Author’s response to reviews

Title: Prevention of Acute Radiation-Induced Proctitis by Aloe vera: a Prospective Randomized, Double-blind, Placebo Controlled Clinical Trial in Pelvic Cancer Patients

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Dear Anne Menard,

Thank you for your letter and for the reviewers’ comments on our manuscript entitled “Prevention of Acute Radiation-Induced Proctitis by Aloe vera: A Prospective Randomized, Double-blind, Placebo Controlled Clinical Trial in Pelvic Cancer Patients”, BCAM-D-18-01407. All of these comments were very helpful for revising and improving our paper. We would like to express our great appreciation to you and the reviewers for the comments on our paper. We have studied these comments carefully and have made corresponding corrections. Our responses are given in a point-by-point manner below.

Kind regards,

Ebrahim Salehifar
Reviewers’ Comments:

Tommaso Cai (Reviewer 1)
The study seems interesting and well conducted. I have a comment about the sample size calculation. It is not clear what is the expected difference between the control and treatment group. This is an important parameter to take into account.
We modified according to reviewer comment (page 9, lines 12-17)

Ranjeet Dash, Ph.D. (Reviewer 2)
The authors evaluated the radioprotective effect of Aloe vera in Pelvic Cancer Patients. However, there is a major concern regarding the product used. 1. How was the formulation standardized with respect to the active chemical constituents? No analytical procedures have been described. 2. It looks like the formulation is prepared in lab which is not a GMP facility. It is not ethically accept to use a formulation prepared in a non-GMP certified lab for clinical studies.
Aloe vera powder was purchased from Giah salamat nasim faraz (Fars, Iran), a commercial company that produce lyophilized aloe vera powder. This company represents the related documents of the product for approval of authorization national office. Then, the application of the powder for human and cosmetic use is allowed. Besides, we received analysis documents (in Persian language) representing lack of presence of toxic substances and residues of pesticides which were all negative.
The main components of aloe vera powder are polysaccharides (pectins, cellulose, hemicellulose, glucomannan, acemannan and mannose derivatives) that has been previously identified in literature. Ref# A. Bozzi , C. Perrin, S. Austin, F. Arce Vera Quality and authenticity of commercial aloe vera gel powders. Food Chemistry 103 (2007) 22–30.
Hence, the topical Aloe vera 3% is not available in market, then aloe vera ointment was prepared in faculty of pharmacy by a Professor of Pharmaceutics. In preparation process, formulating the ointment was taken place under laminar flow hood to provide an aseptic environment and to ensure a sterile condition and also avoiding any microbial contamination. At the end of the formulation process, we rechecked microbial quality control based on GMP parameters. It is common to prepare topical medicine for clinical trial that has been firstly evaluated in human in pharmaceutical laboratory of pharmacy faculty, but it is necessary observe for GMP standards and to test for any microbial contamination before usage in human. All of formulation was supervised by Professor Akbari as a Pharmaceutics specialist.

Mohamed A. Imam, MD, Ph.D, FRCS (Tr and Orth) (Reviewer 3)
Prevention of Acute Radiation-Induced Proctitis by Aloe vera: a Prospective Randomized, Double-blind, Placebo Controlled Clinical Trial in Pelvic Cancer Patients. I think this is a well written and conducted study. The manuscript in the current format is suitable for publication.
The authors concluded that Aloe vera ointment was superior to placebo in prevention of ARP. It prevented the symptoms of radiation-induced proctitis, especially diarrhea, hemorrhage, and proctitis. Aloe vera also enhanced quality of life of patients without any significant adverse event. I believe this might change practice.

We really appreciate the reviewer’s comment.

Ferran Torres (Reviewer 4)

The merits of conducting a clinical trial in this scenario are recognized and this initiative is endorsed. However, there is a number of points which should be addressed/clarified before a positive recommendation for acceptance can be issued. Please, answer and discuss, as appropriate, the point-by-point review attached below.

Special Comments:

1. Comment on the exploratory nature of this study. While valuable information may be gathered from exploratory studies, they should be identified so. There are a number of issues that suggest that this trial should be classified so: the limited sample size, the number of endpoints implicated without a clear definition of their priority and the lack a single endpoint predefined and the number of time points without a predefinition of the priority of the assessment.

We modified according to reviewer comment (page 8, lines 3-10, page 9, lines 1-5)

2. In the study design, it is stated: "Patients were allocated to the study group according to Random Number Table.". It appears that investigators were not concealed on the treatment assignment and this would be a violation of the randomisation rule. However, in the Calculation of the number of patients and randomization subsection it is stated that "Patients, treatment team, and the investigator of clinical responses did not know the types of interventions. At the end of the study, the principal investigator decoded the numbers of consumed ointments and assigned each to the appropriate group correctly." Please discuss.

Both Aloe vera and placebo ointment were formulated and supplied by the Pharmaceutics Department of MAZUMS (Sari, Iran) and dispensed in identical tube containers labeled with the randomization code, study protocol directions, and storage conditions. The 6-digit randomization codes were given by principal investigator and only he was aware of two arms of the study. Patients, treatment team (Physician, nurses and radiotherapy department personnel), and the investigator of clinical responses (post-graduate student, Adeleh Sahebnasagh) did not know the types of interventions.

3. Main and Secondary outcomes. Unclear which is/are the main endpoint/s. The primary endpoint should be clearly defined in the endpoint section. Ideally, it should be the incidence of proctitis in both arms of treatment at the end of 6 weeks (please define proctitis). Which is in accordance with the sample size calculation. If the total score of the 4 components is the main outcome, please state so. If it was not predefined and it is a post-hoc, please define so. Also, define whether the total score is a sum or a mean (I assume mean) of the components. In case that a main outcome was not predefined, there might be multiplicity issues associated with the trial design and interpretation, please include a comment in discussion. All the secondary
Endpoints should be defined one by one in this section. Add also when, how and the measurement units of them. Please, arrange tables in concordance with the final review of the outcomes, so that there is a clear distinction on the endpoint priority. As suggested by the reviewer, the correction has been made (page 8, lines 3-10, page 9, lines 1-5).

4. Sample size issue. In the subsection on the calculation of the number of patients and randomization section it is stated: "Because of the high incidence of acute radiation proctitis symptoms up to 75% patients1,2, we calculated that for a power of 0.8, significance level of 0.05, and an allowance of 10% lost to follow-up rate, for detecting a decrease in ARP symptoms by one-half, 40 patients would be enough." First, this in not traceable, for binary outcomes it would be needed also the control rate to calculate the sample size. Secondly, the sample size is normally calculated for the primary endpoint, but this is unclear in this case, presentation of the results is based on the score (not in the incidence) and there is no justification on this point. As suggested by the reviewer, this section was revised (page 9, lines 12-17).

5. Inferential tests and descriptive results. There is an inconsistency between the inferential analysis with the Mann-Whitney non-parametric test and the reporting of "Mean± SD (Standard Deviation)". This is not understood. If the results do not follow a Gaussian distribution, mean and SD should be replaced by median [Percentile 25th - 75th]. We appreciate the comment by the reviewer and reanalyzed the data and revised the statistical analysis section and table (page 9, lines 17-23, page 10, lines 1-4, table 2, page 13).

6. Please describe the use of the logistic regression in the methods section. Please note that "hazard ratios" are not the appropriate risk estimates from this analysis (see section Effect of Aloe vera rectal ointment on symptoms). Have you used an ordinal logistic regression analysis? Otherwise, if you have dichotomised the symptoms score please state and describe it in the methods section. We appreciate the comment by the reviewer. The "hazard ratios" part was removed from the manuscript.

7. Handling of missing data is not reported. We have followed reviewer’s comment; the aforementioned part has been added to the manuscript (page 7, lines 20-22).

8. The results of the primary endpoint should be clearly identified. The incidence of proctitis for every arm should be reported in a prominent position of the results with the 95%. We have followed reviewer’s comment; it has been revised in the manuscript (table 2, page 13).

9. Table 1. P-values for baseline characteristics in a randomised setting are inappropriate. Please delete them. As suggested by the reviewer, p-values were deleted in table 1.

10. Tables 2-3 and figures 2-3 should be re-assessed.
First, they should be unified because of clear redundancies. Sample sizes per arm and time should be reported somehow; if there were not missing data this could be addressed by a footnote. P-values are not understood, do they refer to the last time measurement (supposed to be the main time-point?, although unclear).

I suggest to use a unique results table including the incidence, the baseline and the last measurement timepoint for clinical presentation and symptoms, with the p-value for week 6 between arm comparisons. The odds ratio should be included in the same table. Figures may be maintained showing the complete time evolution but please priorities the order according to the discussion on the endpoint priority and include variability in the plots. If 95%CI are too wide (given the small sample sizes) include then the standard error of mean.

Please include appropriate figure legends and footnotes so that they are sufficiently explicit. The same for all tables, please include everything that is not direct interpretation in footnotes, for instance the use of Mean±SD (or median [Percentile 25th - 75th]) or abbreviations.

We thank the reviewer for mentioning this comment. The figures and tables were rearranged and revised in the manuscript in pages 11-13.

11. Results CRP are only shown partially, with only a p-value: "Quantitative measurement of C-reactive protein (CRP) showed no significant difference between groups (p=0.33), but still favored Aloe group."

Please report descriptive results for this variable, for instance mean±SD per group or median [Percentile 25th - 75th].

We thank the reviewer for mentioning this comment. The values for CRP were added to result section in table 2, page 13 and in result section page 15 lines 5-6.

Chief-editor:

1. In accordance with BioMed Central editorial policies (http://www.biomedcentral.com/submissions/editorial-policies#standards+of+reporting), could you please ensure your manuscript reporting adheres to CONSORT guidelines (http://www.consort-statement.org/) for reporting clinical trials. This is so your methodology can be fully evaluated and utilised. Please include a statement within your manuscript to indicate that your study adheres to CONSORT guidelines and include a completed CONSORT checklist as an additional file when submitting your revised manuscript.

Please complete the checklist in full by inserting the page number/paragraph and section of your manuscript which reports the information that meets the criteria of the checklist. For example “Methods, paragraph 2”. If a criterion is not applicable for your particular manuscript/study, we can accept “N/A”.

Please note that checklists completed incorrectly will be returned for revision as we cannot progress your manuscript to peer review until the checklist has been completed.

We have followed chief editor’s comment; the aforementioned line was added to the manuscript in page 6 line 7 and CONSORT checklist was attached as required by the chief editor.
2. Please have the text edited by a professional language editing service (please see the suggested services under my signature). There are many issues with grammar, wording, spelling, and/or punctuation that will prevent effective peer review of the current version. After revising our manuscript to address the reviewers' comments, we have had it rechecked by a Brazilian writer and poet with many English publication. As a consequence, the grammatical and stylistic edits have been made throughout the text. We hope that this revised manuscript meets your expectations. The certificate of English editing is attached below.

3. At present, we do not feel that there is sufficient evidence presented in your Background section to justify the testing of Aloe vera specifically in patients to prevent Acute Radiation-Induced Proctitis. We would therefore ask you to expand this section to include as much referenced evidence as possible to explain why you would expect this treatment to have an effect in this model. This evidence should come from previous in vitro or animal work. Please note that we are unable to accept traditional medical use as sufficient justification for any studies.

   We have appreciated the comment by the chief editor. More evidence was added in Background Section in page 4, lines 21-22, and page 5, lines 9-12.

4. Please clearly state where all materials were purchased from in the Methods section.

   We have appreciated this comment by the chief editor. This section was added to the method section in page 6, lines 15-18.

5. As Seyed Jalal Hosseinimehr is a member of the editorial board (Associate Editor) of this journal, in order to ensure transparency, please declare this in the Competing Interests section of the Declarations.

   As required by the chief editor, we declare this in the Competing Interests section in page 18, lines 17-19.

6. Please remove the funding information from the Acknowledgements and include it in the Funding section instead.

   We have appreciated the comment by the chief editor. We corrected this part in pages 18 and 19.

7. The Availability of data and materials section refers to the raw data used in your study and presenting tables and figures is not sufficient to state that all data is contained within the manuscript and additional files. Please only use this statement if you have indeed provided all raw data on which your study is based. We strongly encourage all authors to share their raw data, either by providing it in a supplementary file or depositing it in a public repository and providing the details on how to access it in this section. If you do not wish to share your data, please clearly state this in this section along with a justification. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):

   - The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]
   - The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.
• All data generated or analysed during this study are included in this published article [and its supplementary information files].
• The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.
• The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

Please note that if you do wish to share your raw data and do not have consent from all patients to publish this data it will need to be de-identified.

Please also note that if you include your raw data as a supplementary file you will need to provide, after the References, a section titled “Additional files” where you list the following information about each of your supplementary files: * File name (e.g. Additional file 1), * Title of data, * Description of data. All additional files will also need to have been cited in the main manuscript.

We have appreciated the comment by the reviewer. We revised this section on page 18, lines 13-16.

8. At this stage, we ask that you submit a clean version of your manuscript and do not include track changes or highlighting.

Thanks you.

9. Please consider the list of authors as it currently stands with reference to our guidelines regarding qualification for authorship (http://www.biomedcentral.com/submissions/editorial-policies#authorship).

Currently, the contributions of all authors do not automatically qualify them for authorship. In the section “Authors’ contributions”, please provide further clarifications on their contributions, and see our guidelines for authorship below.

An 'author' is generally considered to be someone who has made substantive intellectual contributions to a published study. Authors are expected to fulfil the criteria below (adapted from McNutt et al., Proceedings of the National Academy of Sciences, Feb 2018, 201715374; DOI: 10.1073/pnas.1715374115; licensed under CC BY 4.0):

Each author is expected to have made substantial contributions to the conception OR design of the work; OR the acquisition, analysis, OR interpretation of data; OR the creation of new software used in the work; OR have drafted the work or substantively revised it
AND to have approved the submitted version (and any substantially modified version that involves the author's contribution to the study);
AND to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Acquisition of funding, collection of data or general supervision of the research group, alone, does not usually justify authorship.
If these guidelines are not met, we would request the following change of authorship form be filled out and sent to our editorial office - https://resource-cms.springernature.com/springer-cms/rest/v1/content/7454878/data/v5
Anyone who contributed towards the article who does not meet the criteria for authorship can be acknowledged in the ‘Acknowledgements’ section.
As mentioned by the reviewer, the role of the authors was explained in detail as mentioned in page 19, line 3-13

10. There are currently two sets of tables in your manuscript, please remove one set to avoid duplication.
We appreciate editor in chief comment and corrected as required.