Author’s response to reviews

Title: Suppression of lung inflammation by the ethanol extract of Chung-Sang and the possible role of Nrf2

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Dear reviewers

Thank you for your careful reviews of our manuscript. Per the comments, we revised the manuscript, for which we performed additional experiments. We tried to address the issues raised by the reviewers as possible as we can. And we hope this revision meets the critiques of the reviewers. Our point-by-point responses to the comments are shown below. Revised contents are highlighted red in the revised manuscript. We hope we understood your comments correctly and responded accordingly.
Response to the comments

Reviewer 1: Manuscript entitled "Suppression of lung inflammation by the ethanol extract of Chung-Sang and the possible role of Nrf2" is mainly focused on the preparation of a herbal formulation with five different medicinal plant which showed the anti-bacterial and anti-inflammatory activity. The work has been described well and all responses of previous comments have been made. It will be better if author provide a schematic of mechanism of action. It will give a clear and thorough picture of the mechanism. Finally, the manuscript may be accepted for the publication.

√ Response: A schematic explanation of our study is included as in Fig. 9. Description pertaining to the scheme is in the figure legend and included in the discussion section.

Reviewer 2: 1. The authors should explain why five compounds such as chlorogenic acid, rosmarinic acid, eugenol, 6-gingerol and aristolochic acid I been identified.

√ Response: Appreciate your valid inquiry. We put the notion about it in the result section.

2. The authors report that eCS has anti-inflammatory activity through activation of Nrf2. Although the activation of Nrf2 is closely related to the anti-inflammatory activity, the authors should investigate the effect of inhibition of Nrf2 activation on the anti-inflammatory activity of eCS.

√ Response: We agree to the comment. Although we suggested that activation of Nrf2 is one of the possible anti-inflammatory mechanisms exerted by eCS, it is uncertain to what degree eCS activating Nrf2 contributes to the anti-inflammatory effect of eCS. It is possible that the effect of Nrf2 activation is either significant or just marginal, which warrants further studies as suggested by the reviewer. Since this comment is valid and important, we would like to investigate with Nrf2 KO mice to address the comment as another set of study.

Reviewer 3: 1. The changes in the histology sections, in Figure 6, should be indicated distinctly for better understanding.

√ Response: We added a better explanation about the figure in the result section.

2. The western blot analysis section under the Methods section should be re-framed for better understanding, mentioning the specifications maintained for running the proteins, transfer of proteins, and incubation with antibodies.

√ Response: We elaborated the details of immunoblotting in the Materials and Methods section.
3. The authors have established the activation of Nrf2 along with NQO-1, HO-1, GCLC. If they had estimated the changes in the levels of GSH, GSSG, and GST in lung tissue, it would have enriched the conclusion.

√ Response: We performed a semi-quantitative RT-PCR analysis of lung tissue to measure the levels of Nrf2-dependent genes. In the revision, the results are added to Fig. 6, shown as Fig. 6E. As we described in the introduction section, LPS treatment alone activates Nrf2, which is via the production of ROS that will suppress the Nrf2 inhibitory function of Keap1. Thus, Nrf2 activation elicited by LPS is considered as a regulatory measure to curb excessive inflammatory reaction. Consistent with the results with RAW 264.7 cells, the additional data show that eCS enhanced the expression of Nrf2-dependent genes in mice lungs, with a statistical significance. While not showing to what degree eCS activating Nrf2 contributes to the anti-inflammatory function of eCS, our results collectively suggest that Nrf2 activation is associated with the anti-inflammatory function of eCS. We hope our response addresses the comment properly.