Author's response to reviews

Title: Acute oral toxicity of Insampaedok-san, a traditional herbal formula, in rats and its protective effects against ovalbumin-induced asthma via anti-inflammatory and antioxidant properties

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Author's response to reviews:

<Original comments>: black, <Revised works>: blue

MS: 3830197191152464 entitled "Acute oral toxicity of Insampaedok-san, a traditional herbal formula, in rats and its protective effects against ovalbumin-induced asthma via anti-inflammatory and antioxidant properties" submitted to BMC Complementary and Alternative Medicine.

Dear Tom Rowles

Thank you very much for your editorial decision letter, which also included the important point. We have made the changes as suggested by the editor. The changes are marked in blue in the revised text. We have made during the revision in a point-by-point response to each of the comments.

We hope the revisions made the responses provided are satisfactory, and our manuscript is now acceptable for publication in the BMC Complementary and Alternative Medicine.

Please, let us know if further revisions are needed.

Once again, thank you for all your help. We look forward to hearing from you.

Sincerely yours,

cozy37@gmail.com

Reviewer's report:

Minor Essential Revisions

This study investigated the potential acute toxicity of an Insampaedok-san water extract in rats and the antiasthmatic effects of ISSE in a model of asthma induced by ovalbumin in mice. Generally, this paper was well written. However, the authors will need to add or correct some description, as follows.
Abstract
The 1st sentence of Methods “In a safety study, ISSE was administrated via oral gavage once daily to rats of both sexes at doses of 0 and 5000 mg/kg for 15 days.” must be modified as follows: In a safety study, ISSE was administered orally to rats of both sexes at single doses of 0 and 5000 mg/kg.

#We appreciate your comment. As commented by reviewer, we edited the sentence.

Results, line 2: “activityand” should be “activity and”

Conclusions, line 1: The sentence “ISSE is safe for human consumption” is not true.

#We appreciate your comment. As commented by reviewer, we edited the sentence.

The sentence should be modified as follows: The approximate lethal dose of the ISSE is >5,000 mg/kg in rats.

#We appreciate your comment. As commented by reviewer, we edited the sentence.

Methods
Animals for acute oral toxicity study section, lines 6 and 8: please change “KRICT” to “KIT”

#We appreciate your comment. As commented by reviewer, we edited the sentence.

Under Animals and experimental procedure, the sentence that describes the treatment of each group is confusing. The authors need to include a time line of the events (OVA i.p and challenge, initiation and cessation of treatment, sacrifice, etc) for clarity purpose. A table which describe each group and what they got, when might be more useful than description of procedure.

#We appreciate your comment. As commented by reviewer, we edited these statements.

Why was the dose (100 and 200 mg/kg) of ISSE chosen? The rationale for choosing 100 and 200 mg/kg of ISSE in present study may be explained.

# We appreciate your comment. In preliminary study, animals were administered by oral gavage at doses of 150 and 300 mg/kg of ISSE. The protocols of preliminary study were the same as those of present study. At the scheduled termination, we measured the IgE and inflammatory cells.

In addition, the authors used "montelukast" as a positive control drug. However, there was no description that montelukas is used as a positive control drug. The authors would explain why montelukast is used as a positive drug.

# We appreciate your comment. Montelukast was developed as a cysteinyll leukotriene (cys-LT)-1 receptor antagonist [1] and was introduced into the market after successful clinical evaluation in patients with aspirin-sensitive asthma, nocturnal exacerbation of asthma, and allergic asthma [2].


The number of mice per group should be presented either in the M&M or in each of the figure legends.

How was eosinophil infiltration or mucus production quantified in the histological studies? The data were not immediately obvious from the histology slides. It may be helpful to readers to highlight the areas of interest in these slides via arrows or other visual aid.

Please describe the post-hoc statistical analysis in detail.

Reviewer’s report:

Major justification which is must for author

1. Abstract are not uniform as well as very much confusing and unstructured

2. Author use whole plant or part of plant mention it’s not mention any where

3. Author use 100 degree centigrade temperature for evaporating the water from the extract. Than author justify how phenol and flavones constituent are there in this extract because it’s decomposes after 55 degree temperature.

4. Which phyto-constituents are responsible for anti asthmatic activity?
Sclerotium, Glycyrrhizae Radix et Rhizoma, Menthae Herba, and Zingiberis Rhizoma Crudus. Several components of ISS, such as Ginseng Radix [18], Aralia continentalis Radix [19], Aurantii Fructus Immaturus [20], Platycodonis Radix [21], and Cnidii Rhizoma [22]. Iss composed 4 marker compounds, ferulic acid (Cnidium officinale Makino), hesperidin (Citrus aurantum Linne), 6-gingerol (Zingiber officinale Roscoe) and glycyrrhizin (Glycyrrhiza uralensis Fischer). These compounds have effects such as, anti-asthma, anti-oxidant, and anti-inflammation.

5. In acute toxicity author follow which OECD guideline which one 420, 423 etc its must.

#We appreciate your comment. As commented by reviewer, we added this statement and the OECD guideline 423.

6. What sort of behavioral activity is indicative for toxicity.

#We appreciate your comment. As commented by reviewer, we edited this sentence ‘We found no treatment associated abnormal clinical signs’.

7. Dose is not selected on the bases of GHS guideline.

#We appreciate your comment. ISSE is herbal medicine, unfortunately we did not selected the GHS guideline. If you comment about GHS guideline, we consider after experiment about GHS guideline.

8. Animal ethical clearance no is not mention by author.

#We appreciate your comment. As commented by reviewer, we added this statement

9. Why author use montelukast its IL-4 Specific antagonist I don’t think its target IL-5 you have to give justification.

# We appreciate your comment. Montelukast is not a IL-4 specific antagonist. In this study, we just use positive control for asthma drug. Montelukast was developed as a cysteinyl leukotriene (cys-LT)-1 receptor antagonist [1] and was introduced into the market after successful clinical evaluation in patients with aspirin-sensitive asthma, nocturnal exacerbation of asthma, and allergic asthma [2].


10. On what bases author selected dose of Montelukast 30 mg/kg.

#We appreciate your comment. We selected the montelukast dose based on published articles such as “Shin IS, Shin NR, Jeon CM, Hong JM, Kwon OK, Kim

11. Proper justification needed for antioxidant activity in anti asthmatic how author correlate this without phyto constituent isolation and which constitute are responsible.

#We appreciate your comment. Many researcher published that oxidative stress is a major feature of asthma and anti-oxidant therapy can treatment of asthmatic effects. ISS is a traditional herbal medicine has a history of thousands of years for Asian country and it has composed 12 herbs. Our present study, just verify of ISSE about anti-oxidant and anti-asthmatic effects. ISS is composed of 12 herbs, Radix Ginseng, Bupleuri Radix, Angelica decursiva Radix, Osterici Radix, Aralia continentalis Radix, Aurantii Fructus Immaturus, Platycodonis Radix, Cnidii Rhizoma, Poria Sclerotium, Glycyrrhizae Radix et Rhizoma, Menthae Herba, and Zingiberis Rhizoma Crudus. Several components of ISS, such as Ginseng Radix [18], Aralia continentalis Radix [19], Aurantii Fructus Immaturus [20], Platycodonis Radix [21], and Cnidii Rhizoma [22], have been reported to possess antioxidant effects. Therefroe, each herb’s constitute component are very important, honestly in this study we just want to know about ISSE effects.

12. Standardization of extract by which method at least formulation it should be must.

#We appreciate your comment. ISS is a traditional herbal medicine has a history of thousands of years for Asian country and it has composed 12 herbs. ISS is composed of 12 herbs, Radix Ginseng, Bupleuri Radix, Angelica decursiva Radix, Osterici Radix, Aralia continentalis Radix, Aurantii Fructus Immaturus, Platycodonis Radix, Cnidii Rhizoma, Poria Sclerotium, Glycyrrhizae Radix et Rhizoma, Menthae Herba, and Zingiberis Rhizoma Crudus. Several thounds years ago, this formulation already established.

13. I think author deign that model so I request author please send the validated parameter because in whole animal model part author is not given any reference. So as per my thinking author deign that animal model. Highly questionable.

#We appreciate your comment. Honestly, we are very hard to understand the referee’s comment. This experimental model established for other researcher’s design with some modification.

14. Discussion is very weak regarded plant constitution

#We appreciate your comment. As commented by reviewer, we edited the discussion.

15. Author must needed to analyst plant properly or need justification how its work on inflammatory path way of asthma.

#We appreciate your comment. In the present our study, we just justify the effects of ISSE. Further study needed the mechanism of asthma.
16. Author saying because of antioxidant property of plant its show inhibition of inflammatory pathway Via ROS and RNS pathway. But my Question is that how its possible without mention the major constituents.

#We appreciate your comment. Many researcher published that oxidative stress is a major feature of asthma and anti-oxidant therapy can treatment of asthmatic effects. ISS is a traditional herbal medicine has a history of thousands of years for Asian country and it has composed 12 herbs. Our present study, just verify of ISSE about anti-oxidant and anti-asthmatic effects. ISS is composed of 12 herbs, Radix Ginseng, Bupleuri Radix, Angelica decursiva Radix, Osterici Radix, Aralia continentalis Radix, Aurantii Fructus Immaturus, Platycodonis Radix, Cnidii Rhizoma, Poria Sclerotium, Glycyrrhizae Radix et Rhizoma, Menthae Herba, and Zingiberis Rhizoma Crudus. Several components of ISS, such as Ginseng Radix [18], Aralia continentalis Radix [19], Aurantii Fructus Immaturus [20], Platycodonis Radix [21], and Cnidii Rhizoma [22], have been reported to possess antioxidant effects. We couldn’t find that sentence ‘Because of antioxidant property of plant its show inhibition of inflammatory pathway Via ROS and RNS pathway. Please, let us know if further revisions are needed.

17. As per me all plants having our own defense mechanism so obviously each plant show some antioxidant property.

#We appreciate your comment. ISS is composed of 12 herbs, Radix Ginseng, Bupleuri Radix, Angelica decursiva Radix, Osterici Radix, Aralia continentalis Radix, Aurantii Fructus Immaturus, Platycodonis Radix, Cnidii Rhizoma, Poria Sclerotium, Glycyrrhizae Radix et Rhizoma, Menthae Herba, and Zingiberis Rhizoma Crudus. Several thousands years ago, this formulation already established.

18. Which type of structural cell arrangement observe by the author example in asthma histology Goblet cells were observe by PAS stain shows structural changes or arrangements like Mataplastic , hyperplastic etc. author is not clarify that point. Histology is not clear and well labeled. And H&E stain is specially use for normal architecture of tissue.

#We appreciate your comment. Figure 3a and 3b showed the H&E and PAS staining, as respectively. We added the mark at the figures.

19. That much eosinophile infiltration shows that how much potent challenge done by author how animal survives?

#We appreciate your comment. At this study, in vivo asthmatic experiments, there was no death animals.

Minor correction
1. Inappropriate referencing in introduction page no three paragraph 2 line no 7 author mention reference no 43 I think author start preparing paper from backside.

#We appreciate your comment. As commented by reviewer, we edited the reference.
2. Improper alignment of manuscript.
   #We appreciate your comment. As commented by reviewer, we edited the alignment of manuscript.

3. Work is not up to the mark.
   #We appreciate your comment. However, we did not understand the exact meaning. Please, let us know the meaning more easily.

4. References are unstructured
   #We appreciate your comment. As commented by reviewer, we edited references

5. Manuscript writing is not uniform.
   #We appreciate your comment. As commented by reviewer, we edited the manuscript of uniform.

Reviewer's report:
Minor Essential Revisions