**Author's response to reviews**

**Title:** Polysaccharides from Liriopes Radix ameliorate streptozotocin-induced type I diabetic nephropathy via regulating NF-kappaB and p38 MAPK signaling pathways

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**Author's response to reviews:** see over
Reply to Editor

MS: 4263971011970871
Title: Polysaccharides from Liriopes Radix ameliorate streptozotocin-induced type I diabetic nephropathy via regulating NF-kappaB and p38 MAPK signaling pathways
Authors: Hung-Jen Lu, Thing-Fong Tzeng, Shorong-Shii Liou, Sheng Da Lin, Ming-Chang Wu and I-Min Liu

Dear Editor,

We appreciate your kind support of our presentation and thanks for your kind agreement. The point-by-point replies for each comment are indicated in the letter to the reviewers. The changes of the revision are highlighted in red. We sincerely hope that this revised version will be suitable to meet your excellent standards of acceptance. Thanks for your kind consideration.

Best regards and hope to have your final decision soon,

Respectfully,
Prof. I-Min Liu,
Reply to Reviewer’s (Referee 1) Comments

MS:  4263971011970871
Title: Polysaccharides from Liriopes Radix ameliorate streptozotocin-induced type I diabetic nephropathy via regulating NF-kappaB and p38 MAPK signaling pathways
Authors: Hung-Jen Lu, Thing-Fong Tzeng, Shorong-Shii Liou, Sheng Da Lin, Ming-Chang Wu and I-Min Liu
Reviewer: Juei-Tang Cheng

Dear distinguished referee:

Thank you very much for reading this manuscript and the helpful comments. The revision has been amended according to your kind suggestions as follows:

1. Provide clarification about the L. spicata var.prolifera (Maidong) used in the study to ensure rigor and reproducibility in future studies.

Reply
Random amplified polymorphic DNA analysis of Liriopes Radix was performed to identify DNA polymorphisms to ensure reproducibility in future studies. Please find it in line 14-15 on Page 4 in the further revision. We are hopeful that this clarification is acceptable and appreciate this helpful comment.

2. State the rationale for evaluating CD4 and CD8 expression in relation to inflammation and macrophage infiltration.

Reply
The rationale for evaluating CD4 and CD8 expression in relation to inflammation and macrophage infiltration has been indicated in the first 4 lines of the 2nd paragraph on Page 16. We appreciate your helpful suggestion and wish this interpretation would be satisfactory.

We hope that this revised version of our work will meet your high standards for acceptance. Also, I wish to express my warmest thanks to you again. Your kind agreement of acceptance will be sincerely appreciated.
Reply to Reviewer’s (Referee 2) Comments

MS: 4263971011970871
Title: Polysaccharides from Liriopes Radix ameliorate streptozotocin-induced type I diabetic nephropathy via regulating NF-kappaB and p38 MAPK signaling pathways
Authors: Hung-Jen Lu, Thing-Fong Tzeng, Shorong-Shii Liou, Sheng Da Lin, Ming-Chang Wu and I-Min Liu
Reviewer: Shih-Liang Chang

Dear distinguished referee:

Thank you very much for reading this manuscript and the helpful comments. The revision has been amended according to your kind suggestions as follows:

1. This is a thorough study testing the responses of a plant extract on type 1 diabetes-induced nephropathy. Is streptozotocin-induced diabetes the best animal model for this study since around 80-90% of all diabetics are type 2?

Reply

Without intervention, approximately 80% of patients with type 1 diabetes and 20–40% of those with type 2 diabetes develop overt nephropathy in 10–15 years. (Dronavalli et al., Nat Clin Pract Endocrinol Metab 2008; 4: 444-452). In the present study, we thus used STZ-diabetic rats as a type-1 diabetic animal model to evaluate the potential of polysaccharides from Liriopes Radix to inhibit the progression of DN and to investigate the possible underlying mechanism of action. Please find it in the 2nd paragraph of page 4. We are hopeful that this interpretation is acceptable and appreciate this helpful comment.

2. Table 1: While the interventions reduced the parameters, these values were not normalized; the text notes only that values were reduced. Should higher doses of the extract have been tested to determine whether normalization is feasible, without marked toxicity?

Reply

The selection of dosage regime for the present studies was according to the previous report that demonstrated administration with PSLR at 200 and 300 mg/kg for 4 weeks exerted potential effect in improving hyperglycemia in diabetic mice (Xiao et al., Int J...
Although PSLR at the chosen doses did not normalize the abnormal values from STZ-diabetic rats, PSLR indeed be effective in the amelioration of the abnormal biochemical parameters in STZ-diabetic rats. We thus believe that PSLR might be considered as potential adjuvant entity for DN treatment. However, the results come from rat studies and thus cannot generalize to human. We will arrange the further study to evaluate the efficacy of PSLR at the higher doses amelioration of the abnormal biochemical parameters in STZ-diabetic rats. We sincerely hope to have your understanding and appreciate this helpful comment.

3. Treated rats exhibited reduced levels of glucose and HbA1c. Does this indicate an overall antidiabetic effect rather than a local (kidney) anti-inflammatory effect of PSLR? The authors should discuss about this issue.

Reply
Role of antihyperglycemia and anti-inflammatory effects of PSLR in the amelioration of DN has been discussed in the last 5 lines in the first paragraph on page 17. We are hopeful that this interpretation is acceptable and appreciate this helpful comment.

The changes of the revision are highlighted in red. We hope that this revised version of our work will meet your high standards for acceptance. Also, I wish to express my warmest thanks to you again. Your kind agreement of acceptance will be sincerely appreciated.
Reply to Reviewer’s (Referee 3) Comments

MS: 4263971011970871
Title: Polysaccharides from Liriopes Radix ameliorate streptozotocin-induced type I diabetic nephropathy via regulating NF-kappaB and p38 MAPK signaling pathways
Authors: Hung-Jen Lu, Thing-Fong Tzeng, Shorong-Shii Liou, Sheng Da Lin, Ming-Chang Wu and I-Min Liu
Reviewer: Wen-Jen Yu

Dear distinguished referee:

Thank you very much for reading this manuscript and the helpful comments. The revision has been amended according to your kind suggestions as follows:

1. The number of independent test in results should be addressed in figure legend or elsewhere.

   Reply
   The number of independent test has been indicated in the line 1-2, 8, 18 on Page 7. We wish this change would be satisfactory and appreciate your kind recommendation.

2. Some symbols to indicate the differences between IHC results should be used.

   Reply
   The symbols (arrows) use to indicate the ED-1-positive cells have been inserted in Fig. 2. We hope this improvement is acceptable.

3. Some descriptions in figure legend should be amended such as in Fig 2 (L4 to L6 “Normal………PSLR”). Similar sentences are appeared in Fig 3, Fig 4, Fig 5.

   Reply
   The descriptions in the legends of Fig. 1-6 have been amended. We are hopeful that the improvement is acceptable and appreciate this helpful comment.

4. The ratios shown in bar figures in Fig 4 (left), Fig 5 (left) and Fig 6 (p-p38 MAPK/p38 MAPK) are not highly correlated to those bands shown in the representative figures.

   Reply
The indicated figures have been amended in the revision. We hope you consider this change sufficient and acceptable.

5. The Y-axis in bar figure of Fig 4 (right) should be amended (#-act in instead of b-actin).

Reply
The Y-axis in bar figure of Fig 4 (right) has been amended in the revision. We hope you consider this change sufficient and acceptable.

The changes of the revision are highlighted in red. We hope that this revised version of our work will meet your high standards for acceptance. Also, I wish to express my warmest thanks to you again. Your kind agreement of acceptance will be sincerely appreciated.