Author’s response to reviews

Title: Effects of ChondroT on Potassium Oxonate-Induced Hyperuricemic Mice: Downregulation of Xanthine Oxidase and Urate Transporter 1

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Author’s response to reviews:

Dear Sir.

This is to resubmit a revised version of the manuscript entitled “Effects of ChondroT on Potassium Oxonate-Induced Hyperuricemic Mice: Downregulation of Xanthine Oxidase and Urate Transporter 1” (BCAM-D-17-01595). We thank editors very much for giving us the chance of resubmission. We cordially appreciate thorough reading and valuable comments of enthusiastic reviewers. Reviewers’ critiques and comments made this revised manuscript more persuasive and logically stronger.

We highlighted the changes to our manuscript within the document by using blue colored text and all the critiques will be answered, item by item, in this response letter. The manuscript was modified by a professional English editor.

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. We have read and understood your journal’s policies,
and we 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. I look forward to hearing from you.

<Our response to the comments and critiques of reviewers>

Vikneswaran Murugaiyah (Reviewer 1):

Abstract:

1. The results should include some data.

2. Indicate the level of significance when describing the results.
   → As you mentioned, we added this part in Abstract.

Introduction:

3. Hyperuricemia itself is not a disease but rather a condition whereby blood uric acid level is elevated. Suggest correcting the first sentence of this section.

   → As you mentioned, we revised this part in the Introduction section. As below, “Hyperuricemia is characterized by an increase in uric acid (UA) blood levels [1].”

Methods:

4. It was mentioned that the extraction was carried out at 100°C for 3 hrs. The temperature seems high for extraction and may cause degradation of thermolabile compounds.
→ We performed extraction of herbal medicines using water solvent. Thus, we was carried out at 100°C for 3 hrs because water has a boiling point of 100°C. In addition, to demonstrate the reproducibility of repeated experimental results, degradation of thermolabile compounds in the extract is inevitable. Also, most herbal plants has more heat stable compounds than thermolabile compounds.

5. The three concentrations mentioned in XOD inhibition in vitro (100, 300 and 500µg/ml) are the initial concentrations. What are the final concentrations measured in the final reaction mixtures.

→ This concentrations shown final reaction concentrations of Chondro T.

6. The number of mice used was 5 per group. Is that sample size sufficient for meaningful statistical analysis? Usually for studies using mice, a sample size of at least 8-10 will be employed.

→ The number of the mice in each group were carried out using the minimum number of mice (N=5). Many other studies use still the number of the mice N=5. Also, these have been show meaningful statistical analysis.

7. In the animal study, potassium oxonate was administered intraperitoneally once daily for seven days. Will that cause stress and pain to the animals? What measures were taken to minimise the stress and pain in the animals.

→ All animal experiments were conducted in accordance with the Institutional Animal Care and Use Committee (IACUC) guidelines. Also, we closely observe all the stress and pain that an animals can receive. Moreover, in order to minimize stress and pain, actions such as minimizing external environment change were performed.

Results:
8. The IC50 for in vitro inhibition was 414µg/ml. How does one categorise the extract potency. Is it considered potent, moderate or weak inhibitor?

   → In our present study, we demonstrated anti-hyperuricemia effects of Chondro T. Also, its five herbal constituents potency showed in the Table 3. This study, Chondro T exhibited significantly inhibitory effects on in vitro XOD as well as in vivo experiments. Also, ChondroT showed a more significant in vivo than in vitro inhibition of XOD. Thus, these effects considered that Chondro T suggest moderate inhibitor.

9. Authors did measure the XOD activity in serum and liver. However, in animals and human there are two kind of enzymes, namely xanthine oxidase (XOD) and xanthine dehydrogenase (XDH). Both are capable to breakdown hypoxanthine to xanthine and further to uric acid. Hence the measurement reported here could be a net activity of both XOD and XDH.

   → We absolutely agree with you and further studies will be required in both enzymes.

10. The concentrations shown in table 3 are the initial concentrations. Authors should mention the final concentrations in the reaction mixture and not the initial concentrations.

   → This concentrations shown final concentrations of Chondro T. We will show the single herbal constituents potency to the next study.

Discussion:

11. In general, there is not much discussion in this manuscript. Authors should include more papers/literature data to support their findings and enrich the discussion.
→ As you suggested, we carefully revised in the discussion section.

12. ChondroT comprises of five plants. Is there any literature/papers describing the uric acid lowering effect of any of these plants? Likewise, is there any literature/papers describing the uric acid lowering effect of any of the compounds identified in chondroT (chlorogenic acid, berberine Cl, nodakenin, isoferulic acid, oxypeucedanin hydrate, decursin, and decursinol angelate).

→ We showed references that lowering effects of uric acid of these each plants (page 14, line 8-10). As below,

“Furthermore, Phellodendri Cortex has been reported to decrease serum UA and liver XOD activity in PO-induced hyperuricemic mice [29]. In addition, Lonicerae Folium and Clematidis Radix showed inhibitory effects against XOD activity in vitro, but these effects were not strong [30].”

Also, As you suggested, we carefully added in the discussion section. As below, “Previous our results suggested that seven reference components in ChondroT, such as chlorogenic acid, berberine Cl, nodakenin, isoferulic acid, oxypeucedanin hydrate, decursin, and decursinol angelate. Chlorogenic acid has been shown to exert anti-gout activity as well as improvement on hyperuricemia and inflammation by inhibiting the XOD activity, serum UA levels, and production of proinflammatory cytokines (e.g. IL-1β and IL-6) [31]. In addition, alkaloid compounds including berberine Cl have been shown inhibition activity of XOD enzymes to reduce uric acid levels [29, 32]. However, further studies are required to determine the functional active constituents that are involved in the anti-hyperuricemia as well as anti-gout activity.”

13. In this study, ChondroT was found to reduce uric acid in hyperuricemic animals (hence having antihyperuricemia effect). Has ChondroT been evaluated in normouricemic animals to rule out any possible hypouricemic effect?

→ We are very sorry but we did not include a group of ChondroT alone administration in this study. Previously, we evaluated the toxicity of ChondroT at high doses such as 500, 1,000, and 2000 mg/kg. ChondroT was confirmed to be safe herb at the high
doses. AST and ALT levels of ChondroT-treated rats are normal value ranges. In addition, BUN levels in ChondroT-treated rats are in normal scores. ChondroT did not show any toxicity. We mentioned the safety of ChondroT in Discussion section.

14. The present study suggests that ChondroT also works as uricosuric agent besides inhibiting XOD. It is well known that currently available uricosuric agents have very limited clinical use. Perhaps authors should highlight this limitation maybe applicable to ChondroT as well.

→ As you suggested, we carefully added in the discussion section. As below, “However, these uricosuric agents limited to use clinical trials, which further studies will be required to determine the safety of uricosurics effects of ChondroT for anti-hyperuricemia.”

Bunleu Sungthong (Reviewer 2):

1. The authors have to mention that the ratio of herbal constituents derived from GHJTY remedy. Otherwise, the readers will question how it derived from.

→ We showed ratio of herbal constituents in the Materials and reagent (page 6, line 10-11). As below, “Briefly, the five herbal constituents, Osterici Radix (Korea), Lonicerae Folium (China), Angelicae Gigantis Radix (Korea), Clematidis Radix (China), and Phellodendri Cortex (China) were combined in a ratio of 6:4:4:4:3 (Table 1).”

2. Please check scientific name throughout the manuscript. They have to write either italic or underline.

→ We checked scientific name throughout the manuscript.
3. For in vitro XOD experiment, the result showed the inhibition of each constituent. The authors have to state how they were extracted or prepared.

→ As you suggested, we revised extraction and prepared methods in the Materials and reagents section. As below, “Briefly, the five herbal constituents, Osterici Radix (Korea), Lonicerae Folium (China), Angelicae Gigantis Radix (Korea), Clematidis Radix (China), and Phellodendri Cortex (China) were combined in a ratio of 6:4:4:4:3 (Table 1). Next, each five herbal constituents as well as ChondroT extracted once using a 10-fold volume of water as the solvent at 100 °C for 3 h. After filtration (180-mesh), the water extract was concentrated using a continuous vacuum evaporator (at approximately 55–60 °C, 670 mmHg), followed by lyophilization using a vacuum drier (720 mmHg) for 8 h. The extraction yield of ChondrT was about 29.5%. The resultant ChondroT and each herbal constituents was dissolved in phosphate-buffered saline (PBS) and filter-sterilized.”

4. Regarding toxicity in discussion part, the authors should give more detail whether the toxicity test was acute or chronic. In page 13 line14, the authors have to specify that it is safe in which model of toxicity testing. Otherwise, readers will misunderstand that it is safe in general. It can be claimed, when there is an evidence on safety consideration. There is also a mistake in page 13 line 13 ay=> at.

→ As you suggested, we carefully revised in the Discussion section. As below, “Previously, we evaluated the toxicity of ChondroT at high doses such as 500, 1,000, and 2000 mg/kg for 4 weeks at Korea Testing & Research Institute (KTR).”

5. Table 3, the explanation of * was missing.

→ We were sorry for any confusion caused. As you suggested, we added the explanation of P values in the Table 3.

6. Table 4, there was some mistake regarding the explanation under the table. For example, ** stands for both P< 0.01 and 0.001.
→ We were sorry for any confusion caused. As you suggested, we revised the Table 4. As below, “##\(P < 0.01\) and ###\(P < 0.001\) compared with NC group; \(*P < 0.05, **P < 0.01,\) and ***\(P < 0.001\) compared with HC group.”

7. Fig 1, there was only note for # and ## symbols. But, in the Fig 1(A) shows ###.

→ We were sorry for any confusion caused. As you suggested, we revised the Fig 1 legends. As below, “#\(P < 0.05\) and ###\(P < 0.001\) compared with NC group; **\(P < 0.01\), and ***\(P < 0.001\) compared with HC group.”

8. Fig 2, there was only note for #. But, there is ### in Fig 1(A). Again with ***, the authors give a note only for * and **. There is no note regarding *** in Fig 2(A).

→ We were sorry for any confusion caused. As you suggested, we revised the Fig 2 legends. As below, “#\(P < 0.05\) and ###\(P < 0.001\) compared with NC group; **\(P < 0.05, **P < 0.01,\) and ***\(P < 0.001\) compared with HC group.”

9. Fig 3, the symbols indicated significant difference were not match with explanation.

→ We were sorry for any confusion caused. As you suggested, we revised the Fig 3 legends. As below, “###\(P < 0.001\) compared with NC group; **\(P < 0.01\) and ***\(P < 0.001\) compared with HC group.”