Author's response to reviews

Title: Antifungal activity of berberine hydrochloride and palmatine hydrochloride against Microsporum canis in rabbits and underlying mechanism

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Author's response to reviews: see over
Antifungal activity of berberine hydrochloride and palmatine hydrochloride against Microsporum canis in rabbits and underlying mechanism

Version: 2
Date: 16 March 2015
Reviewer: Luciana Figueredo

Reviewer's report:
- Major Compulsory Revisions

Many studies have described T. mentagrophytes, as the most important agent of dermatophytoses worldwide on rabbit (Ellis and Mory, 2001; Cafarchia et al., 2010; Hoppman and Wilson Barron, 2007). Considering that, why the authors tested the antifungal activity of the chemical compounds against M. canis?

We thank Reviewer Professor Luciana Figueredo for this comment, The genera Microsporum canis were found both in man and animal and the specie Microsporum canis is zoonotic in nature. Literature revealed that Microsporum canis are major cause of dermatophytosis in pets and rabbits (Băguţ ET et al., 2012). As suggested, we have added this reference in the revised manuscript. Please see Page 2, Line 62.

Reference

The manuscript has several grammatical and spelling errors and should be revised by a native English speaker. Additionally, several scientific names and Latin words (e.g., in vivo and in vitro) are not italicized.

We thank Reviewer Professor Luciana Figueredo for this comment, As suggested, we have made many changes in the revised manuscript. Please check for your kindly help. Thanks so much.

Background section:

We thank Reviewer Professor Luciana Figueredo for this comment, As suggested, we have added this reference in the background section of revised manuscript. Please see Page 2, Line 59.

Reference
Molecular identification and classification of Trichophyton mentagrophytes complex strains isolated from humans and selected animal species.

- Line 61-62 - The sentence “the most common pathogens of dermatomycosis is Trichophyton mentagrophytes and Microsporum etc. Genera Microsporum canis”
is very confusing. Is it about human, animals or rabbit dermatophytoses? Is the “etc” referred to which additional fungi? Microsporum canis is not a genus, it is a species.  
We thank Reviewer Professor Luciana Figueredo for this comment, It should be replaced as animal dermatophytoses. “etc” referred to Microsporum gypseum. As suggested, we have made changes in the revised manuscript. Please see Page 2, Line 59-60.

Reference
Zheng L. The development of the assays for the detection of pathogenic dermatophytes nucleic acid in laboratory. Master Thesis of Hebei Medical University, 2004, in abstract.

Methods section:
- Line 110: The sentence is not clear. Were all Eumycetes isolated or only M. canis?  
We thank Reviewer Professor Luciana Figueredo for this comment, As suggested, we have made changes in the revised manuscript. Please see Page 3, Line 114.

- Line 136-137: Please provide the volume of the suspension inoculated in the Petri dish  
We thank Reviewer Professor Luciana Figueredo for this comment, As suggested, we have made changes in the revised manuscript. Please see Page 3, Line 138.

- The tryptic soy agar is not widely used for culturing dermatophytes (Sabouraud with chloramphenicol and cycloheximide). Why did the authors decide to use this medium? Could the authors add a figure including the photos of the determination of the minimum inhibitory concentration culture?  
We thank Reviewer Professor Luciana Figueredo for this comment, we found tryptic soy agar was suitable for culturing M. canis through many experiments. We are very sorry, we didn’t take the photos at the moment.

- In the figure legends and in the text the DMSO was considered as negative control while clotrimazole as positive control. However in the figures there are two different lines (DMSO # negative control). Please clarify.  
We thank Reviewer Professor Luciana Figueredo for this comment, DMSO and negative control are different groups. DMSO means we used DMSO as a treatment way, while in negative control we didn’t used anything drugs or chemicals. As suggested, we have made changes in the revised manuscript. Please see Results part and figure legends (Fig.3- Fig.11)

Results:
- Line 272 – 313: The text provides the same information contained in the figures 3-11, please reduce it.  
We thank Reviewer Professor Luciana Figueredo for this comment, we modified this section. Thanks so much.
- Figure 1: If possible, please provide the “M. canis quality µg” on 0 hour.
We thank Reviewer Professor Luciana Figueredo for this comment, we are very sorry, we didn’t measure the quality on 0 hour.

- Figure 3-11: The legend is not clear (the squares are very small)
We thank Reviewer Professor Luciana Figueredo for this comment, As suggested, we have made changes in the revised manuscript. Please check with your kindly help. Thanks so much.

- Figure 14: Please provide rows to show the details of the images
We thank Reviewer Professor Luciana Figueredo for this comment, As suggested, we have made changes in the revised manuscript.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Not suitable for publication unless extensively edited

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests

**Reviewer's report**
**Title:** Antifungal activity of berberine hydrochloride and palmatine hydrochloride against Microsporum canis in rabbits and underlying mechanism

**Version:** 2  
**Date:** 7 March 2015

**Reviewer:** Sully Cruz

**Reviewer's report:**
Minor essential revisions

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:**
I declare that I have no competing interests

**Article Report**
Antifungal activity of berberine hydrochloride 1 and palmatine hydrochloride against *Microsporum canis* in rabbits and underlying mechanism

1. Is the question posed by the authors well defined?
Yes, it is, the questions posed by the authors well defined, so in the background is very general, lack focus more on berberine an palmatine as an object of study.

We thank Reviewer Professor Sully Cruz for this comment, As suggested, we have made changes in the revised manuscript. Please see Page 3, Line

2. Are the methods appropriate and well described?
The methods are appropriate and were well described in each section,
however missing references that give sustenance to the methods, may be included where no reference is cited.

We thank Reviewer Professor Sully Cruz for this comment, yes, there is no related references.

3. Are the data sound?
The data are sound.

We thank Reviewer Professor Sully Cruz for this comment.

4. Do the figures appear to be genuine, i.e. without evidence of manipulation?
Yes, the figures appear to be genuine

We thank Reviewer Professor Sully Cruz for this comment.

5. Does the manuscript adhere to the relevant standards for reporting and data deposition?
Yes, the manuscript adhere to the relevant standards for reporting and data deposition.

We thank Reviewer Professor Sully Cruz for this comment.

6. Are the discussion and conclusions well balanced and adequately supported by the data?
Yes the discussion and conclusions well balanced however can be extended more discussion about the activity reported by berberine and palmatine, It alkaloid is procured from roots of B. aristata, B. petiolaris, B vulgaris, B.aquifolium, B. thumbergii, B. asiatica and Among Chinese herbs it’s primary sources are B sargentiana, Phellodendron amurense and Coptis chinensis from rhizomes and bark respectively. The authors only described Phellodendron amurense, however do not mention that they had isolated the alkaloids in the plant. Berberine was demonstrated to have wide spectrum of pharmacological activities like anti hypertensive, anti inflammatory, anti oxidant, anti depressant, anti cancer, anti diarrhoeal, cholagogue, hepatoprotective and has also been used to treat oriental sores, trachoma, CHF. Most important of all its action is antimicrobial activity. So it is not included in the discussions some articles which have already evaluated in vitro antifungal activity.

We thank Reviewer Professor Sully Cruz for this comment, As suggested, we have made changes in the revised manuscript. Thanks so much.

7. Are limitations of the work clearly stated?
Yes, some sections are clearly stated.

We thank Reviewer Professor Sully Cruz for this comment, As suggested, we have made changes in the revised manuscript.

8. Do the authors clearly acknowledge any work upon which they are building, both published and unpublished?
Yes the authors clearly acknowledge any work upon which they are building both published.

We thank Reviewer Professor Sully Cruz for this comment.

9. Do the title and abstract accurately convey what has been found?
Yes, the title and abstract accurately convey what has been found.

10. Is the writing acceptable?
Yes is acceptable but it is necessary to extend the discussion and to include
some articles that reported the activity of the alkaloids. 

We thank Reviewer Professor Sully Cruz for this comment, As suggested, we have made changes in the revised manuscript.