Dear experts and Lian-Sheng Ma,

Thank you for your valuable comments and recognition of our manuscript.

We have performed major revisions upon request and added related content.

Reviewer #1:

Specific Comments to Authors:

1. The major concern is the significance of this study. Little innovation was found in this article since there were too many studies on the regulative effect of berberine on diabetes and intestinal microbiota. In this study, the changes of intestinal microbiota, serum glucose, lipid, etc. were observed after the treatment of berberine, and a simple correlation analysis was conducted. However, it is impossible to prove a causal link between the intestinal microbiota and the above metabolic parameters or to illustrate the mechanism behind the whole events. Besides, how do you estimate the credibility of the correlation analysis?

Although there were some studies on the regulative effect of berberine on diabetes and gut microbiota, our research varies from other studies. For example, we chose GK rats, and other studies chose Wistar rats or other breeds of mice as the diabetes model. In our research, we found that berberine has an effect on the gut microbiota. We further demonstrated through Spearman's correlation and correlation network analysis that the gut microbiota are related to the improvement in metabolic parameters, which may be a positive finding. Additionally, we observed the insulin, HOMA-IR, and GLP-1 levels. The improvement in these indicators shows that the glucose metabolism indicators have been adjusted, instead of just observing the indicator for blood glucose. The results have been further verified.

Gut microbiota play an important role in the regulation of multiple host metabolic pathways (e.g., glucose and lipid homeostasis) through the modulation of gut farnesoid X receptor and/or the G-protein-coupled receptor activity, which has been discussed previously other researches. There may be other mechanisms as well. There is a causal link between the gut microbiota and the above metabolic parameters. Due to insufficient funding for scientific research, we did not explore this link further. I will continue to conduct future research on this topic in which I have a strong interest.

The greater the absolute value of the correlation coefficient, the stronger the correlation, and the closer the correlation coefficient is to 1 or -1, the stronger the correlation. Under normal circumstances, the correlation strength of the variable is judged by the following value range: correlation coefficient 0.8-1.0 most strong correlation; 0.6-0.8 strong correlation; 0.4-0.6 moderate correlation; 0.2-0.4 weak correlation; 0.0-0.2 very weak correlation or no correlation. The data provided by our research is basically strongly correlated. The results are further verified by Spearman's correlation and correlation network to improve accuracy.

In addition, we have observed that some bacteria are related to the improvement in blood glucose, which has not been reported in other studies and may be another finding of this study.

2. There is a bug in study design: a normal control group was absent. As a result, you could not tell the characteristic of intestinal microbiota in normal rats, making it hard to evaluate those changes after the treatment good or bad. So additional experiments are needed to make up for this defect, if the lab and funds permit.

We fully considered the lack of a control group before we conducted the experiment. In future studies, we will choose a normal control group to adjust for this limitation.

The GK rat is a reliable model to study T2DM as the rats in our study had all of the typical hallmarks of T2DM, such as insulin resistance, defective insulin production, fasting hyperglycaemia and hyperinsulinemia. Some studies have chosen Wistar rats as controls. We found that the results of their blood glucose, blood lipids, and gut microbiota of Wistar rats are completely different from GK rats. This comparison is of little significance to research. In addition, some studies that selected GK rats also did not set up a normal control group, and I think the reasons may be similar.

If Wistar rats are chosen, they will be included as a normal control group in our research design. Another study design involves the use of GK rats as both a normal control group and model group.

3. The study also detected the levels of serum fasting GLP-1 and the histological change of pancreatic islets. However, that was just a simple overlay of different parameters to make this article seems colorful, since the logical link between them

and the whole events was obscure. It is suggested that further experiments are needed to prove the relationship between the intestinal microbiota and GLP-1, or just delete this part.

GLP-1 has the same effect as meal consumption on insulin secretion. Additionally, it exerts other functions in the pancreatic islets, including the regulation of β-cell proliferation and protection of β-cells against metabolic stresses. We observed GLP-1 and the histological change in the pancreatic islets. Improvements in GLP-1 and pancreatic islets have played a positive role in the regulation of blood sugar.

SCFAs as fermentation products of gut microbiota are involved in stimulating the secretion of a number of gut peptide hormones, including PYY, GLP-1, and GLP-2. SCFAs are able to act by activating selected G-protein-coupled receptors that are involved in GLP-1 secretion to improve glucose and insulin sensitivity. We would like to keep this part of the content and add some explanations in the manuscript.

4. The manuscript needs to be revised to improve the English and correct grammar errors, which exist throughout the manuscript.

We have carefully modified the grammar and terminology and have assured the proper usage of the English language.

Reviewer #2:

Language Quality: Grade B (Major language polishing)

We have carefully modified the grammar and terminology and have assured the proper usage of the English language.

Thank you again to the experts and editors. With your help, this manuscript continues to evolve to publication.

Best regards,
Jindong Zhao
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