**Author’s response to reviews**

**Title:** MOK, a pharmacopuncture medicine, regulates thyroid dysfunction in L-thyroxin-induced hyperthyroidism in rats through the regulation of oxidation and the TRPV1 ion channel

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**Author’s response to reviews:**

"The authors' response letter has been included as a supplementary file"

Authors’ Response to the Review Comments

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Title of Paper: MOK, a pharmacopuncture medicine, regulates thyroid dysfunction in L-thyroxin-induced hyperthyroidism in rats through the regulation of oxidation and the TRPV1 ion channel

Authors: Ji Hye Hwang, Seok Yong Kang, An Na Kang, Hyo Won Jung, Chul Jung, Jin-Ho Jeong and Yong-Ki Park

Date Sent: Aug 22, 2017
We appreciate the time and efforts by the editor and referees in reviewing this manuscript. We have addressed all issues indicated in the review report and marked all corrections in our revised manuscript with blue. We believed that the revised version can meet the journal publication requirements.

Thank you very much for all reviewers’ comments, again.

Respectfully yours,

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Reviewer(s)’ Comments to Author:

We have explained as detailed in the point-by-point according to the reviewer’s comments below. All changes were marked in the manuscript with blue. The authors thank you very much for the constructive and helpful comments of the reviewer.

Editor Comments:

Comments to the Author

Please add to chemical characterization of used extract.

Ans) As per your suggestion, we did HPLC analysis for identification of the compounds in MOK extract. We added the explanation about it in Materials and Methods (lines 194-204), Results (lines 296-299), and Discussion (lines 412-421). We also added the figure 9 with the legend (lines 655-656).
Referee: 1 (Hongtao Bi, Ph.D. (Reviewer 1))

Comments to the Author

This study is an interesting and meaningful work. The experimental design is straightforward and properly carried out. The data are clearly presented and convincing. The result is of practical importance for the modern utilization of MOK.

Ans) We added the part of “HPLC analysis” in Materials and Methods (lines 194-204), Results (lines 296-299), and Discussion (lines 412-421) with figure 9. We believed that our analysis can help readers understand the character of MOK pharmacopuncture.

We thank you very much for the constructive and helpful comments of the reviewer.

Referee: 2 MichałBijak (Reviewer 2):

Comments to the Author

The paper presented by Hang et al. is very interesting. Background is very well described and introduce to research objective. Results are clear.

1. But there is a lack of chemical characterization of used extract. In my opinion to publish this paper in BMC Complementary and Alternative Medicine chemical composition must be included.

Ans) As per your suggestion, we did HPLC analysis for identification of four compounds, bilirubin, ursodeoxycholic acid (UDCA), baicalein and muscone in MOK extract. We added the explanation about it in Materials and Methods (lines 194-204), Results (lines 296-299), and Discussion (lines 412-421). We also added the figure 9 with the legend (lines 655-656) as follows:

Materials and Methods

HPLC analysis

To identify the constituents of the MOK extract, high-performance liquid chromatography (HPLC) was conducted using standard compounds. The HPLC apparatus was a WatersDelta600 (Waters, Milford, MA, USA) with PDA (Photodiode Array) Detector. A CAPCELL PAK C18
UG80 column (SHISHEIDO Co., Ltd. Japan) was employed. Chromatographic separation was performed using a gradient solvent system consisting of acetonitrile with 0.1% formic acid (B) and water (A). The gradient program was as follows: 0 min, 0%B; 5 min, 3%B; 15 min, 3%B; 25 min, 10%B; 35 min, 15%B; 45 min, 15%B; 55 min, 30%B; 65 min, 50%B; 75 min, 70%B; and 80 min, 0%. The column eluent was monitored at UV 254 nm. All solvents were subsequently degassed with 0.2 μm cellulose acetate filtering. Chromatography was performed at room temperature with a flow rate of 1.0 mL/min, and a 25 μL volume was analyzed.

Results

HPLC analysis

In HPLC analysis of MOK extract, four compounds such as 3-methylcyclopentadecanone (muscone), ursodeoxycholic acid (UDCA), bilirubin, and baicalein in the MOK extract were identified through the comparison of retention times of authentic standards (Fig. 9).

Discussion

HPLC analysis revealed that MOK extract contained the compounds such as bilirubin, UDCA, baicalein, and muscone. Bilirubin is the main compound of Bos taurus and was reported to have antiinflammation, anticancer and antiviral activities [39]. UDCA, a secondary bile acid, is the main compound of Ursus arctos and was also reported anticancer [40], antioxidation, and liver protective effects [41]. Baicalein is the main flavonoid of Scutellaria baicalensis and was reported the antioxidant [42], anticancer and antiestrogenic effects [43]. Muscone is the standard compound of Moschus berezovskii and has been reported to have the effects of neuroprotection, antioxidation [44], antiinflammation [45], and alleviation of pain and vascular dysfunction [46]. In our HPLC analysis, these compounds might be involved in the therapeutic effects of MOK on hyperthyroidism however, their function responsible for MOK pharmacopuncture remains a problem for further study.

Figure Legends

Fig. 9 HPLC pattern analysis of the MOK extract: (A) each standard compound. Peaks: (1) bilirubin, (2) UDCA, (3) baicalein, and (4) muscone; and (B) MOK extract.
Other minor issues:

1) Results should be presented as mean with SD not S.E.M,

Ans) We presented all data as the mean ± SD of each group (n=5 per group), and it was corrected the legends of figures 1 to 8.

2) How authors preformed procedure allowing to use AnovaTukey?, how normality and equality of variance were established?

Ans) We corrected the sentence in the statistical analysis of Materials and Methods as follows (lines 206-211): The statistical significances were analyzed using one-way analysis of variance (ANOVA) to compare the groups, followed by Tukey’s test.

3) Abstract is too long.

Ans) As per the reviewer’s suggestion. We condensed the Abstract (page 3).

4) Some editorial bugs.

Ans) As per the reviewer’s suggestion, we checked the manuscript, again, and corrected some mistakes like typical errors, spacing words etc.

We thank you very much for the constructive and helpful comments of the reviewer.