**Reviewer’s report**

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

**Version:** 0  **Date:** 28 Sep 2015

**Reviewer:** Oluwadamilola Olaku

**Reviewer’s report:**

**Introduction**

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

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."

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.

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

**Materials and Methods**

The authors did not include any information about sample size calculation

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

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).

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

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

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.

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

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

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/height2.7.

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

Page 7 line 22 should read "…end of experiment."

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

Page 8 line 7 should read "… and cover slip." Line 11 should read "Enzyme-linked immunosorbet 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)…."

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

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 ….."

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.
on their description, the LVMI cannot be measured before treatment because the rats were still alive.

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."

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."

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

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…." Line 17 should read"…T-wave of the mice was elevated after DOX administration."

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.

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…."

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….."

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.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.
No

**Does the work include the necessary controls?**
If not, please specify which controls are required in your comments to the authors.

Yes

**Are the conclusions drawn adequately supported by the data shown?**
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Yes

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I recommend additional statistical review

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