

Answer to reviewers point by point

Manuscript number: pharmaceutics-2118677

Manuscript entitled: "Polyphenolic profile of *Herniaria hemistemon* aerial parts extract and assessment of its anti-cryptosporidiosis in murine model: *In silico* supported *in vivo* study"

By: Mosad A. Ghareeb, Mansour Sobeh, Tarek Aboushousha, Marwa Esmat, Hala Sh. Mohammed, Eman S. El-Wakil

Dear Respected Editor and Reviewers,

First, we would like to thank all reviewers for devoting their valuable time for reviewing rigorously our manuscript. All comments/suggestions are valid and useful to improve the quality of our manuscript.

Please find enclosed our rebuttal replay for reviewers' comments/suggestions point by point. All modifications in the revised file are marked in red colour.

Reviewer #1:

1) In the "Polyphenolic profile of *Herniaria hemistemon* aerial parts extract and assessment of its anti-cryptosporidiosis in murine model: *In silico* supported *in vivo* study" manuscript I really like the design of the study and the experimental part. This part does follow a well-established procedure and makes perfect sense, showing some potentially interesting results. Actually, I also like the computational part, as the docking has been performed using also well-established methods and since the docked compounds are small and similar to oxamic acid, there is a high chance that obtained results are accurate. One minor thing I don't like is to move the computational methods to supplementary data, as they are rather short and definitely belong to the main part of the manuscript.

We are trying to keep our manuscript concise; therefore, we kept at the suppl. file. Based on your wise recommendation, this part has been moved to the main file:

2.5. Docking-based virtual screening

The annotated compound of *H. hemistemon* aerial parts extract were virtually screened for their abilities to inhibit lactate dehydrogenase (PDB codes: 4ND1) [26] using AutoDock Vina software [27]. Detailed methods are provided at the supplementary file.

2) There are, however, two main problems with this study. The smaller drawback is the very short Introduction, which describes nicely the biology but fails to give some of the important information about e.g. the mode of actions of NTZ.

We added the mode of action to the introduction section.

3) The bigger problem is, however, this sentence: "Searching for essential protein targets for *Cryptosporidium parvum* in Protein Data Bank (PDB) resulted in choosing

lactate dehydrogenase (PDB ID: 4ND1) as a probable target." which for me is completely not convincing and does not actually show why this particular protein was chosen as a target. There are 63 structures of proteins from *Cryptosporidium parvum* Iowa II in the RSCB database and authors should explain much better why this particular protein was chosen; particularly given the fact that the mode of action of NTZ is known and it does not involve lactate dehydrogenase. That said I find this study interesting and I'm inclined towards accepting it, though it's does provide only preliminary results. For now, however, I recommend a major revision.

In order to putatively find out the probable protein target for the annotated compounds in *H. hemistemon* aerial parts extract, we subjected their structures to molecular inverse docking experiments using the idTarget platform (<http://idtarget.rcas.sinica.edu.tw>). This platform can dock the query structures against almost all proteins hosted in Protein Data Bank (PDB), and then retrieve the results as a list of binding affinity scores. Accordingly, we found lactate dehydrogenase (PDB ID: 4ND1) as a probable target for gallic acid, hydroxy benzoic acid, and methyl gallate with affinity scores of -9.35, -8.89, and -8.73, respectively. As a validation step, the whole annotated structures were then re-docked into the enzyme's active site using AutoDock Vina.

We hope that we have satisfactorily handled all the concerns highlighted. Thank you so much for everything and I hope to accept my manuscript in this form.

Best regards,

Associate Professor/ Mosad Ahmed Ghareeb

Department of Medicinal Chemistry, Theodor Bilharz Research Institute, Kornaish El Nile, Warrak El-Hadar, Imbaba (P.O. 30), Giza 12411, Egypt

E-mail: m.ghareeb@tbri.gov.eg

Tel.: 00201012346834