Author's response to reviews

Title: Identification of nuclear factor-kappaB inhibitors in the folk herb Rhizoma Menispermii via bioactivity-based ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry analysis

Authors:

Dan Sun (sundan@nankai.edu.cn)
Ge Meng Zhou (765206890@qq.com)
Hui Xu Ying (378432449@qq.com)
Feng Bin Cheng (chbinfeng@163.com)
Qi Yan Han (824297655@qq.com)
Yan Nie (52089698@qq.com)
Yuan Yuan Hou (houyy@nankai.edu.cn)
Gang Bai (gangbai@nankai.edu.cn)

Version: 3 Date: 11 September 2014

Author's response to reviews: see over
Dear Editor:

Thank you for giving us the opportunity to revise our manuscript ID 4225727651356119 entitled “Identification of nuclear factor-κB inhibitors in the folk herb Rhizoma Menispermi via bioactivity-based ultra-performance liquid chromatography/quadrupole time-of-flight mass spectrometry analysis”. We appreciate the detailed suggestions provided by you and reviewers, which helped us to improve our manuscript. We have carefully considered the suggestions, and have revised our manuscript accordingly.

We have provided our point-by-point response to the comments. Although this makes for a rather lengthy cover letter, the reviewers’ comments have been included verbatim in italics, followed by our comments and revisions in bold, which we believe will provide you and the reviewers with the best explanation for the changes we made. In the revised manuscript, the changes are indicated by using the “Track Changes” option.

Please consider the following point-by-point replies to the reviewers’ comments and suggestions.

To reviewer #1:

Thank you very much for patiently reviewing our work. We reviewed our manuscript and also found a number of grammatical mistakes. We have made appropriate correction carefully.

Major points:

1. There are some grammatical errors in the text, so the manuscript should be carefully checked and improved.

Response:

Thank you for your suggestion. The revised manuscript has been polished by a professional Elsevier language editing service company for SCI papers. The amendment has been marked in red of my manuscript (P.3, Line 34, 47; P.6, Line 91, 93; P.9, Line 154).
2. *The font of the herbal drug “Rhizoma Menispermi” should not be italic.*

**Response:**

Thank you for your helpful and reasonable suggestion. The font of the herbal drug “Rhizoma Menispermi” was revised in roman. The amendment has been marked in red of my manuscript (P.1 Line; P.3, Line 32, 48; P.4, Line 68).

3. *Abbreviation for the first mentioned in the text should have full name, and then write the abbreviation directly when it appears again.*

**Response:**

Thanks for your suggestion. Now we have revised the manuscript according to the reviewers’ comments. The amendment has been marked in red of my manuscript (P.4, Lines 55, 58; P.5 Line 80).

4. *The format of references should be checked, for example, the name of magazine should have full name*

**Response:**


To reviewer #2:

Thank you very much for your patient reviewing work. We are deeply grateful to you for supporting our manuscript. We have carefully considered your suggestions and revised the manuscript accordingly.

**Major points:**
1. There are many kind of pneumonia models, to better interpret the relaxant characteristics of RM on the treatment of lung infections, the authors may want to introduce why the P. aeruginosa PAK strain induced mouse inflammation model was used in this study in the section of introduction or discussion.

Response:

Thank you for your suggestion. Now we have revised the manuscript according to the reviewers’ comments. As suggested, we added the following content in the introduction section (P.5 Lines 83-90). *Pseudomonas aeruginosa* PAK strain acute pneumonia mouse model has been widely used in studying infection and host immune responses [1, 2]. PAK is a clinical isolate, which is highly virulent and can quickly cause tissue damage during lung infection [3, 4]. Besides, the LPS shed by this bacterium intensify the immune response [5, 6]. Thus, we utilized PAK induced pneumonia in our study.


2. The authors found seven alkaloids in RM as the NF-κB inhibitors, and confirmed the activities in vitro. However, an analysis of the lung tissue distribution of these components was recommended to further validate bioactivity.

Response:

Thank you for your suggestion. The reviewer’s advice is appreciated. Yes, it’s necessary to further validate the biological activity of these compounds in vivo. Actually, it has been reported that sinomenine and magnoflorine, two of the seven alkaloids we obtained by HPLC, have anti-inflammatory effects in the in vivo experiments [1-4]. However, there are no commercial products available for the other five alkaloids and the yields of five compounds from HPLC are very low and not enough for in vivo experiments. As pointed out by the reviewer, we will continue to separate the other five alkaloids from RM, until there is enough for in vivo experiments for our further study.


3. Antibiotic therapy is better for various diseases caused by bacteria, although the NF-κB inhibitive effect of RM was confirmed in the present study, the bacteriostatic effect of RM and the chemical constituents was suggested to investigate in this model in the future study.

Response:

Thank you for your suggestion. Now we have revised the manuscript according to the reviewers’ comments. The reviewer’s suggestion is well taken. In our mouse pneumonia experiments, P. aeruginosa infection strongly elicited the production of TNF-α, IL-1β and chemokines, resulting in sever inflammation. The large amount of TNF-α led to activation of NF-κB pathway and recruitment of immune cells, which further strengthened the inflammation. Our results in this
study demonstrated the anti-inflammatory effects of RM, which might protect mice from detrimental inflammation. Antibiotics inhibit or interfere with bacterial metabolism, for example, penicillins and cephalosporins suppress bacterial growth by inhibiting bacterial cell wall biosynthesis [1,2]. It is possible that RM might have bacteriostatic or bactericidal effects. As pointed out by the reviewer, we will examine the effects of RM in our further study.


I am looking forward to your reply. For any questions, comments or suggestions please contact to me.

All best regards!
Yours Sincerely,
Yuanyuan Hou

College of Pharmacy, Nankai University,
College of Pharmacy, Nankai University, Tianjin 300071, P. R. China
Phone/Fax: +86-22-23506930
E-mail: houyy@nankai.edu.cn