Author’s response to reviews

Title: Inhibitory effects of ChondroT and its constituent herbs on RANKL-induced osteoclastogenesis

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Author’s response to reviews:

Dear Editor:

This is to submit an original research article entitled “Inhibitory effects of ChondroT and its constituent herbs on RANKL-induced osteoclastogenesis” (BCAM-D-19-00853) for consideration by “BMC Complementary and Alternative Medicine”, coauthored by Rui Hong Guo and others. We thank editors very much for giving us the chance of revision. We changed our manuscript according to your recommendations and all the critiques will be answered, item by item, in this response letter. We highlighted the changes we made in the manuscript using colored text. This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. In addition, the manuscript received English language editing service by the ‘HARRISCO’ group. We have read and understood your journal’s policies and believe that neither the manuscript nor the study violates any of these. There are no conflicts of interest to declare.

Thank you for your consideration. We are looking forward to hearing a positive response from you.

Sincerely,

Young Ran Kim, PhD, Professor
College of Pharmacy and Research Institute of Drug Development,
Editor's comments:

1. At present, we do not feel that there is sufficient evidence presented in your Background section to justify the testing of ChondroT and its constituent herbs in an animal model. We would therefore ask you to expand this section to include as much referenced evidence as possible to explain why you would expect this treatment to have an effect in this model. This evidence should come from previous in vitro or animal work. Please note that we are unable to accept traditional medical use as sufficient justification for animal studies.

Answer: Thanks for your comments. We changed the description in the Background section as “Ganghwaljetongyeum (GHJTY), a traditional decoction composed of 18 herbs, has been used to treat joint pain, limitation of motion, fever, and inflammatory processes associated with arthritis [14, 15]. We selected five effective herbal constituents from GHJTY with greatest potential to improve the efficacy and convenience of pharmaceutical prescription through bioinformatics analysis and pharmacologic activity tests [16]. The resulting concoction named as ChondroT, which comprised water extracts of Ostericum koreanum (Maxim.) Kitag., Lonicera japonica Thunb., Angelica gigas Nakai, Clematis manshurica Rupr., and Phellodendron amurense Rupr. in a 6 : 4 : 4 : 4 : 3 ratio [17]. ChondroT exhibited more significant chondroprotective effects and anti-inflammatory processes related to arthritis than GHJTY did [17]. ChondroT also showed significant anti-osteoarthritic effects in a rat model of monosodium iodoacetate- or collagenase-induced osteoarthritis [14, 18]. In addition, the efficacy and safety of ChondroT on knee-osteoarthritis were evaluated by randomized, double-blind, placebo-controlled, multicenter clinical trials [19]. Recently, we demonstrated the anti-hyperuricemic effects of ChondroT by regulating xanthine oxidase activity and kidney mouse urate transporter 1 in potassium oxonate-induced hyperuricemic mice [20]. To further investigate the efficacy and action mechanism of ChondroT as a therapeutic potential herbal drug, we evaluated its function on bone diseases in this study. Among its five constituent herbs, Ostericum koreanum (Maxim.) Kitag., Angelica gigas Nakai and its major active decursin have been reported to possess anti-osteoclastogenic activity in bone marrow cells isolated from mice [21-23]. Recently, the component phellodendrine from Cortex Phellodendri Chinensis has been reported to have an obvious inhibitory effect on osteoclast differentiation and function [24]. Therefore, the complex herbal medicine ChondroT has potential beneficial effects against osteoclastogenesis”. Hence, the present study investigated anti-osteoclastogenic effects of ChondroT and its five constituent herbs in RANKL-activated primary precursor cells and the underlying signaling pathways involved.

2. Please clarify your method of euthanasia/sacrifice. You have listed to different methods in your Methods section. Please clarify if one was anesthesia rather than euthanasia.
Answer: Thanks for your comments. As suggested, we changed the description of the euthanasia/sacrifice method as “Mice were anesthetized with Carbon dioxide (CO2), and then were sacrificed by cervical dislocation in accordance with IACUC guidelines”.

3. Please provide details in your Methods section on who undertook the formal identification of the plant material used in your study. Please also confirm whether a voucher specimen of this material has been deposited in a publicly available herbarium, and include this information in your manuscript. A deposition number should be included, if available.

Answer: Thank you for your comments. We provided the details of the plant materials in the Methods section as “ChondroT was prepared using a previously described method (doi: 10.1186/s12906-016-1211-0). We purchased five herbal medicines from Omniherb (Yeongcheon, Korea) as shown in Table 1, and their origin was confirmed taxonomically by professor Jong-Kil Jeong in the Department of Herbology at the college of Oriental Medicine, Dongshin University. Voucher specimens (Kyr2014-020) were deposited at the college of Pharmacy, Chonnam National University”.

4. In the interest of full transparency, please include the full, uncropped western blots in the supplementary material. Failure to do so many prevent us from moving ahead with your manuscript.

Answer: Thank you for your comments. We have submitted the full, uncropped Western blots in the supplementary material according to your suggestion.