Author's response to reviews

Title: Evaluation of wound Healing and anti-inflammatory activity of the rhizomes of Rumex abyssinicus J. (Polygonaceae) in Mice

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Author's response to reviews: see over
Dear Editor,

Thank you for giving us an opportunity to revise our manuscript. We also thank the reviewers for their constructive criticisms. We have looked at the comments forwarded and provided a point-by-point response as described below. We hope that the MS is now in a good shape after an extensive revision and is suitable for publication in your esteemed journal.

Kind regards
Ephrem Engidawork (PhD)

RESPONSE TO REVIEWERS

1. REVIEWER ONE (Andrea Aro)

Major Compulsory Revisions:

Comment: The purpose of the study was to evaluate the potential activity of the Rhizomes of *Rumex abyssinicus* J. in wound healing and in inflammation. Considering that the subject of the manuscript is very interesting, I believe that additional analyses should be included to become the manuscript more consistent and appropriate for publication.

Response: Efficacy of traditional medicinal plants for wound healing is evaluated using a host of parameters, including, among others, wound contraction, epithelization, tensile strength and hydroxyproline measurement. We have determined these parameters using two well established models (excision and incision). In addition, we also determined the anti-inflammatory activity of the plant in support of the plant’s wound healing activity. Thus, we are of the opinion that necessary parameters to meet our objective have been determined.

Abstract section:

1) The tissue that was analyzed was not cited in the abstract. Please, include this information in this section and in the title of the manuscript.

- Accommodated

2) The analyses that were used for the characterization of the healing activity of the plant in vivo were not described in this section. This information with the respective result could become the data more interesting if it was included.

- Accommodated
Introduction section:
1) Line 61: correct the word “coomon” for “common”
   • Corrected
2) Line 67: the correct form is “at least”
   • Corrected
3) A description of the main molecular events of the wound healing in skin should be included, such as the collagen synthesis, pro-inflammatory cytokines participation and biomechanical aspects of the tissue during this process. These information could clarify the choice of the techniques used for the evaluation of the healing potential of the plant and facilitating the data discussion.
   • Included

Material and Methods
1) Why animals of either sex were used? I believe that the hormonal variation of the female can interfere in the result, especially when it is compared with the result from male animals.
   • Of course there may be influence of hormones but the animals were first wounded and randomly assigned to different groups to avoid such influence
2) Please, describe the composition of the base of the ointment.
   • A table provided (Table 1)

Results section:
1) Please, include the time of wound healing process that was analyzed in each Table.
   • Time included in each Table as appropriate.

Discussion section:
1) (Line 324): It is very speculative to attribute the role of *R. abyssinicus* in the increasing in percentage closure of excision wounds as a result of the induction of macrophage cell proliferation, since no test was done for this. Various other components of the extracellular matrix have to be produced to close the excision wounds that should be considered in the discussion of this result.
   • This section is elaborated taking into account other factors that could contribute for the observed phenomena (see Line 290-301 and 317-325). Macrophage cell proliferation effect of the plant was reported from our institute by Getie et al (see reference 21).
2) (Line 304): Likewise, it is not possible to suggest that the plant has the ability to facilitate the proliferation of epithelial cells, enhancing wound contraction by enhanced epithelial migration. Histological analysis should be done for evaluating this result.

- Wound contraction facilitates epithelization and the extract is shown to increase contraction. Epithelization in turn is facilitated by proliferation and migration of epithelial cells around the wound bed. The extract has decreased epithelization period. Obviously, this effect could be due the extract’s ability to increase proliferation or viability of epithelial cells. We have reframed the discussion in such a way. Issues suggested by the reviewer can be taken up in the follow up mechanistic studies on the plant. This study was done to see whether the plant has an activity or not.

3) (Line 314) “by removing unwanted things”…what does it mean?

- Deleted and modified… see line 332-334

4) (Line 359) “the extract could be the result of its ability to inhibit the action of bradykinin and/or prostaglandins” …again is very speculative.

- It is well established that inflammation mediated by carrageenan has three phases and the mediators for each phase have been delineated. We do not think linking the effect observed based on the timing is pure speculation.

5) (Line 366) “The probable explanation for increased tensile strength could be due to the increase in both remodeling of collagen, and the formation of stable intra- and intermolecular crosslink”…why a simple staining with HE or some more appropriate dye for the observation of collagen organization (Ponceau S, ponceau SS, xylidine ponceau) was not done? A simple analysis under light microscopy could be done to prove the result, improving largely the data discussion.

- We do not disagree with the referee about doing such a work could improve the discussion. However, each study has its own objective and we did use methods that help us achieve our objective. We have included a phrase “it remains to be seen how the extract produced such effect but could involve such and such mechanisms (see line 298-301).

2. REVIEWER TWO (Priyanga Ranasinghe)
Minor Revisions - The language in the manuscript requires significant improvement. Numerous typographical and grammatical errors are present throughout the manuscript. Suggest to get the manuscript corrected by an English speaking person/expert if possible

- The manuscript has been extensively edited and we hope now it is in a good shape.

3. REVIEWER THREE (Mary T. "Terry" Loghmani)

Major Compulsory Revisions

Some major compulsory revisions will need to be made to determine the appropriateness of this manuscript for publication. It needs to be re-written with grammar and spelling errors corrected to make it easier/possible for the reader to follow.

- We overhauled it if that term is correct to use it for wa writing.
- Title should somehow reflect the objective of the study. There are several findings in this study and all them reflect the wound healing and/or anti-inflammatory activities of the plants. Hence, we prefer to keep the title with some rewording.
- The purpose and design needs to be more clearly described to make the research procedures and related findings more decipherable.

- Rephrased and corrected

The type of wound (s) under consideration needs to be specified clearly. The types of treatments and their rationale for selection were not clearly described. Of significant concern, is that the research groups need to be clearly defined, and better rationales provided for the difference in the types of animals for the different experiments and the methods/rationale for the outcomes selected. A chart listing the groups may help with this. Presenting the data as graphs with significance differences between groups would be helpful to more quickly interpret the findings. This needs to be tied to the discussion. Limitations need to be listed.

- The style of writing has been modified so that the manuscript could have consistency and coherence.
- There are reasons why you select certain animals for a particular study. For e.g., rabbits are more sensitive for skin irritation (hypersensitivity reactions) and rats
are preferred for dermal toxicity over mice, as the effect is better observed probably
due to their size. These are known by the scientific community working in this area.

Overall, there needs to be greater clarity, consistency and coherence between all elements of the
manuscript in order for its scientific merit to be better communicated and evaluated.

I could not find several of the references, either because my library does not have access to
some/many of the journals or the references were from a book, or of significant concern, is that
some of the articles simply could not be found on PubMed as cited.

- **All journals are not cited in the Pubmed, as you are aware. As much as possible, we
  have now included journals that are indexed in the said database.**

Minor Essential Revisions

Figures of the wound healing between groups would be very helpful and interesting. I could not
fully discern if the authors were building on some of their own, previous work. In conclusion,
since traditional plant-based medicine could have significant global health implications, and their
efforts/interest in this area are commendable, I would encourage the authors to revise and re-
submit.

- **Unfortunately, our camera used to take all he pictures was out of order and we tried
to provide pictures taken with the mobile of one of the investigators.**