Reviewer’s report

Title: Water extract of Cnidii Rhizoma suppresses RANKL-induced osteoclastogenesis in RAW 264.7 cell by inhibiting NFATc1/c-Fos signaling and prevents ovariectomized bone loss in SD-rat

Version: 1 Date: 27 Aug 2018

Reviewer: Yuankun Zhai

Reviewer's report:

The present study demonstrates that the extraction of Cnidii Rhizoma (CR) Bacitracin, an herb medicine, could suppress RANKL-induced osteoclastogenesis in RAW 264.7 cell in vitro, and prevents ovariectomized bone loss in SD-rats. The interpretations are in accord with the results obtained. However, there still have some problems and need some substantive revisions before this paper be accepted.

(1) In Figure 8A, the growth plate looks different among that 5 groups, E2, CR-L and CR-H with a light blue, but Sham and OVX have a dark blue, please give an explanation. Is CR effects on the chondrocytes or the growth plate, and then inhibit the bone loss?

(2) The author need show the micro CT data which is more accurate than HE staining.

(3) In Figure 6, PCR reaction shown there have no bands of RANK and CTR after supplemented with CR at 250μg/ml to 1000μg/ml, but the authors also shown quantity analysis data. Please make sure the quantity analysis is corresponds to the bands.

(4) In the results, "CR increased the femur weight in the OVX rat model", the authors described that the weight loss of tibia and femur, but in Figure 6 and its legend, there have no Figure 6C and 6D, please make sure that Figure 6 is correct.

(5) The author need supply biomechanical testing data (such as 3-bending test) to show the improvement of bone quality.

(6) The author need check bone metabolism parameters (such as the level of BALP, TRAP, PICP and β-CTX in serum) to confirm the effects on bone quality after supplemented with CR. Please see some literature.

(7) In Figure 8, please make sure the format of scale is same in every HE figures. They should in same length and same color, please modify the scale in Sham group.

(8) The author need supply a cartoon to illustrate the main mechanism of CR during the prohibition of osteoclast formation and the inhibition of bone loss in OVX rats.
(9) In table 1, please add the Gene bank number, and the PCR product size.

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
If not, please explain in your comments to the authors.

No

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

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Needs some language corrections before being published

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