

## Peer Review File

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### Review comments

#### Reviewer A

Mostafa Elfawal et al. show the effects of Artemisia and its major compounds against *Babesia microti*.

This is a very interesting study which shows that an effect observed for a plant or a standard molecule is not valid for all pathologies.

Some remarks:

Comment 1: l88: Specify the species. I think you are talking about *Artemisia Annua*.

Reply 1: No, we were introducing the subsequent sentences regarding specific examples involving both *A. annua* and *A. afra*, so in this instance stating *Artemisia* was correct.

Changes in text: None.

Comment 2: l1138: Put extracts of *Artemisia annua*.

Reply 2: Extracts is not specific enough, so we clarified.

Changes in text: "...candidiasis was warranted. Here we report on the response of six *Candida* species and human *B. microti* to AN, AN derivatives, and to *A. annua* extracts and orally gavaged leaves (DLA), respectively." Pg 4; ln 142-143.

You show perfectly the dose effects of your samples. However you do not talk about the cytotoxicity of these samples.

Comment 3: Have you done LDH, FACS, Counting tests?....

If yes, can you mention which one and the result?

Reply 3: We did not do any cytotoxicity tests in these experiments. First, in the *Candida* experiments, the solvent control acts as a cytotoxicity control. It was the death of the *Candida* cells beyond that of the solvent control that was the experimental objective. For the rodent experiments, we had already reported on the lack of any adverse effects on mice in prior studies using DLA.

Changes in text: We added this statement to the Discussion: "Animals treated with DLA suffered no adverse effects similar to our prior rodent studies (4, 44, 45)." Pg 8; ln 336-337.

#### Reviewer B

This paper demonstrates the inefficiency of ART and derivatives on protozoa and fungi. This study presents interesting. However, in my opinion, the paper has some shortcomings regarding the use of references, and the discussion deserves improvements.

The study is generally well designed and the manuscript is well written. but the way the study was done is poor and needs to be improved. The methodology did not were described rigorously and with reproducible details. I suggest only a few modifications, It needs to be more detailed

and divided into topics. The reviewer feels the manuscript can be accepted after some minor revisions. The science and the results obtained deserve publication. Although I have some concerns about its real potential.

Comment 4: Key Words needs to be improved: it should not contain the same words in the title  
Reply 4: We changed the key words.

Changes in text: Key words now read as follows: artesunate, artemether, dihydroartemisinin, babesiosis, dried leaf *Artemisia annua*, dried leaf *Artemisia annua* extract Pg 2; ln 74-75.

The abstract does not make it clear the study. they were not investigated by the authors.  
Therefore, I suggest modifying

Comment 5: The review has not indicated what is unclear in the abstract, nor what needs modifying.

Reply 5: We do not understand what the reviewer is trying to indicate that needs modifying.  
Changes in text: None.

If the authors cannot make the changes requested, I believe it would be best to reject the article so as not to compromise the quality of the journal.

The quality of the introduction is poor. It is not clearly set out and the issues addressed are not discussed in depth. The manuscript tackles an interesting subject, however, this is not a qualified introduction on " ART ", for the reasons set out below.

Comment 6: the authors need to add that ART won the Nobel Prize for Medicine in 2015

Reply 6: We added a reference to Tu's 2015 Nobel Prize.

Changes in text: The 2015 Nobel Prize for Medicine was awarded to Dr. Tu for her isolation and validation discovery of the antimalarial molecule in *A. annua* (1). Pg 3; ln 82-83.

Comment 7: The work needs to insert an immunological mechanism: this article needs to be explored artemisinin and its derivatives: towards use in immunomodulatory approaches

1- <https://doi.org/10.1080/21691401.2018.1505739>

2- <https://pubmed.ncbi.nlm.nih.gov/32939611/>

Reply 7: We reviewed the above suggested references and disagree about their inclusion; the cited references have no relevancy to this study. We note that one author is listed on both as well as on the next 3 suggested references. We did add a comment on the immunomodulatory effects of both ART and DLA in rodents.

Changes in text: Both AN and *A. annua* also have immunomodulatory effects, e.g. on TNF- $\alpha$  and IL-6, as recently shown in rats (18). Pg 3; ln 87-88.

Comment 8: Recently, observed the biological effects of this plant and also attributed it to artemisinin, and other authors suggested that this compound may be useful as an alternative for the treatment of many diseases in animals ---

3- DOI: 10.5897/jmpr2016.6318

4- DOI: 10.1016/j.tbdis.2018.04.004

5- DOI: 10.1016/j.scitotenv.2020.143851

Reply 8: While we agree the references 3-5 noted above are suggesting *A. annua* may be useful in treating veterinary parasitic diseases, we do not agree that they should be cited in this report.

The focus was on human diseases: Candidosis and babeiosis. Furthermore, there is again the same name that appears as a co-author on all of the 5 publications suggested by this reviewer. It is unethical to force authors to cite papers that are irrelevant to the primary topic of the manuscript. We did not include any of them in our discussion or in the cited reference list. It is also unethical to force authors to cite publications that are unrelated and seem to be all from one author (who may be this reviewer).

Changes in text: None.

Comment 9: The reviewer worked with ART before and knows its half-life is short. When using extract, it appears to be a zero-order release. The authors should discuss such property enhance the effects in clinical practice.

Reply 9: ART delivered via the plant material appears to have a longer half-life along with far greater bioavailability than pure ART. The PK and PD are quite different for plant delivered ART. Although we do not feel it is appropriate to delve into the PK/PD of ART in this negative study, we briefly mention it in the Discussion related to the animal part of the study and hope that assuages the reviewers' concerns.

Changes in text: We added this: "One might argue that AN was not bioavailable enough to achieve an adequate serum concentration in the mice. Although poorly bioavailable as a pure compound, when delivered via the plant as DLA, AN is >40 fold more bioavailable than pure AN (18, 45, 51). Furthermore, the half-life ( $t_{1/2}$ ) of pure AN in mice is about 18.8 min (52), but from the plant is about 51.6 min (45), indicating that DLA more than doubles the AN half-life in rodents." Pg 9; ln 362-366.

Comment 10: authors need to improve the writing of the conclusion and insert a future perspective in addition, guide new studies ..... It is important to describe the views of the authors, rather than simply gather findings from previous research, and this authorial view is barely seen in the manuscript.

Reply 10: The extensive conclusions section covers what is crucial for what is essentially a Discussion (relevance to the current literature) and Conclusions (outcomes from this study).

Changes in text: We renamed our Conclusions as "Discussion and Conclusions" as more recently indicated as being acceptable in the author checklist. Pg 8; ln 330.

Comment 11: Make sure that ALL references cited throughout the text are in the final list of references and vice versa.

Reply 11: we used Endnote to manage the numbered references for this article.

Changes in text: Any newly cited references have been added to the reference list.

Comment 12: Finally, I would like to remind the authors that the submission of the manuscript is the RESPONSIBILITY OF ALL THE AUTHORS, who must, therefore, analyze the adequacy of the text before submitting it.

Reply 12: As the corresponding author of my many publications over my >45 yr career, I always have ALL authors respond to any and all iterations of a manuscript. Nothing moves out of my or my co-authors laboratories to publication without the input and agreement of all authors.

Changes in text: None.

**Reviewer C**

*Artemisia annua* L. is a well-known medicinal herb used for thousands of years to treat parasites and fever-related ailments caused by various microbes. Although the plant is effective against many infectious

it seems fair to point out its lack of action. It is rare for scientists to report the results of studies that may prove ineffective in an application. I agree that the authors should report the lack of success of *Artemisia annua*. The paper is written properly, the graphs clearly illustrate the results of the analyses.

Comment 13: I am not a native speaker, however, I noticed minor linguistic remarks, as seen attached the pdf file.

Reply 13: We made the suggested edits.

Changes in text: See Pg 11, *Candida* species in Table 1 changed to *Candida* sp.; pg 13, ln 421; pg 14, ln 461.