Author's response to reviews

Title: Antimalarial activity of plumbagin in the in vitro and animal models

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Author's response to reviews:

Summary of Response to Reviewers' Comments

Title: Antimalarial activity of plumbagin in the in vitro and animal models
Version: 1 Date: 8 October 2013
Reviewer: Sittiporn Pattaradilokrat

Major compulsory revisions:

1. In Materials and Methods, the authors stated the plumbagin treatments were performed at high doses (e.g. 100, 200 and 500 mg/Kg). However, in Results section, the authors only reported that results from treatment with 100 mg/Kg plumbagin by which plumbagin exerted no toxic effect to animals' health. For some reasons, no description of toxicity or effect on animals' health at high doses was reported. This hidden part of study is the "real" toxicity test.

Response: The information on description of signs and symptoms of mice receiving a single oral doses of 500 and 200 mg/kg body weight including the multiple dosing of 100 and 50 mg/kg body weight for 14 days have been added in the Results section.

2. In Result section (line 177-178 and line 182-184), the authors reported results of gross examination (size of organ) and cell morphology. But, clear methods were not described anywhere in the text. Detailed methods for behavioral observations were described, neither.

Response: The description of gross and histopathological examination of vital organs of mice have been added in the Materials and Methods section.

3. Conclusions (line 40-42) and (line 241 – 244) looked totally different. It seems that the authors concluded results from two pieces of independent work.

Response: The conclusions of the study results in both the Abstract and the main content have been revised.
4. Drug concentrations in the in vitro experiment should be converted to uM or nM; to facilitate comparison of the drug activities to other published work.
Response: The IC50 values for all drugs have been reverted to nM unit.

Minor essential revisions:
1. There are numerous grammatically incorrect English usages. See below, for some example.
   (1) Line 48-49:
   “Malaria caused by Plasmodium falciparum is the most virulent….species” should be “Malaria is caused by Plasmodium falciparum, one of the most virulent and most widespread malaria parasite species....”
   Response: The meaning of this sentence is that Plasmodium falciparum is the malaria species (among the five species that infect human) that is the most virulent and most widespread in the world.
   (2) Line 63-64: this is not a sentence.
   Response: The sentence has been revised as........” It is a naphthoquinone that occurs in plant roots as a colorless combined form that can be processed to plumbagin by acid treatment.”
   (3) Line 80: “The authentic” should be removed.
   Response: This has been removed.
   (4) Line 103: “The 96-well drug plate was dosed with plumbagin at eight final concentrations” should be “Plumbagin (in PBS/DMSO/Tween-20?) was added to the malaria parasite culture at 8 concentrations of 0.039, ...”
   Response: The sentence has been revised as...” Plumbagin (dissolved in DMSO and diluted with RPMI 1640 to final concentration of 1%) was added to the malaria culture at eight final concentrations of 210, 420, 840, 1680, 3360, 6720, 13440 and 26880 nM. Chloroquine (3.89-498.15 nM) and artesunate (0.39-50.0 nM) were used as standard antimalarial drugs.

2. In Discussion, the authors proposed the use of lipid-soluble solvent to prepare plumbagin. I would recommend the authors’ to repeat the in vivo assays with DMSO or other solvents. It may improve the efficacy of the test compounds.
Response: For the in vivo study, plumbagin was initially dissolved in DMSO and further diluted with 20% Tween-20. The final concentration of DMSO is <1%.
DMSO (100%) as well as other dissolving solvents is toxic to animals.

3. There are other examples of relevant work that conducted similar in vitro and
in vivo assays, which should be of interest to the authors. See, Yuan et al., 2011 Science. http://www.ncbi.nlm.nih.gov/pubmed/21817045, for example.
Response: This reference has been included as suggested.

Level of interest: An article whose findings are important to those with closely related research interests
Quality of written English: Needs some language corrections before being published
Statistical review: Yes, and I have assessed the statistics in my report.
Declaration of competing interests:
I hereby have declared that no competing interests exist.

Reviewer's report
Title: Antimalarial activity of plumbagin in the in vitro and animal models
Version: 1 Date: 10 November 2013
Reviewer: Porntip Chavalitshewinkoon-Petmitr

Comments
1. Why did IC50 of ethanolic extract of plumbagin from Plumbago indica Linn in this study (IC50=0.11 mg/ml) on 3D7 P. falciparum greatly differ from previous study by Simonsen et al., 2001 (IC50=17 mg/ml)?
Response: The IC50 value reported by Simonsen's paper is for the crude ethanolic extract of Plumbago zeylanica, whereas that reported in the present study is for the pure compound plumbagin.

2. One more table should be added to show IC50s of all tested drugs.
Response: The Table has been added as Table 1.

3. In Results (p.8, line 180), it should be Table 2 not Table 3 as mentioned in the text.
Response: This has been corrected.

4. In Figure 2, obviously there is a reduction in body weight of male mice compared with the control but authors mentioned that there was no abnormality in body weight (p.8-9).
Response: The reduction in the body weight of male and female mice both in the acute and subacute toxicity tests was not statistically significant. This has been clarified in the text.

5. Table 1 and 2 can be combined. What is the % of Tween-80 used in the control group in Figure 2? Please correct it.
Response: The two tables have been combined as suggested. The percentage of Tween-80 used is 20%.
6. There are some confusing sentences and difficult to understand such as on p.7 lines 146-148 so please correct it.

Response: The sentence has been revised as..." The positive and negative control groups were fed with the antimalarial chloroquine at oral daily doses of 10 mg/kg body weight of plumbagin and 20% Tween-80, respectively."

7. Authors should try to discuss why plumbagin was more potent against chloroquine-resistant P. falciparum than the sensitive parasite in term of their drug targets.

Response: The discussion on this issue has been added.

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

Quality of written English: Needs some language corrections before being published

Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

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