Author’s response to reviews

Title: Mapping the evidence of hepatoprotective properties of Moringa oleifera from sub-Saharan African countries: a systematic review protocol

Authors:

Tambwe willy Muzumbukilwa (tambwemuzu@yahoo.fr)

Mukanda Kadima (gedeonkadima@gmail.com)

Manimbulu Nlooto (Nlooto@ukzn.ac.za)

Peter Owira (owirap@ukzn.ac.za)

Version: 1 Date: 28 May 2019

Author’s response to reviews:

AUTHORS RESPONSES TO REVIEWERS COMMENTS/ BMC Systematic reviews

REVIEWER#1:

Comment 1: This is a good protocol for medical treatment in recent years. But the evaluation of liver enzymes only not sufficient. You must evaluate the oxidative cascade in serum. It includes the natural antioxidant and the free radical formation. The natural antioxidant includes 1-super oxide dismutase (SOD) 2- catalase (CAT) and 3- glutathione reductase (GR). The free radical formation represented by malondialdehyde (MDA). This for a full evaluation of the effect of moringa on the liver tissue.

Response 1: Thank you for the comment. The suggestion made by the reviewer has been accepted and incorporated in the text as follows: “In vivo studies will include those in which the activity of the serum levels of hepatic enzymes Alanine Transaminase (ALT) or serum glutamate-pyruvate transaminase (SGPT), Aspartate Transaminase (AST) or Serum Glutamic oxaloacetic transaminase (SGOT), Gama-Glutamyltransferase (GGT), Alkaline phosphatase (ALP). In addition, studies measuring as primary outcomes the oxidative cascade in serum including the natural antioxidants [superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR)] and the free radical formation [malondialdehyde (MDA)] will be included. The secondary outcome will consist of studies in which the levels of hepatic enzymes activities of ALT, AST, GGT or ALP, SOD, CAT, GR, and MDA in serum after administration of sub-Saharan African Moringa oleifera were measured for a full evaluation of the effect of Moringa on the liver tissue. Refer to page 2 of the clean version as well as the version with highlighted sections.

The following research question has been improved and read as follows: “Does the administration of substances inducing liver damage increase the levels of hepatic enzyme
activities (ALT, AST, GGT or ALP, SOD, CAT, and GR), and MDA. Refer to page 4 of the clean version as well as the version with highlighted sections.

And its objective read as follows: “To establish whether substances inducing liver damage increase the levels of hepatic enzyme activities (ALT, AST, GGT or ALP, SOD, CAT, GR, and MDA).” Refer to page 5 of the clean version as well as the version with highlighted sections.

The secondary outcome has been improved and read as follows:” The secondary outcome will consist of studies in which the levels of hepatic enzymes activities of ALT, AST, GGT or ALP, SOD, CAT, GR, and MDA in serum after administration of sub-Saharan African Moringa oleifera were measured. “Refer to page 6 of the clean version as well as the version with highlighted sections.

REVIEWER#2: No comments.

REVIEWER#3:

Comment 1: This protocol seems to target an interesting research question and there is no systematic review in the topic. I think the findings of this review may help designing the first human trial or avoiding it! So I encourage the authors to improve their protocol based on the following suggestions.

1. GENERAL

1.1. The paper should be edited by a native editor.

Response 1: Thank you for the comment. The editing services have been done by a native English, Ms. Carrin Martin.

Comment 1: 1.2. In the cover letter, the authors consider Egypt as a Sub-Saharan country. I do not think it is correct.

Response 1: Thank you for the comment. This was a mistake in the cover letter; thus, Egypt has been removed in the Sub-Saharan countries’ list. Refer to the additional file 3, table 1 “search strategy in PubMed.”

Comment 2: 1.3. I recommend the authors to study and follow the guidelines introduced by CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies) http://www.dcn.ed.ac.uk/camarades/ for any systematic review including studies on animals.
Response 2: Thank you for the comment. The guidelines introduced by CAMARADES (Collaborative Approach to Meta-Analysis and Review of Animal Data from Experimental Studies) will be followed when the screening data stage of this systematic review will be undertaken. Refer to page 9 under Assessment of risk of bias section of the clean version and highlighted on the track changes version.

2. ABSTRACT

Comment 3: 2.1. Methods in abstract copied into the system is incomplete and un-informative. It should at least include some info on databases, eligibility criteria, quality assessment, and final analysis/synthesis.

Response 3: Thank you for the comment. Information about databases, eligibility criteria, quality assessment, and final analysis/synthesis have been added in an abstract and read as follows:

“In vivo studies will include those in which the activity of the serum levels of hepatic enzymes Alanine Transaminase (ALT) or serum glutamate-pyruvate transaminase (SGPT), Aspartate Transaminase (AST) or Serum Glutamic oxaloacetic transaminase (SGOT), Gama-Glutamyltransferase (GGT), Alkaline phosphatase (ALP). In addition, studies measuring as primary outcomes the oxidative cascade in serum including the natural antioxidants [superoxide dismutase (SOD), catalase (CAT) and glutathione reductase (GR)] and the free radical formation [malondialdehyde (MDA)] will be included. The secondary outcome will consist of studies in which the levels of hepatic enzymes activities of ALT, AST, GGT or ALP, SOD, CAT, GR, and MDA in serum after administration of sub-Saharan African Moringa oleifera were measured for a full evaluation of the effect of Moringa on the liver tissue.

Search engines will include MEDLINE, CINAHL, PubMed, Google Scholar, SABINET and WHO/ African Index Medicus. The screened results will be grouped according to any noteworthy grouping variable, such as study characteristics.

Data will be analyzed using Stata statistical software (Stata Corp V.14, Texas, USA).

Study data will be quantitatively synthesized by first assessing heterogeneity to examine whether the estimates from included studies could be pooled. Heterogeneity will be assessed by the Chi-squared test on Cochran’s Q statistic, which will be quantified by I-squared values. Refer to page 2 under the Abstract section of the clean version and highlighted on the track changes version.

Comment 4: 2.2. Discussion is missing from the abstract copied into the system. Last sentence of the cover letter is suitable to be used is discussion in abstract.

Response 4: Thank you for the comment. The discussion has been added in the abstract copied into the system. Results from this protocol will give new insights into the Moringa oleifera plant for developing effective hepatoprotective drugs against liver damage. Refer to page 2 under the Abstract section of the clean version and highlighted on the track changes version.
Comment 5: 2.3. Abstract in the system is not matching the abstract in the paper. They should be exactly the same.

Response 5: Thank you for the comment. The abstract in the system has been matched with the abstract in the paper.

Abstract

Background: One of the most challenging health problems is liver disease, which can be caused by medications, toxic substances, and excessive consumption of alcohol. Liver problems can also be caused by infections, autoimmune disorders, and food. This study aims to establish evidence of the use of Moringa oleifera sub-Saharan African countries to manage liver damage, conditions in animals.

Methods: In vivo studies will include those in which the activity of the serum levels of hepatic enzymes Alanine Transaminase (ALT) or serum glutamate-pyruvate transaminase (SGPT), Aspartate Transaminase (AST) or Serum Glutamic oxaloacetic transaminase (SGOT), Gama-Glutamyltransferase (GGT), Alkaline phosphatase (ALP), superoxide dismutase (SOD), catalase (CAT), glutathione reductase (GR), and malondialdehyde (MDA) were measured after administering substances that induced liver injury as a primary outcome.

The secondary outcome will include studies that measure the serum levels of hepatic enzymes (ALT, AST, GGT and ALP, SOD, CAT, and GR); and free radical formation represented by MDA after administering the sub-Saharan Moringa oleifera, decreases in their levels indicating the improvement of their activity. Search engines will include MEDLINE, CINAHL, PubMed, Google Scholar, SABINET, WHO/African Index Medicus. The screened results will be grouped according to any noteworthy grouping variable, such as study characteristics.

Data will be analyzed using Stata statistical software (Stata Corp V.14, Texas, USA).

Study data will be quantitatively synthesized by first assessing heterogeneity to examine whether the estimates from included studies could be pooled. Heterogeneity will be assessed by the Chi-squared test on Cochran’s Q statistic, which will be quantified by I-squared values.

Discussion: Results from this protocol will give new insights into the Moringa oleifera plant for developing effective hepatoprotective drugs against liver damage.

Systematic review registration: CRD42018084698. Refer to page 2 under the Abstract section of the clean version and highlighted on the track changes version.

3. METHODS
Comment 6: 3.1. Exclusion of studies based on language is not acceptable. It is easy to find a
volunteer to check the eligibility and/or extract data via posting a task in
taskexchange.cochrane.org which is open to all systematic reviewers.

Response 6: Thank you for the comment. Exclusion of studies based on language has been
corrected. The exclusion criteria read as follows:

- Articles on human or animal study other than rats and mice.
- All articles without available full text.
- Articles not addressing the hepatoprotective activity of Moringa oleifera.
- Articles using co-administration with another plant.
- Articles using Moringa oleifera not harvested in sub-Saharan Africa.
- Case-control group of treatment with another plant than Moringa oleifera. Refer to page 6
  of the clean version as well as the version with highlighted sections.

Comment 7: 3.2. The authors need to consult a search expert librarian or information specialist in
their institute about the choice of databases, how to search them and how to manage the search
results. For example, I wonder why the authors are not searching for any African database or
Africa-focused WHO databases as the most relevant resources to the topic.

Response 7: The authors have consulted an expert librarian specialist in health sciences at the
University of KwaZulu-Natal with regards to the choice of databases. She has provided relevant
information on the inclusion of grey literature on this topic. African database such as SABINET,
and WHO/African index Medicus will be used as the most relevant resources to the topic. Refer
to page 7 under Information sources and search strategy for identifying relevant studies section
of the clean version and highlighted on the track changes version.

Comment 8: 3.3. The reason for limiting the research to sub-Saharan African countries is not
known. I suggest the authors to remove this geographical limitation. There might be valuable
studies from the other countries as the plant is available in India, Pakistan, and Bangladesh. This
also may provide insights about the trend of research in other countries and/or differences among
species of this plant.

Response 8: Thank you for the suggestion. This study is targeting the Moringa oleifera growing
and harvesting in sub-Saharan Africa. The study on Moringa worldwide could be a future study.
Comment 9: 4. REGISTRATION: I see from the PROSPERO that authors are now in screening step. They might need to reconsider parts of the protocol before proceeding. This includes searching for African resources.

Response 9: Thank you for the comment and suggestion made in the inclusion of African resources. In this systematic review research, data extraction has not yet started.

Comment 10: 5. KEYWORDS: Hepatotoxicity, hepatoprotective, liver disease or any other relevant term should be among the keywords.

Response 10: Thank you for the comment, a relevant term related to this research have been added among the keywords. Keywords: Moringa oleifera, alanine aminotransferase, aspartate aminotransferase, Gamma-glutamyltransferase, alkaline phosphatase, superoxide dismutase, catalase, glutathione reductase, malondialdehyde, hepatoprotective, sub-Saharan Africa countries. Refer to page 2 of the clean version as well as the version with highlighted sections.

Comment 11: 6. BACKGROUND: While mentioning the clinical properties it is much better if you say in RAT or in ANIMALS or etc. i.e. "activities [in rats/animals] [9]" rather than "activities [9]". Same applies for 'leakages IN RATS [15].

Response 11: Thank you for the comment. It has been corrected. Diuretic and hepatoprotective activities in rats [9]. The leaf extracts reportedly reduce serum intracellular enzymes levels by stabilizing the cell membrane, which prevents enzymes from leakages in rats [15]. Refer to page 4 under the background section of the clean version as well as the version with highlighted sections.

Comment 12: 7. RESEARCH QUESTIONS: I do not think the question 1 is directly relevant to this review. I understand that the authors are interested in knowing the answer to this question however the answer to this question may require a separate systematic in the review in which the authors focus on finding all the studies on substance-induced liver damage not limited to Moringa oleifera. Apparently, substance-induced liver damage can be the subject of many studies, not just the ones that intervene with Moringa oleifera.

Response 12: Thank you for the comment. This review will be looking only at papers where Moringa oleifera would be administered to rat and/or mice for treating liver damage induced by a certain substance; furthermore, question 1 will be related to the intervention and to the primary outcome.

8. EXCLUSION CRITERIA
Comment 13: 8.1. The reason for including rat and mice and excluding human and other animals is not clear. Since the number of comparative studies with two groups is limited enough I suggest the authors now to limit the review to rat and mice unless they provide the rationale.

Response 13: Thank you for the comment. Most of the research testing the hepatoprotective of Moringa oleifera, researchers used rats and mice. On the authors’ point of view, to include human and other animals in this study could not bring any change in the expected results.

Comment 14: 8.2. There is no rationale in excluding Letters, reviews, and comments as they all can have reports of comparative studies.

Response 14: Thank you for the comment. The Letters, reviews, reports, and comments have been included in this study as requested by the reviewer. The new list of inclusion criteria is read as follows:

• Research published from 2009 to 2019.
• In vivo research on rats and mice treated with sub-Saharan African Moringa oleifera to manage the liver damage.
• Where AST, ALT, GGT, ALP, SOD, CAT, GR, and MDA are measured in the animals’ serum.
• Articles, letters, reviews, report, and comments will be included in this study.

(Refer to page 6 under the inclusion criteria section of the clean version as well as the version with highlighted sections).

Comment 15: 8.3. Excluding studies where the full text is not available has no reason. It is usually possible to contact the authors and request a copy of their paper or ask the library to order it.

Response 15: Thank you for the comment. The authors have taken the suggestion of contacting the authors’ paper or the library whenever the full text of the needed paper will not be available and the criteria of excluding studies where the full text will not be available have been removed. The new statement is read as follows: “When the full text is not available, we will contact authors to request a copy of the paper or we will ask the library to order it” (Refer to page 8 under Selection of studies section of the clean version as well as the version with highlighted sections).

Comment 16: 8.4. Again, the authors should provide the reasons for excluding a study where 'Moringa oleifera not harvested in sub-Saharan Africa'. Also, it should be 'harvested'.

(Refer to page 7 under the exclusion criteria section of the clean version as well as the version with highlighted sections).
Response 16: Thank you for the comment. The choice of “Moringa oleifera” harvested only in sub-Saharan Africa was made following ecological reason such as the difference of soil, weather and most focused in sub-Sahara African plants. In addition, the word “harversted” has been corrected and replaced to “harvested” (Refer to page 6 under exclusion criteria section of the clean version as well as the version with highlighted sections).

Comment 17: 9. SEARCH DATE: Based on Systematic Reviews journal's guideline the search should not be more than 14 months old and at the time that you are receiving this review and making the revisions you should run an update search.

Response 17: Thank you for the comment. An update search has been made following the systematic reviews journal’s guideline and read in the following statement as “Research published from 2009 to 2019” (Refer to page 6 under inclusion criteria section of the clean version as well as the version with highlighted sections).

Comment 18: 10. COUNTRIES: The authors refer to '54 sub-Saharan African countries'. To the best of my knowledge, there are 54 countries in Africa and only 46 of them are Sub-Saharan.

Response 18: Thank you for the comment. This was a mistake from authors. The authors agreed that there are 54 countries in Africa and only 46 of them are Sub-Saharan. The new statement is now read as follows “Individual country names for the 46 sub-Saharan African countries will also be used as additional key search terms for more abstracts on the subject” (Refer to page 7 under Information sources and search strategy for identifying relevant studies section of the clean version as well as the version with highlighted sections).

Comment 19: 11. DATA ANALYSIS: It is 'Cochran's Q statistic' not 'Cochrane's Q statistic'

Response 19: Thank you for the comment. This has been corrected accordingly (Refer to page 8 under Data analysis including assessment of heterogeneity section of the clean version as well as the version with highlighted sections).

Comment 20: 12. RISK OF BIAS: I wonder why the authors mention Cochrane's risk of bias tool instead of SYRCLE's risk of bias tool. This paper lists 30 tools (https://doi.org/10.1289/ehp.1206389) but it is published in 2013. Please check the recommendations by CAMARADES as well. They may have a better tool.

Response 20: Thank you for the comment. The authors have replaced SYRCLE’s risk of bias tool that will be used in this study instead of Cochrane’s risk of bias mentioned previously. Furthermore, the Systematic Review Centre for Laboratory Animal Experimentation (SYRCLE) risk of bias tool will be used to assess publication bias, addressing assessments for random sequence generation, concealment of allocation, blinding, the outcome measurements, and completeness of outcome reporting. Each bias criterion will be assigned a value of the low, high,
or unclear risk of bias for each included study. Quality of studies will be assessed using the CAMARADES checklist (Refer to page 9 under assessment of risk bias section of the clean version as well as the version with highlighted sections).

Comment 21: 13. GRADE: You may refer and follow this paper for GRADE


Response 21: Thank you for the comment. The paper titled ‘The use of GRADE approach in systematic reviews of animals’ studies” will be followed. After going through the suggested paper GRADE, the new statement is read as follows “Quality rating of overall evidence will be downgraded according to the following factors: Risk of bias, indirectness, the inconsistency of result, imprecision, and publication bias [23]” (Refer to page 9 under Assessment of methodological and evidence qualities section of the clean version as well as the version with highlighted sections).

Comment 22: 14. DISCUSSION: Using MO in discussions seems unnecessary. Either you should use the full form all over the paper or if you want to abbreviate you should use the full form and abbreviation in Background for the first time and then use MO for the rest of the paper.

Response 22: Thank you for the comment. The abbreviation “MO” has been removed and replaced by the full form “Moringa oleifera” in the discussion section. Refer to page 9 under the discussion section of the clean version as well as the version with highlighted sections.