Reviewer's report

Title: A Chinese Herbal Decoction, Jian-Pi-Yi-Shen, Regulates the Expressions of Erythropoietin and Pro-inflammatory Cytokines in Cultured Cells

Version: 0 Date: 18 Sep 2017

Reviewer: Yu-Tse Wu

Reviewer's report:

This study aimed to explore the role of JPYS on regulating hematopoietic and immunological functions, and the authors concluded that JPYS could regulated erythropoietin expression and the expression of pro-inflammatory cytokines in cultured cells. However, the HPLC analysis was not validated to ensure the reliability of the results. In addition, study design should be improved to confirm the safety and effects of the herbal decoction. Several problems and comments were listed.

Comments

Introduction

* Please cite the reference of the statement "For over 20 years…resolving stasis".
* The author could include the indication of JPYS written in the official pharmacopeia.
* The author states that there is a need to develop an alternative medicine owing the side effects of widely used rHuEPO. It is not a proper way to include any extent of prejudice, and even the aim of the study is not to develop an alternative medicine.
* In the cited study [3], the result of increase in serum hemoglobin level could be attributed to the destruction of red blood cells and hemolytic process. Can the author give some description on the relevance between two studies?
* Is there any reference for beneficial effects of JPYS on HIF signaling?
Methods
* The references, mineral oil acting as hypoxia mimicry (as well as LPS and the glucocorticoids) and the validation of HPLC method, should be provided.
* The detailed information of treatment groups in the section of cell culture and treatment should be listed.
* Why did the authors use mineral oil layering the positive control, instead of the marketed drug to assess the effect of JPYS?
* Please add the section of Statistical analysis.
* On Page 6 of line 28. The authors should detail the selections of internal control for normalization and positive control. Why the control (no treatment group) was not included in the experimental design?
* The full name of each abbreviation should be defined.

Results
* How can the author indicate the chemical markers in HPLC chromatogram without the respective standard?
* Figure 1. How did the authors confirm the selectivity of the analytical methods? Did authors conduct method validation before the determination of JPYS?
* Why the internal standard method was not applied to determine JPYS?
* Page 9. The rationale for selecting echinacoside, salvianolic acid B, and rhein as chemical markers should be clarified. How did the author determine the minimal requirement for the chemical components and guarantee their biological results?
* In figure 2, the positive control showed the increasing about 80% and 150% for EPO and HIF-1α mRNA, but the author did not discuss the correlation between positive control and JYPS groups.
* Regarding the dose-dependent effect, please examine any correlation factor to give evidences for the effect.
* The positive control groups should be included in the experiments of figures 3 and 4.
* The cell toxicity was still the important issue. The author did not mention any toxicity information of JYPS.
Discussion

* Please rephrase the deduction "These chemicals may also contribute...need to be further studied".
* Please elucidate why studying those target pro-inflammatory cytokines.
* Please give a comprehensive explanation on the bi-directional immune-modulatory roles of JPYS expressed in different studied macrophages.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.
No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.
No

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.
Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.
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