Reviewer’s report

Title: Water extract from processed Polygonum multiflorum modulate gut microbiota and glucose metabolism on insulin resistant rats

Version: 2 Date: 24 Oct 2019

Reviewer: Reviewer 2

Reviewer's report:

PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses a testable research question(s) (brief or other article types: is there a clear objective)?

Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

Not sure - key details are missing from the manuscript

EXECUTION - Are the experiments and analyses performed with technical rigor to allow confidence in the results?

No - there are major issues

STATISTICS - Is the use of statistics in the manuscript appropriate?

Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

No - there are major issues

OVERALL MANUSCRIPT POTENTIAL - Is the current version of this work technically sound? If not, can revisions be made to make the work technically sound?

Maybe - with major revisions
PEER REVIEWER COMMENTS:

GENERAL COMMENTS:

Minor issues

* What was the age of rats when experimentation began?
* MET is not defined prior to first use (Page 6 line 113 and 118) but is presumably metformin. Please define for clarity.
* Since the one-way ANOVA reported is a multiple comparison test, was any post-hoc test such as Tukey used? Please specify.
* There are some minor stylistic issues throughout, but I have not listed them here as my opinion has specifically been sought to assess the methods and validity of the results.

Major Issues

* I am concerned about how the experiments on every 2nd week were planned. If blood had to be collected from the ocular vein every two weeks as shown in figure 1, why was a small sample from this procedure not used to measure fasting blood glucose as well? I don't understand why the authors chose to test FBG using blood the tail vein. This additional procedure adds unnecessary stress to the animals (both during restrains and blood collection). The only scenario I can think of where conducting both procedures (ocular and tail vein blood collection) becomes necessary is if the authors also intended to perform an oral glucose tolerance test (OGTT) which typically uses tail vein blood samples at 0, 30, 60, 90 and 120 minutes. However, neither the method nor the data for OGTT have been presented.

If OGTT was attempted, this complicated the data. Ocular blood sampling every two weeks then would coincide with the OGTT timeline. In such a scenario, the animals will be extremely stressed and may negatively impact the results. Typically, such scenario can be avoided by having a larger number of animals per group (perhaps 12 or 15) and dividing them for blood collection and OGTT. Doing two blood collection procedures on the same animals so frequently in such a short span is not recommended. The negative impact of such a procedure (if any) may be observable in body weight change before and after the procedure. Was this record kept? I know from the experience in our laboratory that metabolic parameters, including insulin and glucagon concentrations and especially insulin, can be compromised in animals that are stressed due to frequent blood collection (both ocular and tail vein) although this may reflect in blood glucose concentrations only under extreme stress. So FBG concentrations can be more or less reliable, but I am concerned about insulin concentrations. I cannot say with certainty if gene expressions or microbiota data are affected by these procedures. (The issue with blood collection was also raised by the 2nd reviewer, but the response did not clarify these issues)
Were the H and E staining done in different batches or different camera settings used? The dye uptake seems to vary considerably between panels A, B, E, and C&D. Such variation is not expected. Panel B (MOD group) also show signs of portal inflammation. However, this is not mentioned in the results. Also, this representative slide does not show increased vacuolated lipid droplets, as claimed by the authors. Lipid vacuoles are typical round. I can see more fatty deposits in Panel D (treatment group) than in Panel B.

I must stress that these comments do not warrant that this paper is rejected. I urge the editor to seek clarifications on these issues and if satisfied this paper can be a useful addition to the existing literature on Polygonum multiflorum.

REQUESTED REVISIONS:

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**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

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Please complete a declaration of competing interests, considering the following questions:

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