Author’s response to reviews

Title: Iranian propolis efficiently inhibits growth of oral streptococci and cancer cell lines

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Author’s response to reviews:

Dear Dr. Liam Messin

Editor-in-Chief of BMC Complementary and Alternative Medicine

This file was prepared according to the reviewer’s comments on a manuscript entitled “Quercetin as a major compound of propolis efficiently inhibits growth of oral streptococci and block cancer cells proliferation, A431 and KB cell lines as a model” with Manuscript Number: BCAM-D-18-01321 submitted to BMC Complementary and Alternative Medicine. All reviewer's comments have applied in revised manuscript using a different font colour and here is given a list of responses to the comments. Please let me know about the final decision on this manuscript as soon as possible on your career.

Sincerely yours,

Dr. Hamid Reza Nouri,
**Statements with blue color font are applied in revised manuscript**

A list of responses to the reviewer # 1 comments

1. The language is really not acceptable for publication. The manuscript needs extensive revision (native speaker) regarding the language.

Response: In response to this comment, it should be noted that our manuscript was edited by a professional English writer and all of the grammatical and spelling errors that seemed in the submitted manuscript are amended and applied in the revised manuscript.

2. The title should be changed: The authors tested the total propolis extract. I would recommend the following title "Iranian propolis efficiently inhibits growth of oral streptococci and cancer cell lines"

Response: We strongly agree with the reviewer comment, therefore he title changed to "Iranian propolis efficiently inhibits growth of oral streptococci and cancer cell lines"

3. Add the aim of the study to the background.

Response: In response to this comment, it should be said that the aim of this study was added in the background section of the Abstract revised version of manuscript as mentioned below.

“The present study investigated the quantification of quercetin (Q) in Ardabil ethanol extract of propolis (AEEP), and then compared its anti-bacterial, anti-biofilm and cytotoxic effects on cancer and normal cell lines.”

4. The biofilm formation was tested (not killing of already formed biofilm). This has to emphasized in the abstract.

Response: We strongly agree with the reviewer comment, therefor that statement in abstract was replaced with “the biofilm formation was assessed through the crystal violet staining and MTT assays “.
5. The conclusions section has to be rewritten. The authors just repeated the results. What is the clinical relevance of the results?

Response: In response to this comment, we rewritten the conclusion section with relying on clinical relevance. “The results indicated that the synergistic impact of main components of AEEP was related to the inhibition of the cancer cell proliferation, cariogenic bacteria and oral biofilm formation. It may play a promising role in the complementary medicine and, it is suggested to be used as food additives.”

6. How did the authors identified the streptococcal isolates: more details!

Response: More details on streptococcal isolates was added to the bacterial strains in Methods section.” Identification of oral streptococci was performed according to standard methods, included colony morphology, gram positive cocci, record haemolytic reactions, catalase test, fermentation of different carbohydrates and finally confirmed with Microgen Strep ID”.

7. A part of the used methods needs an Ethics approval (page 7, lines 19-23). The authors need a retrospectively approval of the Ethics Committee of their university.

Response: Thank you for pointing this out. Since, we used of the stored fibroblast in our laboratory that was previously approved by the Ethics Committee of Babol University of Medical Science, so this statement was added in the method section and highlighted with yellow color. Permission of fibroblast isolation was mentioned in reference 27 in revised manuscript.

“The Ethics Committee of Babol University of Medical Science previously approved isolation of fibroblast [27].”

8. How can the difference of the effects on cancer cell line and fibroblasts be explained?

Response: It is quiet common with natural substances to show different effects in normal and cancerous cell lines (1, 2). The intracellular oxidant/antioxidant equilibrium could be differently affected by these substances resulting different growth/death responses. A well-known example for this phenomenon is oxidative stress induced by high doses of some agents which have intrinsic antioxidative properties! This dual effect has been shown in our recently published article in another cell line (MCF-7) and was mentioned in the discussion section. However, if two groups of cells (cancer cells and normal cells) lay beside each other, treatment of the cells helps to kill the cancer cells and stimulate proliferation of normal cells during the treatment. By this method may be the dead cancer cells can be faster replaced with the normal cells.
Minor points

1. Page 9, line 10: Streptococcus mutans in italic.

Response: Thank you for raising this point. In Page 9, line 10: Streptococcus mutans was italic.

2. Throughout the manuscript: Streptococcus mutans and not Streptococcus mutants

Response: In response to this comment, Streptococcus mutans were corrected in the manuscript.

A list of responses to the reviewer # 2 comments

1. The document complies with most all the parameters of an article, only missing include the titles of figures from 5 to 8, which even without title is understandable, but nevertheless must include the information or image.

Response: Thank you for this comment. We added the titles of figures and figure legends in the revised manuscript.

A list of responses to the reviewer # 3 comments

1. This is a flat study showing preliminary data but not the mechanism of such activity. The conclusion is based on only MTT assay. The authors should confirm such activities of AEEP and Q by using few more assays and explain their mechanism of action.

Response: Thank you for raising this point of view. In response to this comment, it should be say that many studies have shown that oxidative stress is a possible mechanism involved in apoptosis and necrosis induced by propolis. Polyphenols concentration determines the ROS-scavenging action or the occurrence of oxidative damage. Production of ROS plays a key role in the chemotherapeutic activity of anticancer drugs, resulting the activation of apoptotic caspases in cancer cells (3).

Accordingly, to evaluate AEEP mechanism of action, we have recently published data, showing intracellular ROS induction mechanism involved in the anticancer effects against, MCF-7 cell line using a flow cytometric method. We addressed to this mechanism in discussion section and our recently published data could be referred on the respected editor in chief request.
Reference:

