Author’s response to reviews

Title: Protective effect of Sheng-Mai Yin, a traditional Chinese preparation, against doxorubicin-induced cardiac toxicity in rats

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Version: 1 Date: 07 Oct 2015

Author’s response to reviews:

Reviewers' comments:

Reviewer #2:

Introduction

1. Page 4 line 4 should read "Unfortunately, clinical applications of this drug are restricted ….."

Answer: Thanks for this suggestion. We have revised this sentence according to this suggestion.

2. Page 4 line 17 should read "Hence, further therapeutic agent development especially the…..in complementary and alternative medicine (CAM) have been regarded as the focus in the amelioration of DOX induced cardiac damage.”

Answer: Thanks for this suggestion. We have revised this sentence according to this suggestion.

3. On page 5 lines 9-11 the authors mentioned the anti-apoptosis effect of Shengmai injection via the reduction of Bax protein and increase in Bcl-2 protein. Apoptosis is one of the mechanisms by which chemotherapeutic agents control cancer. This suggested mechanism of Shengmai injection may potentially have a negative effect on tumor control. I suggest the authors should address this issue.

Answer: Thanks for this suggestion. We have revised this sentence according to this suggestion.
Answer: Thanks for this interesting speculation. Two factors that may have contributed to our explanations about this seemingly contradictory problem include the following: (1) As known, the basic way of the effect of Chinese medicine including Chinese herbs and acupuncture is regulating the body’s condition, characterized by whole regulation and dual directional regulation [1]. The dual directional nature refers that the regulation between two systems is dual directional, and the regulation can be positive or negative. The divergent nature refers that an environmental change can cause regulatory reaction of multiple systems. The polymerized nature refers that a cell can receive a variety of control signals from different systems. We use Radix ginseng, one component of Shengmai as an example. Low dose of ginseng could make the blood pressure of anesthetized animals slightly increased, but the high dose decreased the blood pressure [2]. Similarly, we have reason to speculate that Shengmai Yin also has dual directional regulation for tumor apoptosis, which means that in a certain dose range, Shengmai Yin may not inhibit tumor apoptosis. (2) The given reference about the reduction of Bax protein and increase in Bcl-2 protein referred to the response of myocardial cells to the Shengmai Yin, not tumor cells. Different type of cells may have different response to the herbs.

4. Page 5 line 20 should read "The study protocol was approved by….."

Answer: Thanks for this suggestion. We have revised this sentence according to this suggestion.

Materials and Methods

1. The authors did not include any information about sample size calculation

Answer: Thanks for this suggestion which we have already answered in the first revision. This time, we included this answer in the manuscript (shown in the page6, line 3-7).

2. Page 6 line 1 should read "Thirty adult Sprague-Dawley male rats (SPF) grade weighing 235 ± 20g……"

Answer: Thanks for this suggestion. We have revised this sentence according to this suggestion.

3. On Page 6 lines 3 - 6, the authors described the number of rats in each group. The authors did not explain how many rats were administered low, medium and high DOX+SMY. They only mentioned the total in the group (10).

Answer: Thanks for this suggestion. We have included this information in the manuscript (shown in the page6, line 9-11).

4. The authors mentioned that the herbal materials to prepare SMY were obtained from Heyanling Chinese Herbal Medicine Co Ltd. The quality of this study can be enhanced by describing how the mixture was standardized in their laboratory. Readers need to know that authors have made all efforts to reduce bias by making sure there are no contaminants in the herbs. Secondly, the contents of the SMY mixture must be similar. This will improve the internal validity of the study.
Thanks for this advice. Heyanling Chinese Herbal Medicine Co Ltd. (http://heyanling.com/) acquired Chinese herbal medicine GMP certification in Beijing in November 2011, which ensured no contaminants in the herbs. To keep the consistency of the herbal chemical ingredients, all of the herbal components were originally obtained from the standard native sources as stated above with GAP grade. In addition, the liquid of SMY was sterilized by 0.22 μm filter before the administration. This process also ensured no contaminants in SMY. Also, all of these herbal materials were identified by School of Pharmacy, Tianjin University of Traditional Chinese Medicine. A voucher specimen of each species was also deposited at Tianjin University of Traditional Chinese Medicine. The above efforts were also included in the manuscript (last paragraph in page 6)

5. Page 6 line 16 should read ".....SMY was 0.375g/ml..."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

6. Page 6 line 21 should read "....over a 2 week period (total six injections)...."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

7. Page 7 lines 1 - 3 describes the dose used in the experiment. The authors should mention the adult dose and the equivalent in rats. In addition, they need to explain the rationale for choosing the low and high doses.

Answer: Thanks for this advice. We have included the information in the manuscript (page 7, line 5-6 and page 7, line 10-12)

8. Page 7 line 4 should read "Control animals received the vehicle (saline) only."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

9. Page 7 line 6 should read "All the rats had echocardiographic assessment and were sacrificed the day after the last SYM administration.

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

10. On page 7 the authors seem to use Left Ventricular Mass index and Left ventricular weight index interchangeably. The literature refers to Left Ventricular Mass index (LVMI). In addition, the measurement of the LVMI is LVW/Body surface area or LVW/height^2.7.

Answer: Thanks for this advice. We have revised LVWI as LVMI throughout the manuscript (shown in the track changes)

11. Page 7 line 17 should read "The echocardiography...."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.
12. Page 7 line 22 should read "…end of experiment."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

13. Page 8 lines 2 - 3 should read "hematoxylin-eosin staining (H&E) and Masson staining in each group were conducted by standard histological techniques."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

14. Page 8 line 7 should read "… and cover slip." Line 11 should read "Enzyme-linked immunosorbent assay (ELISA). Line 13 should read 12,000 rpms for 20 min…." Line 14 should read "….procollagen type 1 (PICP), amino terminal propeptide of procollagen type III (PIIINP)."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

15. Page 9 line 13 should read "The sequences of primers were shown as follows…"

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

16. Page 10 line 1 should read "…centrifugation at 12,000 rpms…" Line 16 should read "….CK-MB in DOX group which suggests cardiac injury." Lines 17 and 18 should read "…high dose significantly inhibited the level of BNP in a dose dependent manner, with the reduction of…." Line 20 should read "Meanwhile, SMY also suppress CK-MB content in a dose dependent manner (Fig. 1B). Based on the ….."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

17. On page 11, the authors should use LVMI rather than LVWI. The authors mentioned that before the treatment the LVWI was the same between different groups (Fig. 2C). In the materials and methods section, the authors mentioned that doxorubicin was given intraperitoneally and the SMY was given via intragastric administration. The body weight was measured daily and the rats were sacrificed the day after the last SMY administration. The heart tissues were excised and the left ventricle was separated from the atria, aorta and adipose tissue. The left ventricle and the heart were weighed. The authors should explain how they measured the LVMI before treatment. This will require excising the ventricle as described in the materials and methods section. Based on their description, the LVMI cannot be measured before treatment because the rats were still alive.

Answer: At present, our echocardiography can only calculate left ventricular weight according to the formula: LVM (g)=[1.04[(LVIDd+PWTd+SWTd)3-(LVIDd)3]]+0.6g, but can not calculate heart weight. Consequently, we had no results of the heart weight index before treatment was administered. Nevertheless, before the treatment, we can evaluate the left ventricular weight cording to the mentioned formula by echocardiography without sacrificing the rats and calculate the LVMI by LVM (g)/ body weight.
18. Page 11 line 13 should read "...increased heart rate, ST segment depression..." Line 17 should read "...characterized by the reversal of LVEDD..." Line 19 should read "Histopathological study (Fig. 4A) showed that rats in the control..." Line 22 should read "...SMY was recovered to some extent."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

19. Page 12 line should read "...found in SMY+DOX groups..." Line 5 should read "...in the rats who received SMY treatment." Line 7 should read "...such as PICP and PIIINP." Line 8 should read "...secretion of PICP..." Line 9 should read "Treatment for 2 weeks by SMY resulted in suppression of PICP and PIIINP contents; with a reduction of 28.75% and 29.05% respectively."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

20. Page 13 line 8 should read "...respectively." Line 21 should read "There are few reported studies about SMY..."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

21. Page 14 line 1 should read "Our study provides..." Line 4 - 9 should read "We showed a dose dependent...infarction and the biochemical...decreased BW, increased heart weight index..." Line17 should read"...T-wave of the mice was elevated after DOX administration."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

22. Page 15 line 11 and 12 should read "...following SMY administration, accumulation of collagen was attenuated. This may be one..." Line 18 should read "...type III collagen is associated with poor left ventricular..." Lines 20 to 22 should read "... and post myocardial infarction [35]. In our paper, we showed that the markers of myocardial fibrosis (PICP and PIIINP) were increased after the DOX.

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

23. Page 16 lines 4 to 6 should read ".... DOX injury significantly decreased......to some extent." Lines 19 and 20 should read "....that the improvement of cardiac function and morphology by SMY may be mediated...."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.

24. Page 17 line 2 should read "......the processes of tissue repair which..." Line 9 should read "Treatment of DOX injured mice with SMY reduced TLR2 mRNA......" Lines 16 to 18 should read "SMY exhibited cardioprotection on.... Further studies are...."

Answer: Thanks for this advice. We have revised this sentence according to this suggestion.
Conclusion

There are a lot of spelling mistakes and grammatical errors in the manuscript. The materials and methods section has improved, however, there are other important information missing or not adequately addressed. I am still concerned about the standardization and quality of SMY used in the experiments. This has an impact on the internal validity of the study. Figure 2C cannot be explained by the methodology described by the authors. The authors did not include any limitations of their study. This manuscript will require a major revision.

Answer: Thanks for this advice. We have revised the spelling mistakes and grammatical errors in the manuscript. The raised problems were also answered as the above. We appreciate the concerns and suggestions by the reviewer points by points.

References
