Author's response to reviews

Title: Water extract of the fruits of Alpinia oxyphylla inhibits osteoclast differentiation and bone loss

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Author's response to reviews: see over
Dear Editor,

**RE: MS: 8947190561343779**

**Title:** Water extract of the fruits of *Alpinia oxyphylla* inhibits osteoclast differentiation and bone loss.

**Authors:** Hyunil Ha, Ki-Shuk Shim, Taesoo Kim, Chung-Jo Lee, Ji Hyung Park, Han Sung Kim, and Jin Yeul Ma

The authors would like to thank the reviewers and the editor for the thoughtful comments to improve the quality of our manuscript. According to the comments of the Editor and the Reviewers, we have made revision which marked in red in the revised version. Our point-by-point responses to the comments are detailed on the following pages. We hope that the revised manuscript is now suitable for publication in *BMC complementary and alternative medicine*.

Sincerely yours,

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Responses to the comments of Editor

Additional revision request: Please update your ethics statement to include the name of the ethics committee that approved your study.

(Response)
The relevant sentence has been changed to read as follows (page 8, lines 169-171): “All animal experiments were carried out according to the protocols (permission number: 12-090) approved by the Institutional Animal Care and Use Committee Guidelines of Korea Institute of Oriental Medicine.”

i) Suggest authors to give a brief account about authentication of Alpinia

(Response)
Thanks for the comment. We have addressed that in the revised manuscript (page 6, lines 117-119; page 7, lines 127-135; page 13, lines 268-280). The raw materials were authenticated by an expert botanist, emeritus Prof. Ki-Hwan Bae (Collage of Pharmacy, Chungnam National University). In addition, chrysin, tectochrysin, and nootkatone, known to be abundant in ethanol extracts of the fruits of A. oxyphylla, were identified in 75% ethanol extract of the fruits of A. oxyphylla used in our study (please see Supplementary Figure 1 in the response to the reviewer #2’s comment 1).

ii) Explain why they chose a high oral dose

(Response)
Thanks for the comment. The following statement has been added to the results and discussion section (page 17, lines 361-368). “Our results clearly showed that WEAO at a dose of 0.25 g/kg twice daily attenuates RANKL-induced osteoclast differentiation and bone destruction in vivo. However, such beneficial effects of WEAO were not observed at a dose of 0.75 g/kg (data not shown). Therefore, further studies are needed to more thoroughly characterize the dose response effects of WEAO on bone metabolism.”

iii) Discuss about possibility of clinical use and information about chemicals of Alpinia

(Response)
We have addressed this issue in the revised manuscript (page 13, lines 268-280). We also found that nootkatone is one of the constituents contributing to the inhibitory effect of WEAO on osteoclast differentiation (Figure 1E–G).
Responses to the comment of Reviewer #1

1. In Fig. 4B, include statistical analysis

(Response)

Thanks for the comment. We have done that in the revised manuscript as suggested.
Responses to the comments of Reviewer #2

1. There is lack of information about the authentication of the fruits of Alpinia oxyphylla. Standardization of the raw materials and the water extract WEAO using HPLC, ULPC or LC-MS is essential for medicinal research. The yield of the extract should be given-

(Response)

Thanks for the comments. We have addressed these concerns in the revised manuscript (page 6, lines 116-135; page 13, lines 268-280). The raw materials were authenticated by an expert botanist, emeritus Prof. Ki-Hwan Bae (Collage of Pharmacy, Chungnam National University). In addition, chrysin, tectochrysin, and nootkatone, known to be abundant in ethanol extracts of the fruits of A. oxyphylla, were identified in 75% ethanol extract of the fruits of A. oxyphylla used in our study (Supplementary Figure 1).

<Supplementary Figure for review purpose only>

Supplementary Figure 1. HPLC chromatograms of 75% ethanol extract of the fruits of A. oxyphylla (100 mg/mL) and a standard mixture of chrysin, tectochrysin, and nootkatone (each 200 µg/mL) at 203 nm.

2. As mentioned before, the systemic RANKL injection model is not a good animal model to mimic clinical events such as estrogen-deficient osteopenia (osteoporosis) or localized bone destruction (osteoarthritis or lytic bone metastasis). They can choose the other model to enhance the clinical value of the paper. Besides, the authors need to justify why they choose oral administration of WEAO (0.25 g/kg twice daily) for 5 days. 500mg/kg per day is quite high for human (HED = 40mg/kg extract).
(Response)
Thanks for the comments. Considering the reviewer’s comments, we have added the following statements to the results and discussion section.

On page 17, line 344: “Increased RANKL activity is involved in bone destruction in various bone diseases [2]. Having found that WEAO inhibits RANKL-induced osteoclast differentiation in vitro, we next examined the in vivo effect of WEAO on osteoclast-mediated bone destruction using a murine model of bone loss by RANKL injection.”

On page 17, line 361: “Our results clearly showed that WEAO at a dose of 0.25 g/kg twice daily attenuates RANKL-induced osteoclast differentiation and bone destruction in vivo. However, such beneficial effects of WEAO were not observed at a dose of 0.75 g/kg (data not shown). Therefore, further studies are needed to more thoroughly characterize the dose response effects of WEAO on bone metabolism. In addition, the beneficial effect of WEAO in bone disease states such as postmenopausal osteoporosis and lytic bone metastasis remains to be investigated.”

3. The authors have put much effort in data elaboration instead of ‘real’ discussion. It is better to discuss (1) what is (are) the main chemical components of the fruit of Alpinia oxyphylla responsible for its biological actions.

(Response)
As shown in Figure 1E–G in the revised manuscript, nootkatone was found to be one of the constituents contributing to the inhibitory effect of WEAO on osteoclast differentiation. The relevant paragraph has been added to the results and discussion section (page 13, lines 268-280).

(2) how their work could be applied and used as preventive medicine (3) and the rationale of whole study design.

(Response)
We have addressed these issues in the background section (page 5, lines 87-97). In addition, the relevant paragraph in conclusions has been expanded as follows (page 19, lines 375-378): “Given the role of excessive RANKL activity in pathological bone loss, our findings suggests that WEAO may be useful for the prevention and treatment of bone metabolic diseases associated with excessive osteoclastic bone resorption.”