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
Dear Dr. Editor of BMC Complement Altern Med

I really appreciate your thoughtful comments about my manuscript (MS1751392721125163). I would like to submit the accompanying manuscript entitled “Rhizoma polygoni cuspidati extract reduced progression of diabetes-induced mesangial cell dysfunction via inhibition of platelet-derived growth factor-BB (PDGF-BB) and its receptor interaction in streptozotocin-induced diabetic rats”. We would like this 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 blue.

I will look forward to hearing from you concerning the acceptability of our manuscript in the future.

With my best regards,

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Reviewer 1: Karim Raafat

Research article comments:

1. Old references: need more recent references.
   Answer: I revised it.

2. The statement: "albuminuria is well characterized in the streptozotocin (STZ)-induced diabetic animal model", needs more emphasis and reference.
   Answer: I added the references in Background section.

3. 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: I revised it.

4. The statement: "In traditional Chinese medicine, rhizoma polygoni cuspidate has been used for anti-diabetic, antibacterial and antiviral effects", needs more emphasis and reference.
   Answer: I added references in Background section.

5. Why did the authors use one-way analysis of variance, although in the experimental part they declared that they used more than one solvent?
   Answer: Reviewer misunderstood the method to prepare the extract of Rhizoma polygoni cuspidate (PCE). PCE is an ethanloic extract of Rhizoma polygoni cuspidate. We only used ethanol as a solvent.

6. 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?
   Answer: Type 1 diabetes mellitus (T1DM) only accounts for 3-5% of diabetes mellitus patients. The vast majority of diabetes is of Type 2 diabetes mellitus (T2DM) (International Journal of Biological & Medical Research 2010; 1:326–329). Thus, the traditional usage of Rhizoma polygoni cuspidate as an anti-diabetic drug has been mainly targeted in patients with T2DM. However, STZ-induced diabetic rat used in this study is an animal model of T1DM. Moreover, metformin, a well-known anti-diabetic drug for T2DM, had no effect on body weight and blood glucose in T1DM (Chinese Medical Journal 2014;127:1298-1303:10, Diabetes Care 2002;25:2153–2158). Similarly, although PCE has been known as anti-diabetic drug, PCE has no significant effect of body weight change in rat model of T1DM.
7. In table (1), 475.9 ± 10.7 is a body weight or BGL?

Answer: It’s a body weight at 24 weeks of age of normal Sprague-Dawley (SD) rats. In our study, 6-week-old male SD rat purchase from the Charles River Laboratory (Waltham, MA, USA) and acclimated for 1 week prior to the study. Our study started using a 8-week-old male SD rats (weight, ~ 200 g) and were monitored for 16 weeks.

I added this sentence in the Material and Methods section.

8. In table (1), 162.1 ± 11. missing figure?

Answer: I revised it.

9. In table (1), apparently the PCE is inactive towards diabetes. Please clarify?

Answer: I already described the reason why PCE is inactive on T1DM in Question 6. Because we used rat model of T1DM, PCE failed to decrease blood glucose in STZ-induced diabetic rat. However, PCE has significant effects on any parameters of renal structure and function without the strong reduction of blood glucose.

10. In figure (1), there is no significant difference between normal and DN in diameter. Please clarify?

Answer: For better understanding, I changed the images for Fig. 1A

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

Answer: Urinary albumin excretion levels were measured by a competitive enzyme-linked immunosorbent assay kit as described manual (ELISA kit, Life Diagnostics, Inc. USA).

I added this sentence for albuminuria assay method in Materials and Methods section.

12. Figure 2 is not clear and need more metrical illustration.

Answer: In order to better understanding, I changed the images with high resolution.

13. How did the authors measure the protein expression in more details?

Answer: I added the detailed method to measure the protein expression in Materials and Methods section.
14. The binding assay declare that there is no affinity. Please clarify?

Answer: In our ligand/receptor binding assay, we showed that PCE inhibited the binding of PDGF-BB to its receptor. This phenomenon can be ascribed to selective affinity of PCE against PDGF-BB or PDGFR-ß. Thus, we performed an additional assay to determine whether PCE can directly bind to human PDGF-BB or PDGFR-ß. I added this result in additional supporting data and Discussion section. As shown in the results of supporting data, PCE has no selective affinity against human PDGF-BB or PDGFR-ß.

15. In Figure, Why PCE-100 is better than PCE-350? Please explain?

Answer: Reviewer misunderstood our results. PCE had the effect on diabetic renal dysfunction in a dose-dependent manner. There is no data that the activity of PCE-100 is better than that of PCE-350.

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

Answer: I changed the high-resolution images in Figure 5.

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

Answer: I re-described the results and discussion in more detail.

18. Is albuminuria alone indication of improvement of DN. Please clarify?

Answer: Unfortunately, in our study, creatinine clearance and proteinuria did not measured in urine. However, albuminuria appeared to be a sensitive marker of end-stage kidney disease and diabetic nephropathy. Proteinuria is usually albuminuria. Proteinuria often assessed as albuminuria (Kidney Int. 2013; 83: 996–998). In addition, mesangial matrix expansion has been considered to be a hallmark of diabetic nephropathy with proteinuria. Because PCE significantly ameliorated mesangial expansion and albuminuria in STZ-induced diabetic rats, we could conclude that PCE improved diabetic nephropathy.

19. With such insufficient findings is the title still valid. Please change the tile to suit the findings.

Answer: I revised the title. The title is “Rhizoma polygoni cuspidati extract reduced progression of diabetes-induced mesangial cell dysfunction via inhibition of platelet-derived growth factor-BB (PDGF-BB) and its receptor interaction in streptozotocin-induced diabetic rats”.
20. Did the authors use emodin. Please clarify. If not what is the evidenced responsible active ingredient in PCE?

Answer : In our results, there is no data using emodin. We only described that emodin is one of the major compound of PCE in Discussion section.

21. Why did the authors use ethanolic extract only of PCE, which is apparently inactive against diabetes?

Answer : The lowering blood glucose level can prevent or delay diabetic complications. However, various anti-diabetic drugs, such as metformin, DPP4 inhibitors, have been successfully repurposed from other clinical indications to treat renal injury. To determine the mechanism of action of these drugs independent of their glucose-lowering effects, animal model of T1DD often used. I already described the reason why PCE is inactive on T1DM in Question 6 and 9. The purpose of this study was also to evaluate the effect of PCE on diabetic nephropathy without the reduction of blood glucose. Our results suggest that even in hyperglycemia, it is possible to attenuate diabetic nephropathy by PCE.

22. The statement: "Although major chemical compounds of PCE include resveratrol and emodin, the most active compound of PCE remains to be identified. Nevertheless, the ability of PCE to protect against renal damage may be due to the effect of these compounds." is scientifically vague. Please illustrate?

Answer : Although major chemical compounds of PCE include resveratrol and emodin, we did not provided the evaluated effect on diabetic nephropathy using the these compounds. However, nevertheless, in previous study demonstrate that resveratrol or/and emodin ameliorated renal function in diabetic rodents, and suppressed high glucose-induced glomerular mesangial cell proliferation by inhibiting NF-kB pathway (Exp Cell Res 2013;319:3182-9, Plos One 2014;9:e93588, Int J Biochem Cell Biol 2012;44:629-38, Int J Endocrinol 2014;2014:289327). Based on previous reports and our in vivo results, the ability of PCE to protect against renal damage may be due to the effect of these compounds.

I added this sentence in Discussion section.

23. The authors declared that the animals were 16 weeks diabetic and these are not illustrated in the results. Authors should show the aggravation or progression of DN.
Answer : Unfortunately, we collected renal tissue samples only at the end of the study. Thus, we can not provide the progression of DN for 16 weeks. However, our previous study showed that hyperglycemia and albuminuria progressed rapidly for 12 weeks in STZ-induced diabetic rat (Phytomedicine, 2014; 21:734-739).

24. English editing should be done with a native expert.

Answer : The manuscript was corrected by an English native speaker in American Journal of Expert Edit Company (AJE).

Reviewer 2 : Hae-Dong Jang

1. Major Compulsory Revisions

1) Fig. 2C: Although PCE-100 and PCE-350 group have similar level values, the results of statistical analysis are different. Please check statistical analysis procedure.

Answer : For better understanding, I changed the images and re-checked the statistical analysis of all figure.

2) Fig. C, D: The statistical analyses by the Student's t-test was done in level of p <0.01. You need to do statistical analysis using other method because there is apparent difference between PCE-100 and PCE-350 group.

Answer : I re-checked the difference between the PCE-100 and PCE-350 group in all Figure and added sentence of statistical significance in figure legends

2. Minor Essential Revisions

1) Which is right the affiliation for Junhhyun Kim, Gacheon university or Korea Institute of Oriental Medicine?

Answer : I revised it.

2) Can you provide some information about resveratrol and emodin profile of your sample, PCE?

Answer : In previous studies, the HPLC profile of Polygonum cuspidatum were already reported The contents of resveratrol and emodin is a 6.05 and 4.33 mg/g of Polygonum cuspidatum, respectively. (J Chromatogr A 2013;1286:102-110, Molecules 2014;19:1258-1272)