paper was revised in language and grammar by native speaker and proofread very closely for mistakes and grammatical errors. The detailed modifications according to your comments have been listed as below.

Once again thank you for your help.

Reviewer 1:

Comment:

This is an interesting study showed the effects of Berberine on diabetic rat, which may act locally at ileum through altering MAPK and GnRH-GLP-1 pathways.

Answer:

Thank you for your great help! The paper was revised in language and grammar by native speaker and proofread very closely for mistakes and grammatical errors.

Comment:

Here is my comments:

Minor Essential Revisions

Background " Over 135 million people are affected...", need a reference to support.

Answer:

We added the reference as follows,


Comment:

It is better to have mean and SD to present table 4, rather only fold effects. It does not have the number of experiments performed.

Answer:
In gene array experiment, there are only 3 samples in each group. So, there is no SD and P value. We added SD in real time PCR experiment.

Comment:
Detailed statistic analysis need to be clarified, especially figure 1B and figure 2A.

Answer:
We added the statistic analysis of Fig 1 and Fig 2 in result section, as follows,

**Berberine decreased fasting blood glucose of DM rats**

The fasting blood glucose (FBG) levels of DM rats and the BerL group and BerH group were significantly higher than those of control rats at week 0 ($P<0.01$), week 2 ($P<0.01$), week 4 ($P<0.01$), week 6 ($P<0.01$) and week 8 ($P<0.01$). FBG in the BerL group and BerH group decreased significantly at week 2 ($P<0.05$), week 4 ($P<0.05$), week 6 ($P<0.05$) and week 8 ($P<0.05$) compared to the DM group (Fig. 1B).

**Berberine moderated the glucose tolerance of DM rats**

The blood glucose levels of the DM, BerL and BerH groups were higher than those of the control group before (0 min, $P<0.01$) and at 30 min ($P<0.01$), 60 min ($P<0.01$) and 120 min ($P<0.01$) after oral glucose administration. Blood glucose levels of the BerL and BerH groups significantly decreased before (0 min, $P<0.05$) and after oral glucose administration ($P<0.05$ all above, Fig 2A). The AUC of OGTT for the DM, BerL and BerH groups increased compared to the control group ($P<0.05$). The AUC for the BerL and BerH groups reduced compared with the DM group ($P<0.05$, Fig. 2B).

**Reviewer 2:**
Comment:
Berberine is the main active component of an ancient Chinese herb Coptis chinensis French, which has been used to treat diabetes for thousands of years. The efficacy of berberine for treatment of type 2 diabetes has been confirmed in several clinical trials, but the exact underlying mechanism is not clear. In this study, the authors investigated the effect of berberine on glucose metabolism in diabetic rats and concluded that berberine may moderate blood glucose and insulin secretion through decreasing GnRH-GLP-1 pathway. It makes sense for the authors to provide such a preliminary explanation for the pharmacological mechanism of berberine.

Answer:
Thank you for your great help! The paper was revised in language and grammar by native speaker and proofread very closely for mistakes and grammatical errors.

Comment:
- Major Compulsory Revisions

1. The authors propose the hypothesis that berberine acts directly in the terminal ileums, and suppresses gastrointestinal movement. Could the authors provide some more direct evidence? such as the observation of gastrointestinal movement after berberine administration, etc.

Answer:
In previous report, Feng et al. found berberine could inhibit myoelectrical activity and gastrointestinal transit in rodents [1]. We added this information in discussion section, as follows,

Furthermore, Feng et al. found that berberine could inhibit myoelectrical activity and gastrointestinal transit in rodents [21].

Because we performed this experiment last year. So, we can not perform gastrointestinal movement experiment now. We hope to do it in future experiment. And we added this information in conclusion section, as follows,

More experiments should been performed in the future to confirm these results (such as
studying gastrointestinal movement and use of Western blot analysis).


**Comment:**

2. The authors have done immunohistochemical straining for the detection of GLP1R and MAPK10. To give more convincing evidence, the authors should provide the western blotting results of the examined proteins.

**Answer:**

Thank you for your good advise. But, we performed this experiment last year. The tissues of rats have been ran out. So, we have no tissues to do western blot now. As you seen, we performed immunohistochemical straining for the detection of GLP1R and MAPK10. And we found that, there was a statistically significant increase in the immunoreactivities of GLP1R and MAPK10 in the BerH group. We added some information as in the paper as follows,

More experiments should been performed in the future to confirm these results (such as studying gastrointestinal movement and use of Western blot analysis).

**Comment:**

- Minor Essential Revisions

The spelling mistakes or figure should be revised, the following are some examples:

1. p.3 “glucosereducing” should be “glucose reducing”
2. p.11, (FDR=1.01-13). “-13” should be superscript.
3. In Figure 4, the size of the immunostaining pictures for GLP1R and MAPK10 should be adjusted to the same.

**Answer:**
I corrected these points one by one, and re-drew the Figure 4.

If there are any problems, please do not hesitate to tell us.
Once again, we would like to thank you for your great efforts of helping us to improve the quality of present study.

Sincerely yours,
Qian Zhang