Author's response to reviews

Title: Analysis of the effect of the active compound of green tea (EGCG) on the proliferation of peripheral blood mononuclear cells

Authors:

Farid Saleh (dadaland@gmail.com)
Raj Raghupathy (raj@hsc.edu.kw)
Sami Asfar (sami@hsc.edu.kw)
Medhat Oteifa (drmedhat.oteifa@hotmail.com)
Noha Al-Saleh (drnono105@hotmail.com)

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Author's response to reviews: see over
Responses to Reviewers’ Comments

Reviewer: Nisaudah Radenahmad

1. **Comment**: No major or minor revision needed.
   
   **Response**: We thank the reviewer for his comment.

2. **Comment**: An article of outstanding merit and interest in its field.

   **Response**: We thank the reviewer for his comment.
Responses to Reviewers’ Comments

Reviewer: Junpeng Wang

We thank the reviewer for his time and for the very objective and useful comments. We tried to respond to each comment, and to do the required changes in the manuscript. We think now it is in a better shape, and the credit goes to him.

1. Comment: More mechanisms on cell proliferation and cytokine production from PBMC should be further measured.

   Response: We agree that it would be of interest to examine effects of EGCG on the proliferation of lymphocytes as well as the production of other cytokines; however, this was a preliminary study aimed at assessing the effect of EGCG on lymphocyte stimulation and the production of only one cytokine, IFN\(\gamma\).

2. Comment: In the whole manuscript, the “IFN#” or “IFN-#” should be consistent.

   Response: We have revised the manuscript to refer to interferon-gamma as IFN-\(\gamma\) consistently.

3. Comment: In the methods, PBMCs were stimulated using breast cancer peptides for six days; whereas, supernatants were collected 96 hours. Why did the culture use different times? Are there different culture times for the proliferation? Please clarify it.

   Response: We apologize for this error and thank the reviewer for pointing it out. Supernatants of peptide-induced proliferation were collected after 6 days of culture. Supernatants of mitogen-induced and anti-CD3-induced proliferation were collected after 4 days of culture. This has now been corrected, as shown on page 5.

4. Comment: Should the statistical analysis be one-way ANOVA?

   Response: We used ANOVA as cytokine data are generally not normally distributed.

5. Comment: Should the figure or table for optimal EGCG and antigen peptide concentrations on proliferation added?

   Response: We suggest that the optimization data be referred to only as text; we may not need another table to present optimization experiments as these are part of the standardization.

6. Comment: The author mentioned that they used anti-CD3 or PHA as positive control. Thus, the antigen-specific proliferation and IFN- release should be given more description; whereas, CD3 or PHA on cell proliferation and IFN# release should be deleted.
Response: By “positive control” we imply that we expect that the lymphocytes WILL respond to mitogen and anti-CD3, but we would not have been able to predict the EXTENT of the response. Therefore, we submit respectfully that we need to include the effects of anti-CD3 and PHA on cell proliferation and IFN release.

7. Comment: In the discussion, Wilasrusmee [38] and Zvetkova [39] show that tea extract, not, EGCG affect murine lymphocyte proliferation and neopterin production. Please clarify them.

Response: Various studies have established that the most abundant catechin in green tea is EGCG, which represents around 50% of catechins found in green tea. Therefore, any effect of green tea extract is attributed to this catechin. These two statements are now added to the text on page 10, sentence 5 of the manuscript.

8. Comment: An article of insufficient interest to warrant publication in a scientific/medical journal

Response: We submit that this paper presents interesting data on the suppressive effects of EGCG and that it is worthy of consideration. EGCG has stimulated a great deal of research because of its anti-inflammatory and other properties; our demonstration of its immunosuppressive effects may point to future immunomodulatory potential. Further, two of the three reviewers do support publication of this manuscript in Biomed Central.

9. Comment: Needs some language corrections before being published.

Response: All three authors have reviewed the manuscript carefully, from the perspective of the language and we have made some improvements. We hope that the revised version will be found acceptable for publication.
Responses to Reviewers’ Comments
Reviewer: Muhammad T Sultan

We thank the reviewer for his time and for the very objective and useful comments. We tried to respond to each comment, and to do the required changes in the manuscript. We think now it is in a better shape, and the credit goes to him.

1. **Comment:** The introduction section should be revised significantly. It should not be review of literature and it should focus on the objectives of the research.

   **Response:** We thank the reviewer for his comment. With all due respect, we believe that the introduction was short and direct to the point. It summarized the important points cited in the literature, and which directly relate to the objectives of the research. The latter were clearly stated at the end of the Introduction.

2. **Comment:** The references like "Kang and colleagues" and "Kawai and co-workers" should be numbered according to the journal format.

   **Response:** Both references are now numbered according to the journal format.

3. **Comment:** The introduction should be in symmetry focusing on the importance of the topic as currently it looks that things are just placed without considering their relevance.

   **Response:** The authors did review the introduction, as advised by the reviewer. We find only some minor changes that need to be made. We hope such changes are now acceptable to the respected reviewer.

4. **Comment:** The section results needs revision and one paragraph headings should be avoided.

   **Response:** Similar to the Introduction, we ensured that the Results section was concise, and that it highlighted the important findings in our study.

5. **Comment:** The discussion section should be revised keeping in mind your results and their supportive references. The same problem like introduction that it should be not be merely review of literature. Discussion should be based on logical reasoning and etc.

   **Response:** The authors did review the introduction, as advised by the reviewer. We find only some minor changes that need to be made. We hope such changes are now acceptable to the respected reviewer.
6. **Comment:** The reference database should be checked again for clarity

*Response:* We have doubled checked the references listed in our manuscript against the guidelines stated by the journal. They all comply now with such guidelines.

7. **Comment:** An article of importance in its field

*Response:* We thank the reviewer for his comment.

8. **Comment:** Needs some language corrections before being published

*Response:* All three authors have reviewed the manuscript carefully, from the perspective of the language and we have made some improvements. We hope that the revised version will be found acceptable for publication.