Author's response to reviews

Title: Rhizoma polygoni cuspidati extract reduced progression of diabetic nephropathy via inhibition of platelet-derived growth factor-BB (PDGF-BB) and its receptor interaction in streptozotocin-induced diabetic rats

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Author's response to reviews: see over
I really appreciate your thoughtful comments about my manuscript (MS1751392721125163). I would like to re-submit the revised version of our manuscript entitled “Root of Polygonum cuspidatum extract reduces progression of diabetes-induced mesangial cell dysfunction via inhibition of platelet-derived growth factor-BB (PDGF-BB) and interaction with its receptor in streptozotocin-induced diabetic rats”. We would like this revised manuscript to be considered for publication as a research article in “BMC Complement Altern Med”. I revised every points addressed by reviewers. Thank you for your comments for improving my paper. Please check my revised paper carefully. All changes are highlighted in red.

Thank you for considering our manuscript for publication in your journal.

With my best regards,

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Reviewer: Karim Raafat

Reviewer’s report:

Major:

1. The statement: "albuminuria is well characterized in the streptozotocin (STZ)-induced diabetic animal model", still needs more emphasis and reference.

Answer: Thank you for your suggestion, I changed it as following phrase in Page 4, Line 70-72.

“Hyperglycemia has been is well characterized and albuminuria is considered to be one of the most sensitive markers of renal injury, and albuminuria significantly increased within 6-8 weeks in the streptozotocin (STZ)-induced diabetic animal model [7-10].”

Reference

2. In the title the authors mentioned rhizomes of the plant and in the aim of the work they mentioned roots. Did the authors use rhizomes or roots?

Answer: Thank you for your kindly comment. As reviewer comments, I carefully re-checked my manuscript. We use the root of *Poligonum cuspidatum* in this study. I replaced to “Root” in manuscript
3. The statement: "no difference in body weight between the vehicle-treated diabetic rats and PCE-treated diabetic rats.", is this an indication that the extract is inactive. Please clarify as metformin has other mechanism of reduction of BGL other than mentioned?

Answer: Thank you for your comment. The lowering blood glucose level can prevent or delay diabetic complications. However, various anti-diabetic drugs, such as metformin, dipeptidyl peptidase 4 (DPP4) inhibitors, although it has been successfully work to blood glucose lowering in type 2 diabetes, have been repurposed from other clinical indications to treat renal injury. Actually, Kanasaki et al and Liu et al investigated the anti-fibrotic effect of linagliptin, DPP4 inhibitor, in type 1 model of diabetic nephropathy [26, 27]. These studies demonstrate that an insulin-deficient model of diabetes enable the evaluation of the effects of the DPP4 inhibitor independent of glycemic control and body weight. Also, these studies provide information regarding renal benefit of DPP4 inhibitor independent of glycemic control and body weight. Moreover, metformin, a well-known anti-diabetic drug for type 2 diabetes mellitus (T2DM), had no effect on body weight and blood glucose in type 1 diabetes mellitus (T1DM) [28, 29]. As reviewer comment, our data shows that PCE inactivated on blood glucose and body weight in STZ-induced diabetic rat, type 1 diabetes. However, our study demonstrated that PCE has effect on the reno-protective action. Therefore, PCE possibly has reno-protective effect independent body weight and hypoglycemic effect.

References


I added this sentence in Page 14-15 Line 291-302.
4. In table (1), apparently the PCE is inactive towards diabetes. Please clarify showing why the authors used type I model for a type II study?

Answer: Thank you for your comment. As I mentioned in Background section, anti-diabetic property of *Polygonum cuspidatum* was already reported (Evidence-based complementary and alternative medicine 2013:208349). The aims of our study were to investigate the effect of PCE not on blood glucose but on diabetes-induced renal impairment (diabetic nephropathy) without affecting hyperglycemia. For this reason, we used animal model of T1DM. STZ-induced diabetic rat is well known as a model of diabetic nephropathy (J Renin Angiotensin Aldosterone Syst 2008 9(4):189-95)

5. How did the authors measure the albuminuria in more details?

Answer: Thank you for your suggestion, I revised it as following phrase in Page 7, Line 148-159.

“Urinary albumin excretion levels were measured using a sandwich enzyme-linked immunosorbent ELISA assay kit according to the manufacturer’s manual (Life Diagnostics, Inc., PA, USA). Rat albumin present in the urine sample was captured by an anti-rat albumin antibody that had been pre-adsorbed on the surfaces of microtiter wells. After sample binding, unbound proteins and molecules were washed off with washing buffer, and a biotinylated detection antibody was added to the wells to bind to the captured albumin. Streptavidin-conjugated horseradish peroxidase (SA-HRP) was then added to catalyze a colorimetric reaction with the chromogenic substrate 3,3′,5,5′-tetramethylbenzidine (TMB). This colorimetric reaction produces a blue product, which turns yellow when the reaction is terminated by the addition of stop solution (0.1 M H₂SO₄). The resulting, yellow reaction products were read at 450 nm using a microtiter plate reader (Bio-Tek, Winooski, VT, USA).”

6. Figure 5 is not completely clear and need more metrical and scientific illustration.

Answer: Thank you for your suggestion. To provide the evidence for the mesangial proliferation, the immunofluoresence staining for proliferation markers, such as α-SMA and
PCNA was shown in Figure 4. To provide the concrete evidence, we performed double immunostaining for PCNA and Thy 1.1 (a mesangial cell marker). In order to better understanding, I changed the high-magnification images in Figure 5.

7. The results and discussion sections need to be more detailed and clear

Answer: Thank you for your suggestion, I amended the manuscript as your comment and the manuscript was re-corrected by an English native speaker.

Thank you for your kind suggestion and constructive comments.