Author's response to reviews

Title: Evaluation of antimalarial activity of leaves of Acokanthera schimperi and Croton macrostachyus against Plasmodium berghei in Swiss albino mice

Authors:

Tigist Mohammed (tgdeg43@yahoo.com)
Berhanu Erko (berhanue@yahoo.com)
Mirutse Giday (mirutseg@yahoo.com)

Version: 3  Date: 24 May 2014

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

Thank you for sending us comments of three reviewers on our manuscript entitled ‘Evaluation of antimalarial activity of leaves of Acokanthera schimperi and Croton macrostachyus against Plasmodium berghei in Swiss albino mice’ (MS: 3660756141193311). We have gone through the paper and made the necessary revision. Please, take note of the corrections and modifications or explanations in responses to the comments of the reviewers as shown below. Responses to the reviewer are given in bold letters.

Reviewer: Fabrice Boyom
- Do not italicize “in Swiss” in the title.
Corrected

Abstract
1) Results section
- Given that parasitaemia was suppressed in dose dependent manner at all dose levels, authors should delete the sentence ‘The highest suppression was observed at the higher dose tested (600mg/kg).’
Statement has been deleted.

2) Conclusion
The conclusion is not consistent, please work on this.
Some modification has been made on it.

Background
- 1st paragraph
- Delete “in sub-Saharan Africa” in the first sentence.
Phrase deleted as recommended.

- 2nd paragraph
I suggest that the following two sentences be formulated into one: “Ethiopia is also one of the most malaria epidemic-prone countries in Africa. Rates of morbidity and mortality increase 3-5 folds during epidemics [5].” For example: “Ethiopia is also one of the most malaria epidemic-prone countries in Africa where rates of morbidity and mortality increase 3-5 folds during epidemics [5].”
Sentences combined as suggested.
Today, herbal products are being used worldwide in a variety of healthcare settings, and as a home remedies [8]. What do you mean by “and as a home remedies”?

These are remedies that are made at household level and are used to treat household members.

- 4th paragraph:

1) A study reported that methanol extracts of C. macrostachyus have shown substantial antimalarial [...] with IC 50 value of 0.94µgm/ml [14]. Give details about the plant parts used in the study.

The plant part used in the study is now indicated in the manuscript.

- Authors should also make mention of previous studies on related plant species [ex. Croton zambesicus- Boyom et al., J.Ethnopharmacol. 123, 483-488 (2009)].

This reference has now been included in the manuscript.

2) An in vivo study conducted “in Kenya” on Acokanthera oppositifolia, a taxonomically related species to A. schimperi, demonstrated an interesting antiplasmodial activity on P. falciparum [16]

- If you chose to provide such details, please do throughout!

We have now done it that way.

- The highlighted on in [antiplasmodial activity on P. falciparum] should not be italicized.

Correction has now been made.

Methods

- Plant material collection sites

Authors should give precisions on the plant part collected (young?, old? Fallen leaves?).

Young leaves of the plants were used for the test and this has now been indicated in the manuscript.

The reference identification numbers of the voucher specimens at the Institute should be provided.

The reference numbers of the voucher specimens has now been indicated in the manuscript.

- Preparation of crude plant extracts

1) The powdered plant materials were packed in plastic bags and kept in a clean and hygienic place so that no contamination occurred until extraction. What do you mean by hygienic place?

What type of contamination could occur? I don’t think such details are necessary…

The phrase ‘and kept in a clean and hygienic place so that no contamination occurred’ has now been removed from the manuscript.

2) The coarsely powdered plant materials were weighed using sensitive balance and repeatedly extracted in water and methanol solvents in maceration flasks.
3) I suggest that authors give the starting plant material quantity instead of this descriptive approach; Delete solvents.

The quantity of the starting plant materials has now been provided in the manuscript. ‘Solvents’ was deleted as suggested.

4) Justify the 24 hours and 72 hours extraction for water and methanol.

These two different hours were used following methods used in previous studies; reference has now been cited in the manuscript.

5) The methanol extract was concentrated at 40°C with a rotary evaporator (Buchi Rota vapor,) in distillation flask to eliminate methanol from the crude extract and concentrated further to dryness in a water bath.

- Please delete the highlighted text; what was the temperature of the water bath?

Highlighted text was deleted. Water bath temperature is now provided.

6) Then, the extracts were stored in tightly closed bottle containers in a freezer at 4°C… Did you mean a refrigerator?

It is to mean a refrigerator. Correction has now been made in the manuscript.

Experimental animals and parasite inoculation

Experimental animals

1) Provide information on mice sex and weight.

Information on mice age and weight has now been provided in the manuscript.

2) On weekly basis, the parasites were maintained by serial passage of blood from infected mice to non-infected ones.

This should be based on parasitaemia levels. Please elucidate.

Serial passage of blood was done when parasitaemia levels in infected mice reached 20-30%. This is now indicated in the manuscript.

3) Parasite inoculation

Albino mice previously infected with P. berghei having variable parasitaemia…. What do you mean by variable parasitaemia?

It is to mean different percentages of parasitaemia. Correction has now been made in the manuscript.

In vivo toxicity test of the crude plant extracts

1) The crude methanol and aqueous extracts of Croton macrostachyus and Acokanthera schimperi intended for the antimalarial test against P. berghei…

Please delete the highlighted text.

Highlighted text deleted as suggested.

2) The mice were starved 3-4hrs…

Please be consistent. Either you use hours or hrs throughout the manuscript.

‘Hours’ is now used throughout the manuscript.
3) Then, the mice in group 1 were given orally 2000 mg/kg body weight in single dose volume of 0.2 ml of the extract. This sentence should be edited. For ex: Then, the mice in group 1 were given orally 0.2 ml of 2000 mg/kg body weight of the extract in single dose in dH2O. **Correction was made in the manuscript as suggested.**

4) The mice in the control groups received 0.2 ml of respective vehicle of the extract (dH2O). Please delete respective. **Correction made as suggested.**

5) For sub-acute toxicity studies, [...] were used for measurement. Please add an “s” to measurement. **Correction has been made as suggested.**

*For this particular study, how were animal divided into groups? And how many per group?*

**The animals were grouped randomly, five mice per group. The same statement has been included in the manuscript.**

In vivo antimalarial screening
1) I suggest that authors describe their approach in a concise stepwise way to make it easy to understand and repeat. **Attempt has been made to modify statements for easy understanding repeatability.**

2) Formulas of % Suppression and % Parasitaemia should be well written. **The presentation of the formulae has now been improved.**

3) On the fifth day (D4), blood samples were collected from tail snip of each mouse [23] and thin smears were prepared and stained with 10% gemisa solution. It should be giemsa and not gemisa!! **Correction made as suggested.**

4) Packed cell volume measurement was done before infection on day 0 and on day 4. Blood was collected from tail of each mouse in heparinized microhaematocrit capillary tubes filled up to 3/4th of their length. Then, the blood was centrifuged using microhematocrit centrifuge and measured using a hand scale reader [26]. Such a description cannot enable someone to repeat your experiment!! Please provide a concise stepwise description. **Some modification has been made**

Results
Acute toxicity test
1) In the in vivo acute toxicity studies of the plant extract,....
Add an “s” to extract. **Done.**

2) Sub-acute toxicity test
Methanol and aqueous extract of Croton macrostachyus…
Add an “s” to extract.

Done

3) In sub-acute toxicity studies of methanol and water extract…
Add an “s” to extract.

Done

4) Antimalarial activities
Activities and not aactivities.

Corrected

5) PCV and body weight measurements on day 4 indicated that both methanol and aqueous extracts of Acokanthera schimperi…
Add an “s” to extract.

Done

6) page 12, 2nd paragraph:
- The mean survival time of treatment group ranged from 7.00±1.73 up to 10.60±0.51, ….
Please add the units.

The units (days) has now been added.

- The mice treated with the extracts at 600mg/kg survived longer than those in the negative control group for both solvents (P<0.05).
This should be highly discussed!!

Better description of results has now been provided

Discussion
1) Page 12, last sentence: Redundant, remove!

Statement deleted as recommended.

2) Page 13: The antimalarial activities exhibited by the extracts could perhaps be attributable to the possible presence of active compounds.
This statement is useless and should be removed.

Statement removed as recommended.

3) Page 13, 2nd paragraph:
The antiplasmodial activity has been linked to a range of several classes of the secondary plant metabolites including alkaloids, […] of which alkaloids have been the most important and have shown very interesting antiplasmodial activities [7].
The highlighted antiplasmodial is redundant, remove!

Done

Reference [7] is not enough for this, expand!

Attempt has been made to expand the discussion
Croton spp. generally contain diterpenoids, triterpenoids, alkaloids, flavonoids, lignoids and proanthocyanidins [27], which have strong antiplasmodial activity.

Please expand on this!

Some description has already been given preceding this statement.

Page 13, 3rd paragraph:
A compound is considered active when reduction in parasitaemia is ≥ 30% [24]. Croton macrostachyus can be considered active, its methanol extract exhibited 34.33% reduction in parasitaemia at 600mg/kg and its aqueous extract showed 30.5% and 50.53% parasitaemia reduction, at 400mg/kg and 600mg/kg, respectively.
You cannot argue on compounds and conclude on extracts. Please revise this comparison and be consistent in your approach.

In our case, compounds may not necessarily refer to pure compounds. They may also refer to compounds contained in crude plant extracts and fractions. Therefore, we consider our argument as a valid one.

Page 14, 2nd paragraph:
Your discussion should be mainly based on findings on plants of the Acokanthera and Croton genera. Search for such studies to reinforce your discussion.

One more statement has now been included in the manuscript.

Page 14, last paragraph:
Please provide further discussion on the issue of extended animal mean survival time of test samples compared to negative controls.

Extended description has now been provided in the manuscript.

Page 15
The comparison of plant extracts with chloroquine potency is irrelevant and should not be a highlighted part of the discussion.

That part of the discussion has now been removed from the manuscript.

Conclusion
The authors should not overestimate their findings. They should indicate directions for further investigation of the candidate extracts.

Comment accepted and modification in the manuscript has been made accordingly.

Why has this study been limited to the suppressive effect of plant extracts?

It was because of shortage of laboratory materials.

References

Errors have now been corrected.
Tables
1) Authors should add the unit for body weight in tables 1 and 2.
   Done

2) The data presented in the 6 tables can fit into two, joining data for body weight and PCV in one, and suppressive effect of extracts in the another.
We maintained the tables as they are so as to avoid overcrowding and provision of too much data in a single table.

Reviewer: Ouattara P Lamoussa Paul
MINOR ESSENTIAL REVISION:
Background
1. First paragraph, third line: update the data on malaria burden. The authors can find data more recent on the website of WHO
   Recent data is now included.

2. Last paragraph and fifth line: the authors should give the kind of Plasmodium falciparum strain used. Was it chloroquino sensitive or not. The authors should specify also the place where the study was carried out.
   Information related to strain used and place of study has now been provided as suggested.

Results
“Antimalarial aactivities” address this error in the title.
Error corrected.

Where are the data on WBC and Haemoglobin?
Why the authors did not show all the hematologic parameters such as the WBC, the rate of haemoglobin. They could in a table shown these data even if there is no difference with the control group

Regarding sub-acute toxicity testing in non-infected mice, we have the data for WBC and hemoglobin but not provided in the manuscript due to consistency problem. We have data on WBC for Croton macrostachyus along with that of body weight and PCV but the same plant does not have data for haemoglobin. Similarly, we have data on haemoglobin for Acokanthera schimperi along with that of body weight and PCV but the same plant lacks data on WBC. This problem happened due the lack of the instrument used to measure WBC during the time of sub-acute toxicity test for Acokanthera schimperi and lack of the instrument used to measure haemoglobin during the time of sub-acute toxicity test for Croton macrostachyus.

The authors should give the conditions of breeding of the mice: temperature, relative humidity, cycle of lighting, access to food and water…
   The requested information has now been provided in the ‘Experimental animals and parasite strain’ section in the manuscript.

The authors should give the mark and the country of the apparatuses used for the experiments
Mark and country has now been indicated.
The authors should give the formula to calculate PVC.

**There is no need for the inclusion of the formula needed to calculate PCV as hand-held hematocrit reader was used, which directly gives the value. The formula is required when measurement is done using a ruler.**

The authors must improve the manner of presenting the formulas of the % suppression; % parasitaemia by using the mathematical tools in Word.

**We tried to improve the presentation of the formula as recommended.**

**General comments**

Why authors did used only chloroquine phosphate as positive control, because nowadays in several country this drug is remove in malaria policy control. They could also use artemisinine also.

**We used chloroquine phosphate because it was the only drug we had during the time of testing.**

**Reviewer: Protus A Arrey Tarkang**

The authors did not state any work on which they are building. In this regard, they could consider chemosuppression and survival time in established malaria infection as well as subsequent clinical trials if efficacy is good.

**We have now included results of some previous works in the discussion section related to suppression and survival time.**

On the whole the English and the reporting are mediocre and would have to be a bit ameliorated; some of the suggestions have been highlighted and comments included on sticky notes for the appraisal of the authors.

**Efforts have been made to accommodate the given suggestions and comments.**

Find below the minor essential revisions that should be effected and re-submitted for re-consideration:

1. Methods are appropriate but the description and reporting of the results are poor.
   **Attempts have been made to improve reporting of results.**

2. The discussion is not consistent and does not really flow.
   **Attempts have been made to improve the flow.**

3. On sub-acute toxicity testing, the Relative Organ Weight (ROW) could be calculated and presented in the results; this could be discussed as an index of toxicity.
   **Data related to ROW was not collected.**

4. Standardization of scientific units is inadequate: use mL, 200 mg etc.
   **Correction made as suggested.**

5. General formatting of the manuscript; punctuations and spacing.
Improved
6. Footnotes for the tables should be self-explanatory and understandable; they should be revised. (Example: Results presented as mean ± SD or SEM; n=?)

Results are presented as mean ± SD is now indicated in every table as footnote. Sample size has also been indicated for each table.

7. Referencing should be consistent; kindly cross-check.

Checked.