Author's response to reviews

Title: Polysaccharides from the root of Angelica sinesis promotes hematopoiesis and thrombopoiesis through the PI3K/AKT pathway

Authors:

Mo Yang (yangm1091@yahoo.com.hk)
Chang Liu (cliu6688@yahoo.com)
RuiXia Deng (ruixia_deng@yahoo.com.cn)
JieYu Ye (jieyu.ye@googlemail.com)

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Author's response to reviews: see over
Dear Editors,

Thank you very much for the reviewers’ very constructive comments. Please find the point-to-point response to the reviewers’ comments.

Could you exclude that the spleen size increases due to impurities of APS?

Response: The reviewer has not specified what he means as impurities, consequently, we found it difficult to perform experiments to address this comment.

What was the LPS or β-glucan content of your APS preparation? This should be checked by the chromogenic Limulus assay.

Response: We have tried very hard but could not find the microplate reader needed to perform the chromogenic limulus assay. Instead, we used TAL test to answer this question. The methods and results have been added to the text. In short, we detected the activity of LPS or β-glucan to be less than 0.32EU/mg of APS extract, or 0.128 ng endotoxin in 1 mg of APS extracts. Please see the text in the sections Materials and Methods (Page 6, first paragraph from the bottom) and Results (Page 11, first paragraph from the bottom) for more details.

200 mg/ml APS given to M-07e cells seem to me an extraordinarily high concentration. APS should be analyzed at concentrations that are in the range of future clinical dosages.

Response: After checking the raw data, the concentration should be 200 µg instead of mg. We apologize for this mistake and the unit has been corrected in all text.

A clear dose-response study on APS is missing. In one good experiment you should clearly demonstrate the dose-response of increasing concentrations of APS on any biologic system.

Response: Dose-response study of APS has been performed and the corresponding results have been reported in a previous conference. The fact has been described in the text and the relevant results have been provided as supplementary information.

What is the exact chemical nature of APS?

Response: The chemical nature of APS has been studied previously (http://onlinelibrary.wiley.com/doi/10.1002/cjoc.200890189/abstract). The description is added to the 9th line from the bottom of page 4.

Minor

Explain the abbreviation TCM the moment it appears for the first time.

Response: Thanks and this has been done (Page 4, last line of the first paragraph).

Wavelength range 400-4000 nm? What is meant by cm-1?
Response: This is the reciprocal wavelength (cm⁻¹), which is a simple convenient unit for comparing energies when dealing with spectra. More details can be found by Google directly.

Explain what is AKT?

Response: Thanks for the comment and the explanation of AKT is added (Page 16, the first paragraph from the bottom). AKT is a serine protein kinase that is also known as Protein Kinase B (PKB).

Figure 5 must be explained, what is A,B,C,D ?

Response: A, B, C and D indicate different treatment groups. The measurements for control/treatment groups indicated with the same letter are not statistically significantly different, while the measurements for control/treatment indicated with different letters are statistically significantly different. This is a notation used by the JMP software and we found it very useful to describe the measurement differences among different groups.

Review 2:

Abstract - this does not clearly summarise the paper - a re-write is needed. It should follow more closely the contents of the paper. Comparisons with TSP are mentioned but no results are included.

Response: We have modified the main text and hopefully now the abstract summarize the main text better. The TPO is used as a positive control in this paper as the effects of TPO on thrombopoiesis has been well-established. As a result, the effects of TPO treatment are not described in the abstract.

Figure 2. There is a discrepancy in that 2A provides results on red cells but writer of the text refers to platelet count. Also, the text does not correctly describe the contents of the Figure. This needs correction.

Response: We thank the reviewer for very detailed reading of the paper. The descriptions for 2A and 2C were misplaced. They have been placed correctly now.

Other Figures. Again, the text does not accurately describe the contents of the Figures and accompanying tables. Where are the significance values? Additionally, far too much information is provided between the Figs and Tables which is difficult for the reader to follow. Considerable simplification is needed with a full reconsideration of the number of Figs, Tables and the text that goes with it. I think there is interesting data but the reader has to work too hard to take in the information.

Response: Again we appreciate the reviewer’s comments and we are quite ashamed of our sloppy presentation on this part. We have added the significance value and simplified the description in the figure legends. Hopefully this will make the paper easier to read.
Regarding the figs that include flow cytometry, again can the data be simplified so that the actual plots are not needed?

**Response:** To our knowledge, some readers would appreciate the actual plots. So we prefer to keep them in the paper while at the same time the user can choose to skip them.

I have difficulty with the concept of APS reversing the effect of Ly294002. Maybe the authors could explain better what they think is going on?

**Response:** We have added more explanation here. In table 2 and 4, it is clear that the total percentages of R1+R2 cells treated with Ly294002 are greater than those treated with APS alone. And the combined treatment of Ly294002+APS lowered the percentages of R1+R2 cells, suggesting that APS reversing the effect of Ly294002 (page 16, the 6th line from the bottom of the page).

Best regard,

Chang Liu