Format for ANSWERING REVIEWERS

March 8, 2017

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 32465-review.doc).

Title: Anti-apoptotic effect of Banhasasim-tang on the chronic acid reflux esophagitis

Author: Mi-Rae Shin, Hyo-Jin An, Bu-Il Seo, Seong-Soo Roh

Name of Journal: *World Journal of Gastroenterology*

ESPS Manuscript NO: 32465

Dear Editor,

Name of journal: World Journal of Gastroenterology

Manuscript NO.: 32465

Column: Evidence-Based Medicine

Title: Anti-apoptotic effect of Banhasasim-tang on the chronic acid reflux esophagitis-induced esophageal mucosal ulcer

Authors: Mi-Rae Shin, Hyo-Jin Ahn, Bu-Il Seo and Seong-Soo Roh

Correspondence to: Dr. Seong-Soo Roh

Reviewer code: 00504462, 00831621 and 02438888

First decision: 2017-02-23 09:17

Scientific editor: Ya-Juan Ma

Thank you for your comments regarding our manuscript. We have studied the reports of the editor and reviewers carefully, and have revised the manuscript accordingly (the revised parts are highlighted). The responses to the comments are as follows:

Reviewer 00504462 : Dear Sir, I want to congratulate you for your research. It is worth publishing. However, there are some points that would be worth discussing further. For instance, ① it might be worth mentioning the pharmacological actions of the seven herbs in BHSST which are different alone or in combination. ② You may also want to discuss whether the bioactive molecules are metabolised before it reaches the esophageal mucosa. Finally it is worth highlighting research about the anti-inflamatory properties of this formula. There are some questions left unanswered. You have not explained whether the action of BHSST is local or systemic, direct or indirect. Furthermore, it would be good to clarify whether BHSST has any effect on GI motility, gastric acid and pepsin secretion, or on esophageal sensitivity. I understand that these are questions you many not be able to answer at this time, but it would be good for you to clarify this, ③ or at least mention these as limitations of the study. It would also be good if you could outline or discuss future research goals derived from this manuscript. Thank you for sending it to us, and hope to hear from you soon in order to print it. Sincerely (conclusion : minor revision)

Response 1:

① I agree about it profoundly. So, I mentioned the pharmacological actions of the seven herbs in BHSST additionally and inserted new reference associated with such action. [lines 236-240 page 10].
② In this experiment, we administrated BHSST for 15 days consecutively. Namely, this study is not acute reflux esophagitis but chronic reflux esophagitis. Accordingly, I think that it doesn't matter whether the bioactive molecules are metaboished before it reaches the esophageal mucosa. At present study, BHSST treated mice had an enough time for absorption, distribution, and metabolism of BHSST.
③ I thought so, too. I strongly feel a need about further research related to GI motility, gastric acid and pepsin secretion, or esophageal sensitivity. So, we mentioned such limitaions of this study as follows.

However, the relationship between the prevalence of esophageal mucosal ulcer and another factors such as GI motility, gastric acid and pepsin secretion, or esophageal sensitivity is unknown, so further study about this relationship is necessary.

And we added it in this manuscript. (lines 305-308 on page 13)

Reviewer 02438888 : GERD is a common GI disease which can lead to erosion or ulcer in esophagus. Continuous esophagitis may cause Barrett lesion that is considered as precancerous lesion. Therefore, treatment of GERD is of clinical importance. A herbal formula, known as BHSST, is effective in treating GERD, but the specific mechanism is not clear. The authors tried to elucidate the underlying mechanism by a rat model. Their experiments showed that BHSST could abolish the increase of NADPH oxidase subunit due to CRE exposure. BHSST could also regulate some apoptosis-related genes and exert anti-apoptotic effect. Finally, the authors concluded that BHSST can suppress esophageal ulcer via regulating ROS-dependent apoptosis. This study may provide new regimen for patients suffering from esophageal ulcer and help clinical practitioner understand the relevant mechanism. BHSST includes 13 bioactive components, so the therapeutic mechanism of BHSST must be complex. Further studies are needed to elucidate the mechanism thoroughly. (conclusion : accept)

Response 2: I really appreciated your opinion. In the present study, some limitaions existed. So, we mentioned such limitaions of this study and revealed a need of further research in this manuscript. (lines 305-308 on page 13)

Reviewer 00831621 : 1. Has Banhasasim-tang been used to treat esophageal mucosal ulcer now? If it has, some clinical data or images of endoscopy are more convincing. 2. This research just shows us the result of BHSST, but the mechanism of it is not mentioned in the text. For example, why can the BHSST abolish NOX4 and p47phox? What is the mechanism? (conclusion : rejection)

Response 3: 1. Banhasasim-tang (BHSST; Hange-shashin-to in Japanese Traditional Kampo Medicine; Banxia-xiexin-tang in Traditional Chinese Medicine) has been used as an herbal prescription to improve dyspepsia, gastric ulcerative disorders, laryngopahryngitis, colitis, and diarrhea [1-4]. However, the protective mechanisms of BHSST treatment in esophageal ulcer by chronic reflux are not fully understood. Therefore, we investigated the effects of BHSST on rats with chronic acid reflux esophagitis (CRE) to examine its ameliorating effect. Based on our data, BHSST treatment was showed the amelioration of esophageal mucosal ulcer which is one of chronic GERD symptom via regulating

ROS-dependent apoptosis. Therefore, these data may be provide a scientific basis for BHSST to expand the indication in the management of GERD field. Accordingly, we think that this study is a new attempt and outline. Maybe, clinical practitioner can apply for treating the symptom associated to esophageal mucosal ulcer in the future. Then, clinical data or images of endoscopy will be reported afterward. At present, we performed a basic study not a clinical study.

[1] **Kawashima K**, Nomura A, Makino T, Saito K, Kano Y. Pharmacological properties of traditional medicine (XXIX): effect of Hange-shashin-to and the combinations of its herbal constituents on rat experimental colitis [PMID: 15467203]

[2] **Xu G**. Treatment of reflux laryngopharyngitis with modified banxia xiexin tang (Pinellia decoction for draining the heart)--a report of 40 cases. *J Tradit Chin Med* 2006;**26**:127-131 [PMID: 16817279]

[3] **Lee KG**, Cui Xi, Lim JP. Effect of the concurrent administration of Banhasasim-tang with cimetidine on gastric ulcer in rats. *Korean J Orient Physiol Pathol* 2002;**16**:572–576

[4] **Kase Y**, Saitoh K, Makino B, Hashimoto K, Ishige A, Komatsu Y. Relationship between the antidiarrhoeal effects of Hange-Shashin-To and its active components. *Phytother Res* 1999;**13**: 468-473 [PMID: 10479755]

2. Recent studies have reported that oxidative stress has a more important role than acids in the pathogenesis of reflux esophagitis [5,6] So, we focused on such direction. We examined biomarkers associated with oxidative stress in serum and esophageal tissue. As the result, the administration of BHSST significantly reduced both the overexpression of serum reactive oxygen species (ROS) and a excessive formation of thiobarbituric acid-reactive substances (TBARS) in esophagus tissue.

These data are as follows (Table1).

Table 1 Biomarkers associated with oxidative stress

Group	Serum ROS (fluoresence/min/mL)	Serum TBARS (nmol/mg protein)	Esophageal TBARS (nmol/mg protein)
Normal rats	$181.8 \pm 10.0^{**}$	3.6 ± 0.1	0.61 ± 0.01
CRE rats			
Control	$278.6 \pm 25.5^{\#\#}$	$6.6 \pm 0.8^{\#\#}$	$0.75 \pm 0.03^{\#\#}$
BHSST	$133.6 \pm 59.2^{*}$	5.3 ± 0.2	$0.56 \pm 0.02^{***}$

Normal, normal rats; Control, chronic acid reflux esophagitis rats; BHSST, BHSST 1 g/kg body weight/day-treated chronic acid reflux esophagitis rats.

Data are mean ± S.E.M. (n=6) Significance: $^{\#\#}P<0.01$ versus normal rats and $^{*}P<0.05$, $^{***}P<0.001$ versus control rats.

Especially, originally ROS were recognized as being instrumental for mammalian host defense, and

early work led to the characterization of the respiratory burst of neutrophils and originally the nicotinamide adenine dinucleotide phosphate (NADPH) oxidase complex, which is now recognized as a primary source of ROS [7-9]. Accordingly, the abolishment of NOX4 and p47phox (subunits of NADPH oxidase) plays a important role in this experiment. Also, we will conduct further studies are needed to elucidate the mechanism thoroughly.

[5] **Oh TY**, Lee JS, Ahn BO, Cho H, Kim WB, Kim YB, Surh YJ, Cho SW, Lee KM, Hahm KB. Oxidative stress is more important than acid in the pathogenesis of reflux oesophagitis in rats. *Gut* 2001;**49**:364-371 [PMID: 11511558]

[6] **Kim YJ**, Kim EH, Hahm KB. Oxidative stress in inflammation-based gastrointestinal tract diseases: challenges and opportunities. *J Gastroenterol Hepatol* 2012;**27**:1004-1010 [PMID: 22413852 DOI: 10.1111/j.1440-1746.2012.07108.x]

[7] **Feagins LA**, Zhang HY, Zhang X, Hormi-Carver K, Thomas T, Terada LS, Spechler SJ, Souza RF. Mechanisms of oxidant production in esophageal squamous cell and Barrett's cell lines. *Am J Physiol Gastrointest Liver Physiol* 2008;**294**:G411-G417 [PMID: 21525435]

[8] **Genestra M**. Oxyl radicals, redox-sensitive signalling cascades and antioxidants. *Cell Signal* 2007;**19**:1807-1819.

[9] **Cheng HL**, Lee YH, Yuan TM, Chen SW, Chueh PJ. Update on a tumor-associated NADH oxidase in gastric cancer cell growth. *World J Gastroenterol* 2016;**22**:2900-2905.

[Additional Modifications]

1. We revised the number of reference because of the insertion of new references additionally.
The existed references are from 23 to 36. But, 23 changes to 30, 36 changes to 43.

In addition, new reference is added to no. 23 ~29 in our manuscript.

(line 412 [23], 415 [24], 419 [25], 423[26], 426[27], 429[28], 432[29]) on pages 16-17, new reference)

2. Because the title of Evidence-Based Medicine should no more than 12 words, we revised our title as follows.

The existed title : Anti-apoptotic effect of Banhasasim-tang on the chronic acid reflux esophagitis-induced esophageal mucosal ulcer

<u>The revised title</u> : **Anti-apoptotic effect of Banhasasim-tang on the chronic acid reflux esophagitis**

The comments from the editor and reviewers were valuable and helpful to revise the manuscript. We believe that the revised manuscript, being sent herewith, is a marked improvement. Therefore, we hope that the revised manuscript is now acceptable for publication. Thank you very much for your consideration.

Yours sincerely,

Mi-Rae Shin and Prof. Seong-Soo Roh