Author's response to reviews

Title: Earthworm (Pheretima aspergillum) extract stimulates osteoblast activity and inhibits osteoclast differentiation

Authors:

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Version: 2
Date: 21 July 2014

Author's response to reviews: see over
Editor, BMC Complementary and Alternative Medicine,

Dear Dr. Tom Rowles:

Thank you for dealing with the manuscript entitled “Effects of earthworm (Pheretima aspergillum) extract on bone cell activities” (Manuscript Number 2062230324109405). The title has been changed to “Earthworm (Pheretima aspergillum) extract stimulates osteoblast activity and inhibits osteoclast differentiation” following the suggestion of reviewer. The authors would like to express gratitude to you and the reviewers for your constructive and positive comments on the manuscript. The article has been revised and compiled as per the reviewers’ suggestions. Please find enclosed a list of changes and detailed explanations. I hope these explanations along with the revised manuscript will meet your requirements.

Thank you for your time. I look forward to hearing from you.

Best Regards

Sincerely Yours,

Chun-Hsu Yao, Prof.

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For the comments of reviewers

Reviewer: Edwin Cooper

Reviewer's report:

1. Title and Subheadings and figure legends are not descriptive. In fact they are trite and do not convey enthusiasm. Each begins with: "Effects of" This format style should be varied by STATING the effects! Starting with the title the construction: "Effects of" is used 10 times which is excessive and not professional.

A: As per the reviewer’s suggestion, title, subheadings and figure legends have been changed to state the effects of earthworm extract.

2. Next time if possible the authors should return a copy of the paper that has the lines numbered.

A: Following the suggestion of reviewer, the lines have been numbered in the margin of revised manuscript.

3. The paper should be read by someone proficient in English so that too many words (e.g. very) should be removed.

A: As per the reviewer’s suggestion, the manuscript has been revised for the English.

4. The computer can do a spell and grammar check.

A: As per the reviewer’s suggestion, the spelling and grammar errors and typos have been checked and corrected by the computer.
5. Beginning on page 3, 4, 5 there should be sub-headings under the major headings to improve clarity. (Please DO NOT use Effects of). Subheadings should help to guide the reader.

A: Following the suggestion of reviewer, sub-headings have been added to the revised manuscript. (pages 3-5)

6. Similarly on pages 7,8,9, there should be sub-headings.

A: Following the suggestion of reviewer, sub-headings have been added to the revised manuscript. (pages 8-10)

7. Interpretation and conclusions are not clear on page 8.

A: As per the reviewer’s suggestion, interpretation and conclusions on page 8 have been revised in the revised manuscript. (pages 8-9)

8. Figures 2 and 4 are difficult to read with the alphabets lower left and starting with A at the bottom left. In addition the figures would be clearer if arrows were used to point out.

A: Following the suggestion of reviewer, arrows have been placed on Figures 2 and 4 of the revised manuscript. Moreover, the concentrations of earthworm extract have been shown in Figures 2 and 4.

9. Authors should carefully check Pub Med for papers dealing with Lumbrokinase which exerts profound effects and must be discussed.

A: Following the suggestion of reviewer, references about lumbrokinase have been added to the Discussion section. (on page 11, lines 12-15 of revised manuscript)


10. To aid in better writing, authors should read out loud the paper that will usually reveal ways to improve the writing.

A: As per the reviewer’s suggestion, the manuscript has been revised.
Reviewer: Angela Bruzzaniti

Reviewer's report:

Essential revisions

The OB data is convincing and shows dose-dependent effects on the cells.

Some minor comments

1. Page 8 first paragraph. What does the OB data refer to that enables determination of % differences? The cell number data does not seem to be included?

A: The osteoblast data on Page 8 refer to the difference in the number of osteoblasts. Moreover, Figure 1 has been revised. The y-axis indicates the number of osteoblasts.

![MTT Assay (Osteoblasts)](image)

**Figure 1 Earthworm extract enhances the proliferation of osteoblasts.** MG-63 cells were cultured with PBS as a control or different concentrations of earthworm extract. Osteoblast viability was measured by MTT assay after 2 day of culture. (*p < 0.05, **p < 0.01 and ***p < 0.001 vs. control; #p < 0.05 and ###p < 0.001 vs. 3 mg/ml).

2. Osteoclast data: It is not clear if the authors are examining the effect of earthworm on
mature OCs. Please show representative images in the treatment groups similar to OB data. This is important since TRAP is detected in non-OCs too. In addition, page 9 also refers to OC numbers, but no data is shown.

A: Several researches have demonstrated that the formation of mature osteoclasts requires 5-6 days in the presence of RANKL. In this study, RAW 264.7 cells were cultured with RANKL for 6 days to form mature osteoclasts. The effect of earthworm extract on mature osteoclasts was shown in Figure 6. Moreover, Figure 6 has been revised. The y-axis denotes the number of osteoclasts.


![MTT Assay (Osteoclasts)](image)

**Figure 6 Earthworm extract does not affect the proliferation of osteoclasts.** RAW 264.7 cells were seeded in 48-well plates and allowed to adhere for 1 day. Osteoclast differentiation from
RAW 264.7 cells was induced with 50 ng/ml RANKL in α-MEM for 6 days. Osteoclasts were then cultured with PBS as a control or different concentrations of earthworm extract for 2 days. Osteoclast viability was measured by MTT assay.

3. Discussion is somewhat redundant with the results and should be shortened/changed to reflect true discussion of findings.
   A: As per the reviewer’s suggestion, the description of experimental results in Discussion section has been shortened in the revised manuscript.

   A: The grammatical errors in the abstract have been checked and corrected.

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.
   A: As per the reviewer’s suggestion, the manuscript has been revised for the English.

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