

Anti-cancer effects of aloe-emodin: A systematic review

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Anticarcinogenic effects of aloe-emodin: a systematic review

Journal of Clinical and Translational Research

Dear Dr. Lewis,

Reviewers and the editor have now commented on your paper. You will see that they are advising that you revise your manuscript. If you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, the reviewers' and editor's comments are appended below. The material provided by the editor can be downloaded via a secured link, which is valid for 7 days:

<https://filesender.surfnet.nl/?vid=26717b03-e24e-3cc8-0b20-000002f07d91>

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by Dec 16, 2016.

To submit a revision, go to <http://jctres.edmgr.com/> and log in as an Author. You will see a menu item call Submission Needing Revision. You will find your submission record there.

Yours sincerely

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Editor's comments:

Dear authors, thank you for submitting your work to JCTR. The paper is very well written and accessible to a broad readership. As you have seen in the reviewer comments relayed to you via our Editorial Manager system, you are requested to make minor modifications before we can proceed with publishing your work. Please provide a point-by-point response to the issues raised, either confirming that the changes were made or rebutting the critique where you deem fit. In addition to the points raised by the reviewers, I kindly ask you to also implement the following items. Please delete this section before submitting your revision.

1. Please continue working in this draft when preparing a revision. I have not only adapted all the figures, but also made in-text corrections. The track changes function was used to inform you of what has been changed. A secure link was provided to you in my decision email with which you may download the entire package, including this version of the paper + comments and the figures.
2. Introduction section: the phrasing “that enhances biochemical productivity for restoring optimal health called the Michaelis-Menten equation” is elusive, mainly due to the phrasing “enhances biochemical productivity.” Moreover, the MM equation is a suitable model to describe enzyme kinetics where a substrate or multiple substrates are converted to a product and a transporter that traffics a protein across the cell membrane. Indeed, one of the variables in this equation is substrate availability, which is also important in your argument. However, I question the validity of the MM model to explain the fact that concentrating phytochemicals potentially increases their availability and is therefore more practical (i.e., rather than eating 100 oranges you may as well ingest a 1000-mg ascorbic acid capsule). The MM model overcomplicates the principle, and I advise you to omit it.
3. To continue with the above, the MM model is a gross oversimplification of the often very complex pharmacokinetics and physiological processing of xenobiotics (i.e., the majority of food supplements). The breakdown of bioactive compounds may already start in the stomach, as is the case for e.g., lecithin that is enzymatically cleaved by phospholipases in gastric juice. Subsequently, the compounds must transgress the mucosal layer in the intestines, evade xenobiotic phase I-III metabolism in the enterocytes, and be transported apically to enter the circulation. If you for example look at the case of curcumin, the pharmacokinetics are a disaster [Pharmacol Rev 2013;66(1):222-307]. Despite the fact that healthy volunteers and patients were administered 12 g (!!!) per day orally, there were no traces of curcumin in plasma in some patients. In other words, the MM model (or its implied principles in your paper) is a gross oversimplification of the pharmacokinetics and physiology in living systems. There are too many factors that restrict the representability of the MM model with respect to food supplements. This section (2nd paragraph of the Introduction and Figure 1) therefore requires nuance or elimination from the text.
4. In light of the above, a section on the enteral and enterohepatic pharmacokinetics of aloe-emodin would greatly benefit the paper, if this information is available. If yes, then please place the section after the last paragraph (Tongue) before the Discussion.
5. Introduction: the phrasing “the innate mechanisms of defense and repair coded in the genes are augmented and hence more effective against infectious agents and other health threats such as cancer” warrants proper citations. In this respect, food supplements are approached antagonistically by the scientific community. This is (in part) unjustified, but nevertheless underscores the necessity to

corroborate every scientific statement with appropriate references to experimental studies that unequivocally prove what is being claimed in the text. Please ensure that all such statements throughout the text are properly referenced to increase the credence of your work, particularly among the more ‘skeptical’ scientists.

6. The authors are kindly requested to use the following notation system for proteins and genes: human or human cell-derived proteins: all caps, non-italicized; human or human cell-derived genes: all caps, italicized; non-human or non-human cell-derived proteins: first letter capitalized and the rest lower case, non-italicized; non-human or non-human cell-derived genes: first letter capitalized and the rest lower case, italicized. See for further information: https://en.wikipedia.org/wiki/Gene_nomenclature.

7. It would greatly benefit the paper if a figure was included summarizing the 50% lethal dose (LD50 value) per cell line, as was done in Figure 10 in [Pharmacol Rev 2013;66(1):222-307]. This gives the readers an idea of the relative cytotoxicity of aloe-emodin, particularly since very little information on aloe-emodin concentrations used in the described experiments was provided.

8. Please specify the species of the addressed cell lines, such as ...human hepatocellular carcinoma (HepG2) cells...

9. Please number the sections (1. Introduction) and subsections (2.1. Bladder) for better organization.

10. It would benefit the comprehensibility of the paper to readers who are no experts in molecular biology to explain the dysregulation of proteins or genes through biological consequences of that dysregulation. For example, aloe-emodin reduced the degree of PCNA staining, indicating a reduction in cell proliferation. At this point I only commented on the cervical cancer section, but please extend the comments to the other cancer subtypes.

11. Could you please provide a summary of the main results in Table 1, placed after the table before you go into specific cancer subtypes? This should be done from a helicopter view. For example: aloe-emodin exhibits anti-cancer properties through its capacity to cause cell cycle arrest by downmodulating various cyclins. Furthermore, the anti-cancer effects are manifested at the level of cell death induction, which occurs mainly through apoptosis. Etc. Also expand this section with the immunomodulatory properties of aloe-emodin.

12. As reviewer 2 pointed out, the table and text are redundant. Please leave Table 1 as is because its format is clear and elucidating. I'd rather have the text in the Results section modified to encompass more complementary explanation of the data in Table 1, as partly alluded to above.

13. A list of abbreviations would make the text more comprehensible. Please include.

14. If any in vivo data is available, it should be included because these data comprise a higher level of evidence on the utility of aloe-emodin to fight cancer.

15. In the list of limitations, you should include the fact that your review mainly addresses in vitro findings that may have limited translatability to the in vivo setting.

Reviewer #1:

Abstract section:

- 1) Background - clear and precise
- 2) Methods - clear and precise
- 3) Results - clear and precise
- 4) Conclusion - clear and precise

Introduction section:

Introduction section overall - clear, succinct and adequate content.

Methods section:

Results section:

Clear and properly constructed. Contain most of the important published results of aloe emodin.

Discussion and Conclusion section:

Valid and based on the results of the study

Reviewer #2:

MAJOR COMMENTS

The review titled: "Anticarcinogenic effects of aloe-emodin: a systematic review", very interestingly reports the multiple consistent effects of aloe-emodin. The authors, recognizing the importance of alternative treatments in finding an effective therapeutic remedy, performed a systematic search with PubMed. They assert that the results of this review suggests aloe-emodin as a potential therapeutic modality for oncology patients.

However, there are certain points that could be improved:

The Michaelis-Menten kinetics is represented by an equation that relates velocity of a reaction, catalyzed by a constant concentration of enzyme, to substrate concentration. Each enzyme has a K_m value that is optimal for the biological conditions in which it operates, therefore strictly dependent on the substrate and on its concentration.

Therefore concentrations of the aloe-emodin represent an important aspect in in vitro studies. The authors should discuss it and especially should investigate the correlation between dose and effects.

Table 1. Summary of effects of aloe-emodin in relation to cancer. The way the table is built looks like a redundancy of what written in the Result section. There is a risk of losing focus on the key results. Authors should simplify this table (in particular, results section), emphasizing the effects on analyzed pathways. I suggest to rewrite the table to make it more crisp: Results column should schematically show the scope (cell viability, mitochondrial membrane potential, apoptosis.....Wee1, cdc25c....) and the effects under treatment (decreased, induced, inhibited, no change.....) only.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

*****Authors response*****

2nd editorial decision

Date: 15-Apr-2017

Ref.: Ms. No. JCTRes-D-16-00030R1
Anticarcinogenic effects of aloe-emodin: a systematic review
Journal of Clinical and Translational Research

Dear authors,

The editor commented on your paper. You will see that he is advising that you revise your manuscript. If

you are prepared to undertake the work required, I would be pleased to reconsider my decision.

For your guidance, editor's comments are attached to this email. Please continue working in this draft.

If you decide to revise the work, please submit a list of changes or a rebuttal against each point which is being raised when you resubmit your work.

Your revision is due by May 15, 2017.

To submit a revision, go to <http://jctres.edmgr.com/> and log in as an Author. You will see a menu item called Submission Needing Revision. You will find your submission record there.

Yours sincerely,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Reviewers' comments:

Dear authors,

Rather than only instructing you what to do, I have taken the liberty to proofread the manuscript to eliminate all the errors and to guide you in further improving the paper. Please find the modified version attached, where the track changes function was used.

Please make sure that all my comments are addressed.

Thank you and kindest regards,

Michal.

There is additional documentation related to this decision letter. To access the file(s), please click the link below. You may also login to the system and click the 'View Attachments' link in the Action column.

*****Authors response*****

Dear Dr. Heger:

We thank you and the reviewers for the thorough evaluation of our manuscript. As per the requests, we have endeavored to modify our paper to improve its quality and suitability for publication. We have addressed the following comments:

General Comments:

1. Figures were reformatted as suggested.
2. Throughout the entire text, minor edits, changes in sentence structure, and grammatical errors were edited as suggested.

3. Once the final paper is prepared and spacing is certain, the table will need to be split so that the rows are not split by page breaks.
4. Figure 3, cell cycle regulation, was adjusted so that double-stranded DNA is not covered by the “DP E2F1” box.

Specific Comments:

5. *In section 3.3 on Colon Cancer, mRNA of which proteins was downregulated?*

We corrected the sentence and it now reads, “Aloe-emodin downregulated messenger RNA expression and promoter/ gelatinolytic activity of matrix metalloproteinase (MMP)-2/9 as well as decreased Ras Homologue gene family member B (RHOB) expression.”

6. *In section 3.5, a reduction in pH and increased ROS production are in fact pro-proliferative, which is in contrast to what the observed effects (i.e., reduced proliferation rate). Do you therefore agree with my modification that the results are currently elusive?*

With a short literature search to verify the effects of increased ROS on cell proliferation, a biphasic effect exists. With low levels of ROS, cell proliferation is increased, and high levels of ROS induce senescence, apoptosis, and necrosis. In light of this, the statement in our paper as originally written does not warrant the added text (Reference: Day, RM and Suzuki, YJ. Cell Proliferation, Reactive Oxygen and Cellular Glutathione. Dose Response. 2005; 3(3): 425–442. PMID: PMC2475947.)

7. *In the first paragraph of section 3.6, this section needs to be contextualized; in which cells, which compounds were assayed? Why is this study addressed separately from the other five studies mentioned below?*

The cell line and methods used to analyze the cell line are included with track changes. The paragraphs in this section are organized by the authors, and since Lee et al. performed a series of 5 studies they are discussed in the same paragraph, while the other studies are discussed in the paragraph before and after.

8. *Paragraph two of section 3.6, is it increased activity or levels of cytochrome c?*

The authors remark that the change is an increase in the abundance of cytochrome c.

9. *Paragraph two of section 3.6, rather than stating that changes occurred, please indicate what the changes were.*

Rather than list all of the isozymes, we report that they all decreased in varying degrees.

10. *At the end of paragraph two of section 3.6, you included two statements, both on anoikis and apoptosis. If both pathways were induced, please state so.*

Photoactivated aloe-emodin induced both apoptosis and anoikis in this study of H460 cells. This was changed in the opening sentence describing this 5th study.

11. *Paragraph 3 of 3.6, cell death of which cell line?*

This sentence was changed to reflect that the human lung non-small cell carcinoma (H460) cell line was being studied.

12. *Section 3.9, paragraph two, please verify that it describes aloe-emodin-induced cell death of U87 malignant glioma cells.*

This is correct as reported in the paper. Aloe-emodin did arrest the cells in S phase and induced apoptosis via loss of mitochondrial membrane potential.

13. *Section on ovarian cancer, you describe “expression”, of what? Protein? If yes, than please reformulate to read ‘protein expression.’*

Yes it is protein expression, and the sentence was changed accordingly.

14. *In the discussion, please confirm that the 5-FU and aloe-emodin resulted in a marked reduction in cytotoxicity.*

This was deleted. In the study they found that AE was “less cytotoxic to human noncancerous skin cells (pre-malignant keratinocytic HaCaT and fibroblast Hs68) than to non-melanoma cancer cells (epidermoid carcinoma A431 and head and neck squamous cell carcinoma SCC25).” However, 5-FU and AE combined were more cytotoxic than either alone, as observed in another study mentioned earlier. Therefore, reference 33 was cited above in the second sentence of this paragraph.

15. *End of paragraph six of the discussion, could you please include a statement about the lack of aloe-emodin testing in non-cancerous cell lines, i.e., cells that do not proliferate? I presume that you have searched for those as well and found non-to-few. This should be specified here as a shortcoming (of the research field, not your paper) because there is little information on effect selectivity.*

The following statement was included: “Finally, few studies to date have investigated the effects of aloe-emodin in noncancerous cell lines. This could be due to the already small number of studies on aloe-emodin along with the difficulty in studying cell growth or inhibition in typically slow-growing noncancerous cell lines.”

16. *Delete section of paragraph seven of the discussion because it addressed diet, and not necessarily the anti-cancer properties of aloe-emodin.*

This was deleted accordingly.

17. *Delete reference 43 as part of the aforementioned section of the discussion on diet.*

This was deleted accordingly.

Please let us know if you have any additional questions or clarifications, and we look forward to the next review of our paper.

3rd editorial decision

Date: 11-Jul-2017

Ref.: Ms. No. JCTRes-D-16-00030R3

Anti-cancer effects of aloe-emodin: a systematic review

Journal of Clinical and Translational Research

Dear authors,



I am pleased to inform you that your manuscript has been accepted for publication in the Journal of Clinical and Translational Research.

You will receive the proofs of your article shortly, which we kindly ask you to thoroughly review for any errors.

Thank you for submitting your work to JCTR.

Kindest regards,

Michal Heger
Editor-in-Chief
Journal of Clinical and Translational Research

Comments from the editors and reviewers:
