Author’s response to reviews

Title: Water extract of Rumex crispus prevents bone loss by inhibiting osteoclastogenesis and inducing osteoblast mineralization

Authors:

ki-shuk shim (angeloshim@kiom.re.kr)
Jin Yeul Ma (jyma@kiom.re.kr)
Bohyoung Lee (leebh@kiom.re.kr)

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Responses to 1st reviewer’s comments

Rumex crispus is used as Yang-Ti, a common folk herb with the functions of treating various kinds of bleeding, ringworm and hepatitis in China for thousands of years. The present study was aimed to investigate the effect of water extract of R. crispus on preventing bone loss. It is an interesting and meaningful work. The experimental design is straightforward and properly carried out. The data are clearly presented and convincing. Besides these, there is a drawback to be concerned. Antliraquinone is the main active ingredient of R. crispus, so the alcohol extraction is better than water extraction for extracting antliraquinone form R. crispus. According to extraction method and the elution profile of RP-HPLC in the present manuscript, it is obvious that emodin, chrysophanol, and physcion are not the main components of WERC. So, authors should focus on the water-soluble active components of WERC, such as polysaccharides, phenols, flavonoid glycosides, etc, which might be the main active ingredients of WERC for prevention and treatment of osteoporosis.

Response: Thank you for taking your time and effort to provide insightful comments. When we identified the several components within WERC, we referenced the HPLC analysis methods reported by Chiang et al. (Journal of the Chinese chemical society, 2009, 56, 341-350) and Guo et al. (Molecules, 2011, 16, 1201-1210). In Chiang’s study, the boiled water extract of R. crispus was prepared for HPLC analysis and mainly detected the anthraquinone compounds. The components, including emodin, chrysophanol, and physcion, detected in our study have been reported the major anthraquinones of R. crispus, which has shown the various pharmaceutical activities in several references. In this study, we found that WERC showed the anti-osteoporosis-
relative activities, and found that the components (emodin, chrysophanol, and physcion) in WERC regulated bone cell differentiation (Supplementary Figure 1). Thus, it might suggest that these components could be active components contributing WERC activity against osteoporotic bone loss by regulating bone cell differentiation. Although we could not perfectly identify the components of WERC, we will perform the identification of the other active components from WERC including water-soluble components according to your comments for further study.


Responses to 2nd reviewer’s comments

The manuscript entitled "Water extract of Rumex crispus prevents bone loss by inhibiting osteoclastogenesis and inducing osteoblast mineralization" is very well planned, executed and presented. However, considering a few points towards the betterment of the manuscript is suggested as follows.

Response: Thank you for taking your time and effort to provide insightful comments.

1. A preliminary comparative experiment with emodin, physcion, and chrysophanol to identify the major active components regulating the differentiation of bone cells would be a great asset for the present work.
Response: Thanks for your critical comment. We have addressed this comment by conducting osteoclast differentiation and osteoblast differentiation experiments with these components. After incubating bone cells with these components, all components (emodin, chrysophanol, and physcion) inhibited RANKL-induced osteoclast differentiation, but some of them (chrysophanol and physcion) induced osteoblast differentiation. Thus, it might suggest that these components could be active components contributing WERC activity to regulate bone cell differentiation (Supplementary Figure 1).

2. A microscopic picture of undifferentiated and differentiates cells should be provided.

Response: Thanks for your critical comment. We have addressed this comment by providing microscopic picture of TRAP-stained differentiated osteoclasts and alizarin red S-stained differentiated osteoblasts in Supplementary Figure 1 of the revised manuscript.

3. Chemical structures of emodin, physcion, and chrysophanol should be provided so as to enable the chemist readers to gain insight for a structure-function relationship study.

Response: Thanks for your critical comment. We have addressed this comment by providing chemical structure of emodin, chrysophanol, and physcion in Figure 1 of the revised manuscript.

Responses to Editor’s comments

From the HPLC spectrum, these three compounds are not main ones, are these ones are active compounds in water extract? If these three are active compounds, assay it, if not, which is/are active compounds?
Response: Thanks for your critical comment. We have addressed this comment by conducting osteoclast differentiation and osteoblast differentiation experiments with these components. After incubating bone cells with these components, all components (emodin, physcion, and chrysophanol) inhibited RANKL-induced osteoclast differentiation, but some of them (chrysophanol and physcion) induced osteoblast differentiation. Thus, it might suggest that these components could be active components contributing WERC activity to regulate bone cell differentiation (Supplementary Figure 1).