Author's response to reviews

Title: Standardized butanol fraction of WIN-34B suppresses cartilage destruction via inhibiting the production of matrix metalloproteinase and inflammatory mediator in osteoarthritis human cartilage explants culture and chondrocytes

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Author's response to reviews: see over
Dear Editor-in-Chief, BMC complementary & alternative medicine

Thank you very much your revision. I have carefully checked and studied to submit this revision in my effort to improvement the paper. I look forward to hearing from you regarding its suitability for publication.

Sincerely yours,

Dong-Suk, Park
Reviewer's report

Title: Standardized butanol fraction of WIN-34B suppresses cartilage destruction via inhibiting the production of matrixmetalloproteinase and inflammatory mediator in osteoarthritis human cartilage explants culture and chondrocytes

Version: 4 Date: 24 September 2012

Reviewer: YUN JONG LEE

Reviewer's report:

Minor essential revisions

1. The duration of cartilage explants culture was 21 days and the conditioned media was collected at 7, 14, and 21 day. And, GAG and type II collagen assays were done at 7, 14, and 21 day (Figure3). Please, correct the description about the time of these assays in Methods.

Answer) Thanks for your comments. I added the time of GAG and type II collagen assays in Methods (Page 8, line 10 and line 21).

2. Cytotoxicity was determined in cartilage explants using LDH test and in isolated chondrocytes using BrdU test, according to Method section. But, the Legend of Fig 2.says that “cytotoxicity of IL-1b…healthy peripheral blood Mononuclear lymphocytes.”. Does Fig 2B.
present the cytotoxicity effect in PBMC or chondrocytes?

*Answer* Thanks for your comments. I corrected the legend of Fig. 2.

3. To evaluate the effect on MAPK pathways, chondrocytes were cultured and stimulated, according to Method section. However, the legend of Fig.7. says that cartilage explants were used. Which is right? It is important to interpret the results- if Fig. 2B presents the cytotoxicity for CA or MF in isolated chondrocytes, a high concentration of these compounds was toxic to chondocytes. Because changes in MAPK pathways could be affected by other stimuli other then inflammatory IL-1b, comparison between WINI-34 band CA or MF seems not appropriate.

*Answer* Thanks for your comments. I changed chondrocytes culture to cartilage explants culture in Methods, Results, and Discussion (page 12 line 1, page 15 line 12-15, page 18 line 4).

4. Above all, they did not show any scientific evidence on the concentrations used in in-vitro experiment. Because it is a major limitation of this study, the authors should address the issue in Discussion.

1) Ten ng/mL of IL-1β is a very high, supra physiologic level- Fig 3 showed that 1-2.5ng/mL was adequate for the stimulation of cartilage explants.
Answer) In preliminary experiments, to optimize the conditions with which to induce proteoglycan and collagen degradation. 1, 2.5, 5, 10, or 20 ng/ml IL-1β-stimulated articular cartilage and chondrocytes have not shown cytotoxicity by LDH assay and viability test for 21 days.

2) If higher concentrations of the compound are needed to diffuse into the cartilage and make an effect on chondrocytes, further higher levels should be administered in in-vivo study. But, they showed the cytotoxic effect of CA and MF, the major lipophilic component of WIN-34B, in PBMC.

Answer) Thanks for your comments. Previously, I described safety and gastro protective effect of WIN-34B (page 14, line 14-18). I added the analgesic efficacy and safety profile of WIN-34B in clinical study (page 16, line 18-21). In general, combined prescription or total extract have shown the safety rather than single compound or lead compound in vivo and in vitro (Choi et al., Osteoarthritis Cartilage. 2002;10(6):471-8; Huh et al., Int Immunopharmacol. 2009; 9(2):230-40).

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable
Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests

Reviewer’s report

Title: Standardized butanol fraction of WIN-34B suppresses cartilage destruction via inhibiting the production of matrix metalloproteinase and inflammatory mediator in osteoarthritis human cartilage explants culture and chondrocytes

Version: 4 Date: 23 September 2012

Reviewer: Li-DongWu

Reviewer’s report:

The authors have revised the manuscript according to the reviewer’s comments carefully.

However, but several short comings should be addressed.

Minor Essential Revisions

1. the authors assessed the viability of chondrocytes and change the data instead of the PBMC viability data, so date about PBMCs should be deleted (page 5 line 26; page 16 line 18-19; and in the supplementary file FIG2 legend).

Answer) Thanks for your comments. I corrected your point out (page 5 line 26, page 16 line 18-19 and legend of Fig. 2).
2. Why there are 37 references? We only find No.1-35 listed in the article.

*Answer* I have a mistake. Thanks for your comment. I delete the references 36 and 37.

Level of interest: An article whose findings are important to those with closely related research interests.

Quality of written English: Needs some language corrections before being published

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests