Author's response to reviews

**Title:** Effects of beta-sitosterol derived from Artemisia capillaris on the activated human hepatic stellate cells and dimethylnitrosamine-induced mouse liver fibrosis

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**Version:** 3  
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**Author's response to reviews:** see over
Dear BMC complementary and alternative medicine editor,

We appreciate for your interest about our work.

We have tried hard to satisfy the referee’s demands and your expectation.

Please find our attached files, which contains our responses to the revision.

Those are consist of 1) revised manuscript, 2) answer for revision, and 3) New figures 2,3, and 7, those have re-produced according to the reviewer's demand.

We have highlighted all of our changes with ‘Red font’ in our manuscript, and provided point-by-point response to the concerns in this cover letter.

If you have any question or additional comment, please let us know.

Sincerely,

Hyeung-Jin Jang, Ph.D.
**Additional revision request:**

- 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.

(Comment) We purchased the aerial part of *Artemisia capillaris* Thunb. from Kyung Hee Oriental Herbal Medicine Research Center. It is as same plant material as we have used at our last study which published in *BMC Complement Alternat Med* (Jang et al., Anti-lipoapoptotic effect of *Artemisia capillaris* extract on free fatty acids-induced HepG2 cells. 2014, 14:253). According to the editor’s demand, we provided more information about the plant material in our manuscript.

(Correction) Page 4, Line 17-19

From

AC was purchased from Kyung Hee Oriental Herbal Medicine Research Center (Seoul, South Korea). AC water extracts for the chromatography analysis were prepared as described in references [12, 13].

To

The aerial part of AC was purchased from Kyung Hee Oriental Herbal Medicine Research Center (Seoul, South Korea). The herb was cut down in a proper size, and extracted with distilled water (DW) for the chromatography analysis as described in references [12, 13].
Reviewer's report 1
Reviewer: Jae-Young Um

Reviewer's report:
Minor Essential Revisions

Abstract
The sentence ‘a-smooth muscle actin’ in the abstract section must be corrected into ‘α-smooth muscle actin’. And the other sentences that absent ‘α (alpha)’ must be checked throughout the manuscript.

(Comment) We appreciate for your sharp observation on our manuscript. We have corrected the mark whole throughout the manuscript and highlighted with Red font.

Methods
The authors determined the anti-fibrotic effects of β-sitosterol, but the investigation is only focused on the collagen-1 and a-SMA expressions levels.

Authors should explain the significance of the targets in the hepatic fibrosis.

(Comment) As we mentioned in our manuscript, Background section, induced liver fibrogenesis is closely related to the fibrogenic cell differentiation and its extracellular matrix (ECM) accumulation. Collagen-1 and α-SMA are highly accumulated ECM when liver fibrosis was induced by TGF-β, as described in the references (Friedman SL. J Biol Chem 2000, 275(4):2247-2250; Hellerbrand C et al. J Hepatol 1999, 30(1):77-87). And Morrison SJ et al. defined α-SMA accumulation is a marker of liver fibrogenesis (Cell 1999, 96(5):737-749). In this study, we have focused the down-regulatory effects of the herbal drugs on ECM factors during liver fibrosis.

Results
The oral administration of β-sitosterol at 40 mg/kg seems to increased collagen-1 and a-SMA expression. Authors should explain the results. The β-sitosterol treatment seems to affects the collagen-1 and a-SMA expressions in vitro. However, differences between the mRNA and protein expression levels in the cells affected by each dose of β-sitosterol treatment are observed. Authors should explain the results.

(Comment) Differences between the mRNA and protein expression levels in mammalian cell were well-studied issues. With regard to a quantitative description of
gene expression, numerous previous studies comparing mRNA and protein levels concluded that the correlation is poor, because many steps in the central dogma cascade is controlled by gene-regulatory events (Schwanhausser B et al. Nature 2011, 473:337-342). Therefore, analysis of mRNA and protein levels alone cannot provide sufficient information to understand gene expression comprehensively.

We therefore, observed the regulatory effects of the β-sitosterol treatment on both of the mRNA and protein expression levels in DMN-induced mouse liver, and 10 mg/kg of β-sitosterol showed down-regulatory effects on both of the mRNA and protein expression levels. However, 40 mg/kg of β-sitosterol showed less down-regulatory effect than 10 mg/kg as the reviewer mentioned. We assume that the result is because the 40 mg/kg of β-sitosterol was enough to induce known effects on the mouse liver.

**Figures**

The authors use gene name mixed with protein name. For example, ‘Relative MMP-1 mRNA level’ in the Figure 2A must be used as ‘Relative MMP1 mRNA level’. Please clear up the term.

(Comment) We appreciate for your sharp observation on our figure. We have corrected the gene name in the figure 2, 3, and 7.

**Figure legends**

Please provide more information in the figure legends. Especially, Figure 2,3, and 5.

(Comment) We provided additional informations in the figure 2, 3, and 5. They are highlighted with Red font in our revised manuscript.
Reviewer's report 2
Reviewer:Tae Jin Kang

Reviewer's report:

1. There were grammatical errors, which will need to be addressed in the revision.
   (Comment) We respect the reviewer’s comment. We have revised and corrected the grammatical errors throughout the manuscript.

2. They should explain the reason why authors used dimethylnitrosamine to induce liver damage in discussion section.
   (Comment) Dimethylnitrosamine (DMN) is highly toxic carcinogen, especially to the liver. At high doses, DMN is a potent hepatotoxin that can cause liver fibrosis of the rat (George J et al. 2001 Toxicology 156(2–3): 129–138). On the basis of the reference, we induced hepatofibrosis rat model with DMN.
   We couldn’t find any reference in our manuscript about the reason why we used DMN in our study. Therefore, we added the reference at the Method section (page 8, line 4). We appreciate for your check.

3. While 40 mg/kg of β-sitosterol did not affect to the α-SMA mRNA expression level, it significantly decreased the protein expression. It would be better if authors describe the reason or opinion on details in discussion section.
   (Comment) Differences between the mRNA and protein expression levels in mammalian cell were well-studied issues. With regard to a quantitative description of gene expression, numerous previous studies comparing mRNA and protein levels concluded that the correlation is poor, because many steps in the central dogma cascade is controlled by gene-regulatory events (Schwanhausser B et al. Nature 2011, 473:337-342). Therefore, analysis of mRNA and protein levels alone cannot provide sufficient information to understand gene expression comprehensively.
   We therefore, observed the regulatory effects of the β-sitosterol treatment on both of the mRNA and protein expression levels in DMN-induced mouse liver, and 10 mg/kg of β-sitosterol showed down-regulatory effects on both of the mRNA and protein expression levels. However, 40 mg/kg of β-sitosterol showed less down-regulatory effect than 10 mg/kg as the reviewer mentioned. We assume that the result is because
the 40 mg/kg of β-sitosterol was enough to induce known effects on the mouse liver.

4. Page 5, line 19; Affiliation of cell line provider (Dr.Scott Friedman) will need to be written.

(Comment) We appreciate for your check. We provided the affiliation of Dr. Friedman at Page 5, Line 21.