Author's response to reviews

Title: Investigation of the mechanisms of Angelica dahurica root extract-induced vasorelaxation in isolated rat aortic rings

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Author's response to reviews: see over
Revision letter

To the Editorial Officer:

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• Thank you for your advice.

Reviewer 2

Major

Q1. The authors say in material and methods that ACh was added, when tension reached peak, this is not indicated in figures 2 A and 2 B. Though the authors have revised the figure, How did the authors know it is the peak, they should have waited for plateau in the curve and then added ACh. Fig. 2?

Response: We agree with your suggestion. We added Ach when the tension almost reached a plateau. Therefore, we have modified the statement to read as “After contraction with PE…” (lines 107–108). Although we did not add ACh until the plateau was reached, we added ADE when the tension reached a plateau. (Supplementary Figure 1)
Q3. The answer should be included in the manuscript.
Response: We have included this in the Experimental Protocols (Lines 108–110).

Q5. The authors have not mentioned what they used for control. The authors say they used Krebs for control. The authors should mention the solvent in which the lyophilised ADE extract was reconstituted. Was the solvent used Krebs, if not your control must be the solvent.
Response: Normally, methanol or ethanol extracts do not completely dissolve in Krebs-Henseleit (K-H) solution. Therefore, we used dimethyl sulfoxide (DMSO) to dissolve the extracts in K-H solution in the previous studies. However, ADE was completely dissolved in the K-H solution in the present study.
Q6. The authors say that we collected 4 aortic rings from a single rat and used about 2-4 rats for a single set of experiment so if they even use 2 rats for a set of experiment number of aortic rings used must have been 8.

Response: We isolated 4 aortic rings from one rat and used 2 rings as the control group and 2 rings as the experimental group. In Figure 3, the control group comprised of 4 rings, while the ADE-treated group comprised of 8. In other words, 2 rats were used as the control group and 3 rats were used in the experimental group. We conducted various experiments and found that the contractions in the control group were not different from our previous experiments and there are little variations. Therefore, we used only 2 rats (4 rings) in the control group. In the in vivo experiment, many variations were observed in individual animals. However, in ex vivo experiments using aortic rings, there are little variations in individual animals. In Korea, minimum use of animals is recommended for animal welfare. We agree with your comments that more number of rings could be statistically significant. However, our experiments are ex vivo experiments using isolated rat aortic rings, and we believe that our results have statistical significance.

Q7. In the whole study the authors have established that ADE produces vasorelaxation which is independent of the endothelium. In line 191 the vasorelaxant effect is attributed to imperatorin. However in line 56 and reference 22 ' Imperatorin is responsible for the vasodilatation activity of Angelica dahurica var. Formosana regulated by nitric oxide in an endothelium-dependent manner. Chin J Integr Med. 2009;15(6):442–7. The authors should discuss the disparity in the result, it might be that the the ADE extract in the present study was methanol extract whereas in the above reference the extract were cyclohexane and ethyl acetate extracts and in methanolic extract the imperatorin conc. was low and therefore did not produce endothelium dependent relaxation.

Response: Thank you for your advice. Hong et al. suggested that before administration of 10 µmol/L phenylephrine, pretreatment of thoracic aorta rings with L-NAME 1 mmol/L, an NOS inhibitor, for 10 min reduced the imperatorin-induced (28 µmol/L) vasorelaxation” (Imperatorin is responsible for the vasodilatation activity of Angelica dahurica var. Formosana regulated by nitric oxide in an endothelium-dependent manner. Chin J Integr Med. 2009;15(6):442–7.). However, Bertin et al. showed that atropine and L-NAME did not alter imperatorin-induced relaxation (Bertin R, Chen Z, Martinez-Vazquez M, Garcia-Argeaz A, Froldi G. Vasodilation and radical-scavenging activity of imperatorin and selected coumarinic and flavonoid
compounds from genus Casimiroa. Phytomedicine. 2014;21(5):586–94.). Furthermore, Hong et al. used mice, but Bertin et al. used rats. Therefore, our results are more consistent with Bertin et al. than Hong et al. Thus, we did not discuss the disparity in the results, as indicated.

Minor

1. In line 54 define qi.
Response: WHO defined that the Qi is the basic element that constitutes the cosmos and, through its movements, changes and transformations, produces everything in the world, including the human body and life activities. In the field of medicine, qi refers both to the refined nutritive substance that flows within the human body as well as to its functional activities (WHO international standard terminologies on traditional medicine in the western pacific region). Qi is a proper noun and the explanation is lengthy. Therefore, we modified qi to Qi and did not include an explanation.

2. Line 60 it should be probable instead of probably
Response: We have modified the term to “probable” as suggested.

3. Line 76, The solvent in which crude extract was dissolved must be mentioned
Response: As suggested, we have modified the statement to read as “The crude extract was dissolved in Krebs-Henseleit (K-H) solution when applying to aortic rings in the organ chamber.” (lines 76–78).

4. In section Animal (line 81, total number of rats used must be specified.
Response: We have stated that 30 rats were used in the experiment (Line 91).

5. In line 95, 96, the isometric force transducer must be used uniformly.
Response: We have modified as suggested.
Sincerely,

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