Author's response to reviews

Title: Oryeongsan suppressed high glucose-induced mesangial fibrosis

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Response to Review Comments

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We thank reviewers for the invaluable comments and suggestions. We hope this revision has improved the manuscript to a level of their satisfaction. We revised the text in the Introduction, Materials and Methods, Results, and Discussion (red colored part).

Herbarium voucher specimen of Oryeongsan (No. HBH112) was kindly provided from Korea Institute of Oriental Medicine, Daejeon, South Korea. Thus, we ensured its authenticity as an experimental material. We added in the Methods parts.

Firstly, to assess the effect of Oryeongsan on cytotoxicity in mesangial cells, the cells were pre-incubated with various concentrations (1 – 100 µg/ml) of Oryeongsan for 24 h. As shown in Fig. 1, Oryeongsan alone did not alter cell viability at the range of 1 – 100 µg/ml.

In addition, LDH assay was performed. As shown in Fig. 2, Oryeongsan alone did not alter LDL release level at the range of 1 – 200 µg/ml. In addition, Oryeongsan treated with HG (25 mM) did not change LDH release level. Thus, these results demonstrated Oryeongsan itself did not affect mesangial cell cytotoxicity.

Figure 1. Cell viability and cell number

Figure 2. LDH release by Oryeongsan with/without HG
We remove all mRNA data by RT-qPCR assay (Figure 2B and 6B) to improve and emphasize the credibility from the result of realtime RT-PCR. Thus, it is confirmed that Oryeongsan significantly decreased HG-induced ICAM-1 mRNA and protein expression in a dose dependent manner.

We marked arrows to point p-smad-2 translocations in Fig. 5

We improved western blotting of collagen IV in Fig. 2A and TIMP-2 in Fig. 3A. These data suggested that Oryeongsan significantly attenuated HG-induced Collagen IV and TIMP-2 expression. Thus, Oryeongsan suppress high-glucose induced renal fibrosis via the regulation of ECM accumulation in renal mesangial cells.

Actually Oryeongsan suppress high-glucose induced renal fibrosis and TGF-beta expression through regulating the NF-kB pathway. Emodin attenuates HG-induced TGF-β1 and fibronectin expression in mesangial cells through inhibition of NF-κB pathway (Ref 1). In addition, NF-κB/AP-1 pathways was involved in suppression of HG-induced proliferation and extracellular matrix accumulation in mesangial cells (Ref 2). Future study is required to clarify the regulation of IKK-αβγ complex on Oryeongsan.
