Author’s response to reviews

Title: Oryeongsan inhibits LPS-induced production of inflammatory mediators via blockade of the NF-kappaB, MAPK pathways and leads to HO-1 induction in macrophage cells

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Author’s response to reviews: see over
Dear Editor of BMC complementary and alternative medicine

Thank you for reviewing our manuscript. We have added and changed some parts according to reviewer’s comments. Here are answers to reviewer’s comments.

**Manuscript ID:** 1963006468125900

**Title:** Oryeongsan inhibits LPS-induced production of inflammatory mediators via blockade of the NF-kappaB, MAPK pathways and leads to HO-1 induction in macrophage cells

**Answers to reviewer’s comments**

Major concerns and objections

Reviewer 1

1. The English writing in the paper is poor and the paper needs to be re-written by a native English speaker. Please double check grammatical errors and the convoluted sentences, as
well as reproducing the data from the references clearly.

Answer: We checked English by at least two professional editors, both native speakers of English. For reference, http://www.textcheck.com/certificate/7WNmoy

2. The Abstract needs to be re-written, especially in the part of Methods. The author should emphasize what kind of methods they have taken advantage of to test pharmacological and biological effects of OR in RAW 264.7 macrophage cells. The content in Background and Conclusions should be added to illustrate the purpose more detailed.

Answer: We changed and added more description in abstract part. According to reviewer’s suggestion, we changed abstract including Methods part as follows described kinds and application range of assays. Also, we added more detail description in the background and conclusion parts.

3. The part of Background did not provide enough information about OR, inflammation, and related components including LPS, NF-kappaB, MAPK and HO-1 in RAW 263.7 macrophage cells. The author should look for some updated references in this part to illustrate their points specifically.

Answer: We added more information about above components in the background part with references.

4. In the part of Result, there are some misleading points. Firstly, I am not sure why the author chose a concentration of 1000 µg/mL as a standard to test whether the drug is toxic to
cells. Secondly, although over-production of NO is associated with various inflammatory diseases, why did the author think that the suppression of NO by LPS stimulation can demonstrate that OR has inhibitory effects on the production of pro-inflammatory mediators. What is more, why did the author pick NF-kappaB and MAPK as representatives in this work?

**Answer:** In this study, we checked which concentration of OR is nontoxic to the cells. Usually, before testing of pharmacological effect of certain candidate, researcher should do cytotoxicity test first. OR is not single compound and herbal extract. We tested the cytotoxic effect of OR, at the concentration of 1000 µg/ml and found that OR up to 1000 µg/ml didn’t express any cytotoxicity to the cells. We examined the anti-inflammatory effect of OR at the concentrations from 10 to 1000 µg/ml, the concentrations of nontoxic to cells. As shown in Figures, the expressions of some inflammatory mediators were significantly inhibited from 500 µg/ml and the expressions of some factors were inhibited at 1000 µg/ml.

The anti-inflammatory efficacy of most drug candidate was first-tested by NO production in inflammation-related cells including macrophages. We described the importance of NF-κB and MAPK in the inflammation-related research in the background part.

5. The part of Discussion seems to be a conclusion of the Result, but lack basic discussion about the future use of OR or how this work can influence the future study on the similar part. Therefore, the author should more content in Discussion to discuss about the meaning of their work.

**Answer:** We added more detailed discussion about our finding.
6. I personally suggest that the author should rearrange the content of picture, since current forms contain no explaining words and are hard to understand.

**Answer:** As suggested by reviewer, we combined the figure 1-7 images and figure legends in the bottom of manuscript text for reviewer to easily understand the contents.

Reviewer 2

1. The more detail methods for herbal decoction extraction should be described in the “Preparation of herbal decoction OR”. Also amount of each herb were not listed in the manuscript. Authors should consider appear composition and amount of each herb in the manuscript by table.

**Answer:** According to reviewer’s suggestion, we added detail making method of oryeongsan in the preparation of herbal decoction OR. Herbal ingredients and their amount in oryeongsan were listed in Table 1. Also we described detailed methods of making test substance in the preparation of herbal decoction OR.

2. The author measured only TNF-α and IL-6 cytokines? If so authors should consider measure other cytokines such as GM-CSF or IL-1β.

**Answer:** According to reviewer’s suggestion, we did experiment to check the inhibitory effect of OR on another inflammatory cytokine IL-1β production. IL-1β is widely used
as one of inflammatory parameters. The effect of OR on IL-1β cytokine and mRNA expression were added to the parts of “Results”, “Discussion”, “Figure 2” and “Figure legends”.

3. RT-PCR conditions such as annealing temperature in the Materials and Methods should be arranged with sequences of genes in the Table 1.

Answer: According to reviewer’s suggestion, annealing temperatures for TNF-α, IL-6, IL-1β, COX-2, iNOS, HO-1 and β-actin were indicated in Table 2 with its primer sequences.

4. Quality and resolution of the figures (especially figure 6 and 7) has to be increased.

Answer: According to reviewer’s suggestion, we changed Figure 2, 3, 4, 5, 6 and 7 with high resolution and quality.

All other problems of our manuscript were corrected and marked with yellow underline.