Author’s response to reviews

Title: Ginsenoside Rb1 Can Ameliorate the Key Inflammatory Cytokines TNF-α and IL-6 in a Cancer Cachexia Mouse Model

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Author’s response to reviews:

Dear Editor and Reviewers:

Thank you for your letter and for the reviewers’ comments concerning our manuscript entitled “Ginsenoside Rb1 Can Ameliorate the Key Inflammatory Cytokines TNF-α and IL-6 in a Cancer Cachexia Mouse Model” (Manuscript Number: BCAM-D-19-00781R1). Those comments are all valuable and very helpful for revising and improving our paper, as well as the important guiding significance to our researches. We have studied comments carefully and have made correction which we hope meet with approval. Revised portion are marked in red in the paper. The main corrections in the paper and the responds to the Editor’s and Reviewers’ comments are as following:

Editor:

Response to comments:

1. Authors should add details of Ginseng extract. Whether the extract was prepared in lab or procured from market? If prepared in lab, detail method of preparation, source of Ginseng, its authentication details should be mentioned.

Response: Thank you for your comments. We have added details of Ginseng extract in Methods section (line 103-115, page 6-7).
2. Standardization data of the extract by using HPLC or HPTLC should be given.

Response: We have given Standardization data of the extract by using HPLC in Methods section (line 111-112, page 7).

3. Source of ginsenoside should mentioned. Authors should add details of ginseng extract and ginsenosides.

Response: The details of ginseng extract and ginsenosides have been added in Methods section (line 103-118, page 6-7) following the comments.

Reviewer 1:

1. In general cell line procurement from cell bank is preferred. How did authors authenticated C26 colon adenocarcinoma cells.

Response: The C26 colon adenocarcinoma cell line came from commercial cell bank. It was purchased by the college of life sciences in our university then was split into storage tubes and gave our lab as a gift.

2. Ginseng extract and isolates from it have been reported for anticancer and immune-enhancing effects, in such case results of this study are obvious. How would author define novelty of this study? Add in introduction.

Response: Cancer cachexia is a disease that has been defined in recent years, which is different from cancer. Treating cachexia is different from treating cancer alone in clinic. The drug for cachexia treatment is rare and urgent compared the many drugs for anti-cancer. After searching the literatures, current we have not found any studies using ginseng alone to treat cachexia. Although ginseng has anti-cancer and immune-enhancing effects, we are the first to report the use of ginseng alone to treat cachexia. We have added the novelty of this study in Background section (line 84-85, page 6).

3. Further, it is known that Ginsenoside Rb1 attenuate Cytokines TNF-α and IL-6. Thus is it expected that it will reduce their levels in cancer cachexia mouse model also. In such case how much significant is this study.
Response: Our aim is to investigate that the Ginseng alone whether could improve the symptoms of cancer cachexia, so we established a mouse model of cancer cachexia and focus on water extract of Ginseng, ginseng extracts and ginsenoside. Based on TNF-α and IL-6 are the two most typical indicators in the model. Testing the two indicators to determine the therapeutic effect of drug is very meaningful for clinical treatment.

4. In general, for more than one groups, ANOVA is applied which is followed by post hoc test. In this study t-test was performed which is used for comparing 2 groups. Authors should apply proper statistical tests for comparison.

Response: Thank you for reviewer’s suggestion and we have changed all t-test to one-way ANOVA in statistical analysis based on the suggestion.

5. All graphs are showing one side of deviation, in my opinion it is not proper. SD values in the graphs should represent both + and - side.

Response: All SD values have been replaced with both + and – side according the Reviewer’s comments.

6. Author should discuss relation between cytokines levels and cachexia.

Response: We agree with the reviewer’s comment. The relation between cytokines levels and cachexia is indeed an important issue. We had put the discussion about the relationship between cytokines and cancer cachexia in the third paragraph of the Discussion section (line 238-248, page 13).

7. It is not clear from the results if treatment is curing cancer thus resulting in reduction of cytokine levels or treatment is attenuating cytokine levels and thus improving cancer cachexia.

Response: Based our results data, it looks like that “treatment is attenuating cytokine levels and thus improving cancer cachexia”.
Reviewer 2:

1. The conclusions in this manuscript are based just on analyzing serum level of two cytokines. I think the authors need to perform lot more experiments to have a definite evidence of Ginseng extract being effective in cancer cachexia.

Response: In the current manuscript we focus on the line of water extract of ginseng - ginsenoside mixture - ginsenoside monomer to study the therapeutic effect of ginseng on cancer cachexia. The cytokines TNF-α and IL-6 are the most representative indicators, and the levels of these two cytokines are usually tested in many studies to determine the therapeutic effect. Then we chose the two indicators to evaluate whether it is effective.

2. The independent sample t test is applied only when there are only two groups to compare. For more than two groups, the appropriate statistical test in ANOVA followed by multiple comparison to compare the significance among different treatment groups. All the statistical tests need to be performed again using appropriate test and then compare if there is significant difference.

Response: We are thankful for reviewer’s suggestions and all t-test have been changed to one-way ANOVA in statistical analysis.

3. The manuscript shows a lot of negative data than the positive one but there is no good explanation of this negative data discussed in the discussion section.

Response: Thanks for the comments. It is sure that both positive and negative data should be discussed as an article. Negative data had been discussed in Discussion section (line 257-273, page 14) in our manuscript.

4. in the discussion section, first paragraph have statements without any references.

Response: Thanks for Reviewer’s suggestion. We have added references in first paragraph of Discussion section according to the suggestion (line 223-224, page 12).

Other changes:

1. All Figures (Figure 1-6) were corrected.
2. ** P &lt; 0.005 was replaced by ** P &lt; 0.01; ## P &lt; 0.005 was replaced by ## P &lt; 0.01 according to the references.

We appreciate for Editors/Reviewers’ warm work earnestly, and hope that the correction will meet with approval.

Once again, thank you very much for your and reviewer’s comments and suggestions.

Best regards,

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