Author's response to reviews

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

Authors:

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Author's response to reviews: see over
Revise letter

To the Editorial Officer:

BMC Complementary and Alternative Medicine

• Thank you for your advice.

Reviewer 1

1. The authors are in need of increasing the contents of intracellular Ca conc.
2. The relationship of Ca concentration with The vasoactivity is needed to test.

: Thank you for your advice. In the present study, we investigated the vasoactivities of ADE on extracellular Ca\(^{2+}\)-induced contraction via receptor-operated Ca\(^{2+}\) channel (ROCC) and voltage-dependent calcium channel (VDCC) (Fig. 3) and on intracellular Ca\(^{2+}\) release-induced contraction via specific inositol triphosphate receptor (IP\(_3\)R) channel or ryanodine receptor (RyR) channel (Fig. 4) in Ca\(^{2+}\)-free K-H solution. We agree that including data on Ca\(^{2+}\) concentration experiments would help improve the paper. However, we would like apologize since we do not have the equipment for measuring Ca\(^{2+}\) concentration. Thus, it would not be possible for us to include data regarding Ca\(^{2+}\) concentration. Although our study did not show the relationship between Ca\(^{2+}\) concentration and vasoactivity, the experiments performed in our study provided evidence of vasoactivities of herbal medicines on Ca\(^{2+}\) channels.

3. The figures are in poor quality.

: We modified figures 1, 2, and 4.

Reviewer 2

1. Title: The title is very simple and does not represents the major goal of this work. The vasorelaxant effect has already reported for this plant. Therefore, reference the title to either the investigation of mechanisms or even the action on transmembrane calcium influx could be more suitable for the work, and then increase the number of readers and citations.
Thank you for your advice. We have revised the title as “Investigation of the mechanisms of *Angelica dahurica* root extract-induced vasorelaxation in isolated rat aortic rings”. In addition, we have added citations.

2. Line 18: "has been used"
   - We have revised this per your suggestion.

3. Line 20: Please, the sentence needs a revision. As suggestion: "Interestingly, it has been used in the treatment of".
   - Thank you for your advice. We have revised this per your suggestion.

3. Line 24 and 25: This sentence should be revised. "The effects... were assessed..." is better suitable instead of "We assessed the effects...". The sentence "...on several vasorelaxant and vasoconstrictive factors..." does not have any sense to me, considering only vasoconstrictors was used. Then, I suggest report the effects... and its underlying mechanisms on rat thoracic aorta".
   - We have revised the sentence as “The vasorelaxant effects of a 70% methanol extract of the *A. dahurica* root (ADE) on rat thoracic aorta and its underlying mechanisms were assessed.”

4. Lines 28 and 30: I recommend to remove the words "hydrochloride" and "chloride" in this context due to any pharmacological relevance is attributed to this ions in the studied protocols. The, it is not necessary to report along the manuscript, but just in "Chemicals and Drugs" section. Please, revise it throughout the manuscript.
   - We have revised this per your suggestion.

5. Line 49: Please, use another word to correct this sentence, such as "furthermore", "moreover", etc. An English assistance throughout the manuscript might be useful.
   - We have revised this per your suggestion.

6. Line 51: As suggestion: "due to".
   - We have revised this per your suggestion.
7. Line 53: Please, see comments for Line 49.
   : We have revised this per your suggestion.

8. Line 58: Determine the "exact mechanism" imply in additional protocols with calcium channels activators as well as evaluation of expression de proteins or patch clamp measurements. Therefore, I suggest "... the probably mechanisms involved in" is better suitable.
   : We have revised this per your suggestion.

9. Line 60: This sentence is not related to the current protocols in this paper. Then, I recommend to remove it.
   : We have removed this sentence.

10. Line 82: Please write "at the ratio of 6:4".
    : We have revised this per your suggestion.

11. Line 86: Please write "Male Sprague-Dawley rats.....were used". Line 86 and 87: This sentence can be removed from the text.
    : We have replaced this sentence with “Male Sprague-Dawley rats (Narabio, Seoul, Republic of Korea) weighing 240–260 g were housed….” (line 88-89)

12. Line 90 and 91: I recommend transfer this sentence to the next section, "Experimental Protocols", Line 94. Then, the section should be named just "Animals".
    : We have revised this per your suggestion.

13. Line 95: Please, state the name of the solution before the symbol, as: "Krebs-Henseleit (K-H)".
    : We have revised this per your suggestion.

14. Line 100: I suggest "in order to". Please, revise the use of this sentences throughout the manuscript.
    : We have revised this per your suggestion (Line 103, 110, and 137).
15. Line 107 a 110: I suggest "in order to". It is better to connect with the next sentence, because you are justifying it. Then, I also suggest "the contractile activity...... were investigated on endothelium-denuded..... after pretreatment with ADE...." Please, following this recommendations in the whole manuscript, in order to improve the quality of profwritng.

: We have revised this per your suggestion (Line 110, and 113-114).

Reviewer 3

1.- In Abstract, Methods section authors should include the pharmacological model used in the present study.

: We included he pharmacological model used in the present study in Abstract, Methods section. “Isolated rat aortic rings were suspended in organ chambers containing 10 ml Krebs-Henseleit (K-H) solution and placed between 2 tungsten stirrups and connected to an isometric force transducer. Changes in tension were recorded via isometric transducers connected to a data acquisition system.”

2.- In the Results section, authors mentioned that they identified four components by coelution in the methanol-soluble extract, but in the Discussion section ther is not any mention about the phytochemical characterization of the methanol extract of A. dahurica. And besides, the knowledge about the most abundant components in the methanol-soluble extract is missing.

: We added discussion about ADE components. “Oxypeucedanin, oxypeucedanin hydrate, imperatorin, and isoimperatorin are well known components of A. dahurica root [37-40]. In the present study, these four components were also found in ADE and the contents were calculated as 5.9%, 0.23%, 0.63%, and 0.43%, respectively. Imperatorin is a well-known anti-hypertensive compound [41-43]. Therefore, the vasorelaxant effects of ADE might be partly attributable to imperatorin. Although oxypeucedanin was the most abundant component in ADE, its vasorelaxant or anti-hypertensive activities have not been reported yet. Oxypeucedanin hydrate and isoimperatorin also have not been reported to have vasorelaxant activities or anti-hypertensive activities. Therefore, further phytochemical and pharmacological studies of ADE are needed.”

Reviewer 4
Major

1. 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.

: We have revised the figures 2 A and 2 B to show the addition of Ach. In addition, we explained it in the Figure 2 legend. “PE-induced contraction of endothelium-intact aortic ring almost relaxed to baseline after acetylcholine treatment (Ach, 10 µM) (A). PE-induced contraction of endothelium-denuded aortic ring remained unaffected after acetylcholine treatment (B).”

2. The methodology does not state how the data was recorded. A more detailed methodology would be appreciated.

: We included the detailed methodology in Methods-Experimental Protocols section. “Isolated rat aortic rings were suspended in organ chambers containing 10 ml Krebs-Henseleit (K-H) solution and placed between 2 tungsten stirrups and connected to an isometric force transducer. Changes in tension were recorded via isometric transducers connected to a data acquisition system.”

3. What was the rationale of using 0.03, 0.1, 0.3,1 and 3mg/ml concentration of ADE for the present study?

: In the previous screening study, ADE did not relax PE-precontracted aortic rings at dose of 0.001, 0.003, and 0.01 mg/ml. Vasorelaxant effects of ADE was observed at the dose of 0.03 mg/ml. Therefore, we selected these doses.

4. The methodology for Effect on ADE on extracellular calcium and SR calcium release is so ambiguous that it becomes difficult to interpret the result. Nowhere in the Effect on ADE on extracellular calcium, it is mentioned whether calcium free krebs was used or not. The author must respond to these before a decision on publication can be reached. For example, additional necessary experiments or controls, statistical mistakes, errors in interpretation.

: The vasorelaxant effects of ADE on extracellular calcium and SR calcium release were investigated in Ca^{2+}-free K-H solution. We have revised this sentence (Line 114). This method was not described in detail in the present study. We cited previous work here since

4-1. The authors have not mentioned what they used for control.

: Thank you for your advice. We have revised the figure legends to include information regarding control group. Figure 1: Control groups were treated with the same volume of Krebs-Henseleit (K-H) solution without ADE; Figure 3 and 4: Control groups were not pre-incubated with ADE.

4-2. The authors have not mentioned anywhere in the manuscript the number of rats they have used. In the legend they have indicated that n ranges from 4 to 8. If n is the number of aortic rings studied, then 4 is not statistically significant and the results can be suggestive but not conclusive. We get about 8 to 12 thoracic aortic rings from a single rat. If the authors have used only 4-8 rings, it means the animals used was only one for a single set of experiment. This does not give diversity to the study and the results are only pilot studies and can not be authentic.

: We have only 4 organ chambers. Therefore, we collected 4 aortic rings from a single rat and used about 2-4 rats for a single set of experiment (In figure 3, N=4 is control group and N=8 is ADE-treated group). Furthermore, isolated aortic rings have little variation. Therefore, we think that our results could be conclusive and statically significant.

Minor Essential Revisions

1. There are several minor corrections to be made like nowhere in the manuscript full form of ADE is given

: We corrected several mistakes as pointed out by you and the other reviewers. The full name of AED was described in Experimental Protocols [Line 96-97, a 70% methanol extract of the *A. dahurica* root (ADE)].

Sincerely,

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