Author’s response to reviews

Title: Sheng-ji Hua-yu Formula Promotes Diabetic Wound Healing of Re-epithelization via Activin/Follistatin Regulation

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Author’s response to reviews:

Dear Editor,

Thank you for the valuable comments and considerate tips. Those comments are very helpful for revising our paper. I have carefully made a revision and the responses are as following. We are also submitting a clean version of the manuscript that do not have tracked changes or highlighting. Once again, thank you very much for your comments and suggestions.
Editor Comments:

1. Please include the email addresses of all authors on the title page of your manuscript.

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These authors’ emails have been added on the title page of my manuscript.

2. Please revise your Ethics statement for clarity; this is a study on mice, not a study protocol.

“The study on protocol” has been changed to “The study on mice”.

3. Please change the text under Consent to Publish to: "not applicable".

“applicable” has been changed to “Not applicable”.

Reviewer 1:

1. The first sentence of Background has no current information on the world prevalence of diabetes and its English language is not correct: "The world prevalence of diabetes among adults (aged 20-79 years) will be 6.4%, affecting 285 million adults in 2010, and this number is estimated to 439 million adults by 2030". The authors should correct it.
Replace with the latest 2017 documents “In 2015 it was estimated that there were 415 million people with diabetes aged 20-79 years, moreover, the number was predicted to rise to 642 million by 2040 [1].”

2. The authors should check the sentences below (in Methods) and clarify which membrane they used, nitrocellulose or PVDF?:

"The protein was transferred from poly-acrylamide gel to nitrocellulose filter membrane with Bio-Rad transfer system for 2h in ice bath, 100V. The protein was dyed in Ponceau S staining solution for 5 to 10 min and then washed with water. The PVDF membrane with the target protein was tailored according to molecular mass."

” PVDF” has been changed to “nitrocellulose”.

3. There are lots of typographical and English grammatical mistakes which should be corrected by a native English speaker and an expert on technical issues. Some examples:

Background:
"They transducer the signaling through ....." (Paragraph 2 Line 9)

Methods:
"The detection of SJHY Formula was analyzed by Shimadzu was analyzed by LC-MS"

"The caudal is venous blood of mice was drawn on day 1st, 3rd, 5th, 7th, 11th."

"Four groups at a time, and its blood glucose level was measured with Roche ACCU-CHEK ACTIVE Glucometer"

" Dissolved streptozotocin in sodium citrate buffer solution (0.1M,pH=4.5) into 2% solution."

"The detection of SJHY Formula was analyzed by Shimadzu was analyzed by LC-MS"

The detection of SJHY Formula was analyzed by LC-MS.

"The caudal is venous blood of mice was drawn on day 1st, 3rd, 5th, 7th, 11th.""Four groups at a time, and its blood glucose level was measured with Roche ACCU-CHEK ACTIVE Glucometer"
“Vein blood from tail of mice was drawn on day 1, 3, 5, 7, 11. Blood glucose level was measured
with Roche ACCU-CHEK ACTIVE Glucometer.”

"Dissolved streptozotocin in sodium citrate buffer solution (0.1M, pH=4.5) into 2% solution."

Streptozotocin (stz) was dissolved in 0.1 M sodium citrate buffer solution (pH 4.5)

4. The authors should use accepted formatting conventions for gene and protein symbols in a
consistent manner throughout the manuscript.

“NF-kB” has been revised as “NF-KB p50”

“Psmad 2” has been revised as “pSmad2”

5. Lots of times in which abbreviations occur without explanation. A first-time occurrence
should give the abbreviation with the full name supplied, thereafter just the short form. The
list of abbreviations is not complete.

“CSU: chronic skin ulcer; HE: Hematoxylin-eosin; photodiode array (PDA); MMP-3: Matrix
metalloprotease-3; NF-kB: Nuclear factor kappa B; PBS: Phosphate buffer saline; pSmad2:
phospho-Smad2; Streptozotocin: stz; SJHY: Sheng-ji Hua-yu; TCM: Traditional Chinese
Medicine; TIMP-1: Tissue inhibitor of metalloproteinase-1; TGF-β: transforming growth factor-
β; WM; western medicine.” has been revised.

6. The authors should provide keywords from Pubmed MeSH Browser 2017. Some of the
keywords, such as "diabetic wound healing" and "Activin/Follistatin regulation", need to be
changed.

Key word “Sheng-ji Hua-yu Formula, Diabetic wound healing, Inflammation, Re-epithelization,
Activin/Follistatin regulation.” has been revised to “Chinese Herbal, Diabetic Ulcer, Wound
Healing, Inflammation, Re-Epithelization, Activin, Follistatin. ” which following Pubmed MeSH
Browser 2017.

7. I would recommend that the authors give the limitations of this study and further studies in
Discussion.
Conclusion: “It will be interesting to apply topical activin and follistatin treatment during wound healing in vivo and cultivate keratinocytes in vitro to explore how activin/follistatin regulation works and the effect of SJHY Formula on cell growth.”

Reviewer 2:

1. Under materials and methods, there is no mentioning about total groups and number of animals in each group?

Animals: 230 female C57BL/6 (8 weeks old)

Diabetic animal model: Each group was monitored on day 1, 3, 5, 7, 11, 15, 8 mice at every time point.

2. The LC/MS figure (page - 22) is showing many number of peaks (phytochemicals). This TCM drug contains 6 herbals along with two other inorganic ingredients. The beneficial effect of this ointment is due to the combined synergistic activity of all 6 herbals. In order to identify all the phytocompounds, GCMS may be additionally performed.

The HPLC profile we have provided is a matter of establishing the quality standard of the SJHY formula. In view of the high content of the drug, the highest content was chosen as the control standard.

Sincerely

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