Author’s response to reviews

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

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Author’s response to reviews:

Responses to Reviewers:
We would like to thank both of the reviewers for their positive and very helpful comments to improve our manuscript. In response to both the reviewer, we have addressed the comments as follow:

Responses to Reviewer #1_ Yanqiu Liu:

1.There are some minor formatting errors in the article, please careful check and modify.
   » I reviewed some minor formatting errors in the article. The number of the figure and the result was not correct, the mark in the figure was wrong. These errors have been corrected.

2.The authors should give scale bars to all the images.
   » I added scale bar to all images. (Figure 3, 4 and 8)

3.In the methods section, the authors should describe the modeling process in detail. What is the difference between the model group and the sham group?
   » I added the following sentence to manuscript (7th paragraph of methods, line 6-8): To induce postmenopausal osteoporosis model, the ovariectomized (OVX) group removed both ovaries. In addition, the sham-operated group did not remove the ovaries after laparotomy to give the same stress.

4.In the results section, the result description is confusing, such as Figure 6 and Figure 7.
   » I corrected figure numbers in the results part.
Thank you for your comment!

Responses to Reviewer #2_ Yuankun Zhai:

(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?

The reason for the difference in femoral tissue was the presence of over-stained tissue when TRAP staining was performed. I operated TRAP staining again, then figure is replaced with a new image. The lower part of the growth plate was captured, and the captured part was shown on the left image. As a result, it was confirmed that CR did not affect chondrocytes or the growth plate (Figure 8a).

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

Unfortunately, all femoral tissue was sectioned, there is no extra femur. It is impossible to take a micro-CT now. From the next experiment, I will perform bone analysis through micro-CT.

(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.

These results are the mean value of the triplicate experiments. The quantity analysis is not a problem.

(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.

I corrected the figure numbers in the results part as your comments. (7th paragraph of results, line 4-6)

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

Unfortunately, all femoral tissue was sectioned, there is no extra femur. It is impossible to biomechanical test now. From the next experiment, I will perform biomechanical test.

(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.

I measured osteocalcin concentration and TRAP activity in serum of OVX-induced osteoporosis rats. An increase in remodeling rates during estrogen deficiency was reflected by increased levels of osteocalcin (bone turnover marker). CR significantly inhibited the expression of osteocalcin (Figure 7d).

Serum TRAP activity was slightly increased by OVX, but the difference was not significant. However, I founded that the CR group had inhibitory effect of TRAP activity. (Figure 7e.). Although I can’t accurately account for the small difference in TRAP activity between Sham and OVX, it is assumed that the experiment period is short. (8 weeks). In the next experiment, I plan to increase the experiment period to more than 12 weeks. (Attach a reference paper)

The tibia weights in previous Figure 7 were removed because there was no significant change in CR.
Serum analysis was added to the manuscript (Methods, results and discussion). (7th paragraph of methods, line 14-17; 8th paragraph of results; 4th paragraph of discussion, line 11-17)

(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.
   We stained and captured the femur again. The scale bar of the sham was modified. We also added which part was captured at low magnification. (I added an image to the left of the figure.)

(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.
   We created a cartoon about the effect of CR on OVX-rats and added it as supplementary data.

(9) In table 1, please add the Gene bank number, and the PCR product size.
   We have added the accession number and base pair of the primer in Table 1. and we inserted a new table. (end of manuscript.)

Thank you for your comment!