Author’s response to reviews

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

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Dear the Editor,

Please find attached a revised version of our manuscript “Water extract from processed Polygonum multiflorum modulate gut microbiota and glucose metabolism on insulin resistant rats”, which we would like to resubmit for publication in the journal of BMC Complementary and Alternative Medicine.

Your comments and those of the reviewers were highly insightful and enabled us to greatly improve the quality of our manuscript. In the following pages are our point-by-point responses to each of the comments of the reviewers. The modified parts of the article are marked in red font for easy viewing.
We hope that the revisions in the manuscript and our accompanying responses will be sufficient to make our manuscript suitable for publication in the journal of BMC Complementary and Alternative Medicine.

We shall look forward to hearing from you at your earliest convenience.

Yours sincerely, Jie Yu

Response to Peer-reviewer’s Comments

Reviewer #1:

The effect of PPM on gut microbiota and glucose metabolism on insulin resistant rats is reported in this paper. The study is well planned, executed and complete. The results of the study is presented well and the manuscript is relatively free from typographical errors. The paper may be accepted for publication after some minor corrections mentioned below.

* Page-7 paragraph-1: In addition to the CON group, the remaining rats were fed a high-fat diet until the end of the experiment. Rats in the CON group and MOD group were given normal saline. The difference between CON group and MOD is not mentioned clearly

Responds: We agree with what the reviewer described. According to the opinion, the sentence has been adjusted, and the expressions that are not relevant to the key points have been deleted to make them accurate and clear.

* Characteristics and composition of PPM - Page-9: As the composition of PPM has been already established this section cannot be included in the results of present study. Therefore it is suggested that this section may be included in introduction part or discussion

Responds: Thanks to the reviewer's precious advice. According to the opinion, the structure of the article has been adjusted. The part of composition of PPM had been deleted from the manuscript.

Reviewer #2:

This type of adding comments is cumbersome. I have included in the text itself.

* May be some typological error. (Page-1 paragraph-3 line-2)
Responds: Thanks to the reviewer's precious advice. According to the opinion, we found the clerical error, in the sentence that "Administration of the water extract from PPM decreased the level of FBG, TC, and TG, and increased the level of FBG, such reducing the IR index and improving IR", we replace the latter FBG with FGC.

* These changes are related to insulin resistance? Sentences are not clear and does not convey the meaning which the authors want to convey. (Page-1 paragraph-4)

Responds: According to the opinion, the article has been re-described. Our findings showed that PPM reversed the increasing of FBG and the decreasing of IRI, accelerated the expression of glucose metabolism-related proteins and regulated the intestinal microecological balance. Therefore, we hold the opinion that PPM may be an effective option for treating IR.

* When you are using short form IR use it through out except the first sentence. (Page-1 paragraph-4 line-1)

Responds: Thanks to the reviewer's precious advice. The author revised the article and replaced the insulin resistance in the text with its abbreviation.

* Some thing is missing or the sentence needs to be corrected. (Page-3 paragraph-3 line-4)

Responds: Thanks to the reviewer's precious advice. Based on the comments, we re-described the sentence to make it as clear as possible.

* The paper relates the compound for protective effect while the sentence written by the authors does not convey the meaning. (Page-3 paragraph-3 line-7)

Responds: Thanks to the reviewer's precious advice. Based on the comments, we re-described the paragraph to make it as clear as possible.

* When the active constituent of the material is proven to have anti-diabetic activity then what made to conduct this study? Explain the rationale of this study else this paper may not receive the scientific merit and novelty. (Page-4 paragraph-1)

Responds: Thanks to the reviewer's precious advice. PPM is used for the treatment of dyslipidemia, while plenty researches focused on its mechanism. Although TSG (the active component of PPM) is proven to have anti-diabetic activity, limited research pay attention to the possibility of PPM in relieving IR, not to mention its efficacy and potential mechanism. For the first time, we focused on the regulation mechanism of PPM on IR from the perspective of gut microbiota and host regulation.
We also revised the related contents to give a more clear expression about the scientific merit and novelty of our research.

* Introduction is too lengthy but does not high light the importance or need for this study. (Page-5 paragraph-2)

Responds: According to the opinion. On the basis of previous modifications, we further simplified the introduction of IR and PPM, deleted the statements that were not related to the key points, and moved the related researches on IR, glycoproteins and intestinal microorganisms to the discussion section to elaborate, so as to make the background part accurate and clear.

* Why a reference is cited here? (Page-5 paragraph-3 line-3)

Responds: This reference is intended to provide evidence that Metformin hydrochloride is a commonly used positive drug in similar researches.

* This is used for what purpose. (Page-5 paragraph-3 line-4)

Responds: It is used for providing ultrapure water for this research.

* Reference is required. Since this literature is not easy to access, provide brief procedure for preparation of PPM. (Page-6 paragraph-1 line-6)

Responds: Thanks to the reviewer's precious advice. Based on the comments, we briefly describe the preparation process for PPM. At first, the root of Polygonum multiflorum Thunb. were thickly sliced (5-9mm). Then, 1kg sliced root was mixed and steamed with black bean juice (0.25 kg) for 2.5 h. Then, they were dried at 60 °C in the oven for further use.

* What does it mean? Middle doses of PPM? (Page-6 paragraph-2 line-2)

Responds: Thanks to the reviewer's precious advice. Based on the comments, we re-described this sentence and describe how we determine the dose. According to Chinese Pharmacopoeia, the usual clinical doses of PPM for humans was 9 g/kg per day. We converted it to the middle dosage of rat.

* Please specify the method which you adopted for dose conversion. Provide appropriate reference for it. (Page-6 paragraph-2 line-4)
Responds: According to the opinion, the method of dose conversion was reexplained and the formula was added. Based on the dose conversion relationship between humans and rats according to the body surface area (rat dose = human dose *0.018/0.02kg),

* This notation looks inappropriate instead you can use high fat diet group and abbreviate it as HFD.

The content of lard is very less. We use to feed about 40% lard. Mention the source of lard and cholesterol. (Page-7 paragraph-1 line-4)

Responds: Thanks to the reviewer’s precious advice. In this study, we used high-fat diets to feed rats to establish an IR model. However, high-fat diets can also be used to establish other rat models such as obesity, non-alcoholic fatty liver disease or hyperlipemia. Therefore, we consider that using MOD to represent the IR rat model will be more representative.

We are sure that 10% lard can establish the IR model from our previous experience and related literatures (see the below). Certainly, 40% lard may help to shorten the overall duration of the study. Therefore, a relative long period (12 weeks) was preset to ensure that IR model could be established successfully.

For example 1: Gothandam Kirubananthan,Ganesan Vijayan Siva,Ayyasamy Thangaraj et al. Antioxidant potential of theaflavin ameliorates the activities of key enzymes of glucose metabolism in high fat diet and streptozotocin - induced diabetic rats. [J] .Redox Rep., 2019, 24: 41-50. (Diabetes was induced in male albino Wistar rats by feeding them with high fat diet comprising of standard laboratory rat chow 84.3%, lard 5%, egg yolk powder 10%, cholesterol 0.2% and bile salt 0.5% for 2 weeks.)


For example 3: Guex Camille Gaube,Reginato Fernanda Ziegler,de Jesus Patrícia Romualdo et al. Antidiabetic effects of Olea europaea L. leaves in diabetic rats induced by high-fat diet and low-dose streptozotocin. [J] J Ethnopharmacol, 2019, 235: 1-7. (Male Wistar rats were either given normal feed or a high-fat diet (70% standard laboratory feed, 15% sucrose, 10% lard and 5% yolk powder) for four weeks).

For example 4: Yang J, Ba T, Chen L, et al. Effects of metformin and sitagliptin on glycolipid metabolism in type 2 diabetic rats on different diets[J]. Archives of Medical Science Ams, 2016, 12(2):233-242. (The rats were fed for 8 weeks with a high-fat diet (63% of calories as fat), which was prepared by supplementing normal chow with 10% (w/w) lard, 10% (w/w) sucrose, 1% (w/w) cholesterol, and 0.3% w/w bile acid (sodium salt).
* Euthanizing rats by cervical dislocation is not an appropriate method. (Page-7 paragraph-2 line-2)

Responds: Thanks to the reviewer's precious advice. We believe that cervical dislocation is commonly used for euthanasia in small experimental animals such as rats. At the end of the experimental period, the rats were anaesthetized with sodium pentobarbital (40 mg/kg) administered intraperitoneally and euthanized by cervical dislocation. Then, rats were quickly dissected to remove the liver and muscle tissue.

* Is it appropriate to collect almost 10% of blood every two weeks.

Is it ethical to repeat retro-orbital puncture every 2 weeks? (Page-7 paragraph-3 line-1)

Responds: Thanks to the reviewer's precious advice. We attached great importance to the protection of animal ethics and welfare. Usually, repeat sampling at the same site need to be carried out after more than 10 days. We cautiously considered the frequency of blood taken when designing the experiment. Finally, in order to observe the dynamic changes of experimental data, we chose to conduct blood collection every two weeks and minimized the harm during the experimental operation. In order to reduce blood sample amount, FBG were tested every four weeks. During the experimental procedure, samples were taken by skilled personnel and sterile capillaries were used to avoid infection and potential damage around the eyelids.

* How the formaldehyde fixed liver was homogenized. (Page-8 paragraph-1 line-1)

Responds: The liver of the same part of each rat was collected for fixation, and the remaining part was used for homogenization to protein assay. We also revised the related contents to give a more clear expression about the taken and usage of liver samples.

* Comparison should be made with Control and HFD and then HFD with treatment groups. (Fig.3)

Responds: Thanks to the reviewer's precious advice. We clearly marked the difference between Control and HFD groups with * symbol, the difference HFD and treatment groups with # symbol in Fig 3 and Fig 4. In addition, we carefully examined the statistical analysis methods and results of all the data.

* Figure clarity is less. (Fig.3)

Responds: Thanks to the reviewer's precious advice. We reprocessed the graphics file to ensure that the uploaded file is clear and accurate. However, the figure clarity may be reduced after the processing of the submission system.
* AMPK:p-AMPK needs to be shown. (Fig.4)

Responds: Thank you so much for your academic guidance and advice. p-AMPK, rather than AMPK, is a more specific a key therapeutic target in patients with diabetes (see references below). Therefore, only the expression of p-AMPK protein was tested in our research.

Certainly, we highly appreciate your suggestions about giving AMPK:p-AMPK ratio to accurately describe the mechanism of that PPM attenuates IR. Therefore, we will focus on the change of AMPK:p-AMPK ratio in further studies. And more solid evidences will be provided in our future research.

For example 1: Yerra Veera Ganesh, Kalvala Anil Kumar, Sherkhane Bhoomika et al. Adenosine monophosphate-activated protein kinase modulation by berberine attenuates mitochondrial deficits and redox imbalance in experimental diabetic neuropathy. [J]. Neuropharmacology, 2018, 131: 256-270. (In addition, elevated phosphorylated AMPK (p-AMPK), p-SAPK/JNK and diminished p-ERK, p-P38, p-Akt and p-mTOR/p-4EBP1 proteins were observed.)

For example 2: Hu Qian, Li Lingli, Zou Xin et al. Berberine Attenuated Proliferation, Invasion and Migration by Targeting the AMPK/HNF4α/WNT5A Pathway in Gastric Carcinoma. [J]. Front Pharmacol, 2018, 9: 1150. (Our findings indicated that BBR downregulated HNF4α while upregulating p-AMPK.)

For example 3: Chen Han, Ji Yingshi, Yan Xin et al. Berberine attenuates apoptosis in rat retinal Müller cells stimulated with high glucose via enhancing autophagy and the AMPK/mTOR signaling. [J]. Biomed. Pharmacother., 2018, 108: 1201-1207. (Western blot analysis was employed to determine the levels of p-AMPK and p-mTOR, as well as apoptosis-related proteins and autophagy-related proteins in Müller cells.)

Reviewer #3:

The work is interesting and acceptable