**Reviewer’s report**

**Title:** Cold exposure induced oxidative stress and apoptosis in the myocardium by inhibiting the Nrf2-Keap1 signaling pathway

**Version:** 1  **Date:** 27 Sep 2017

**Reviewer:** Jun Ren

**Reviewer’s report:**

Exposure to cold weather is associated with infaust cardiovascular responses, including myocardial infarction and arrhythmias. However, the exact mechanisms of these adverse changes in the myocardium under cold stress are unknown. This study was designed to investigate the mechanisms of cardiac injury induced by cold stress in mice. The mice were randomly divided into three groups, normal control (no handling), 1-week cold stress and 2-week cold stress. The authors observed physiological changes of the mice and morphological changes of myocardium tissues, and they measured the changes of 3'-nitrotyrosine and 4-hydroxynonenal, the expression levels of superoxide dismutase-1, superoxide dismutase-2, Bax, Bad, Nuclear factor erythroid-derived 2-like 2 (Nrf2) and Kelch like-ECH-associated protein 1 (Keap1) in myocardium by western blot. Besides, they detected mRNA of superoxide dismutase-1, superoxide dismutase-2, Bax, Bad, Nrf2, Keap1 by real-time PCR. One-way analysis of variance, followed by LSD-t test, was used to compare each variable for differences among the groups.

Results: Echocardiography results demonstrated left ventricle dysfunction in the groups receiving cold stress. Histological analyses witnessed inflammation, vacuolar and eosinophilic degeneration occurred in left ventricle tissues. Western blotting results showed increased 3'-nitrotyrosine and 4-hydroxynonenal and decreased antioxidant enzymes (superoxide dismutase-1 and superoxide dismutase-2) in the myocardium. Expression of Nrf2 and Keap1 followed a downward trend under cold exposure, as indicated by western blotting and real-time PCR. In contrast, expression of pro-apoptotic proteins Bax and Bad followed an upward trend under cold exposure. The results of real-time PCR were consistent with those of western blotting. The authors concluded that these findings were very significant, showing that cold exposure induced cardiac injury by inhibiting the Nrf2-Keap1 signaling pathway. A number of concerns remain.

1. English expression is a major concern. There are numerous grammatical and stylistic mistakes. Discussion needs to be rewritten.

2. Quality of the figures is low. Two weeks of cold exposure seem to be minimal in the response elicited.

3. Fig. 3 should be quantitated. The cell signaling pathway examined is too superficial and the data are merely associated with the functional change. The authors should perform additional work to suggest Nrf2 is permissive rather than associated with the observed functional change.
4. A couple of references should be discussed (PMID: 22442497; PMID: 22565031).

**Are the methods appropriate and well described?**
If not, please specify what is required in your comments to the authors.

Yes

**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?**
If not, please explain in your comments to the authors.

Yes

**Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?**
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I am able to assess the statistics

**Quality of written English**
Please indicate the quality of language in the manuscript:

Needs some language corrections before being published

**Declaration of competing interests**
Please complete a declaration of competing interests, considering the following questions:

1. Have you in the past five years received reimbursements, fees, funding, or salary from an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

2. Do you hold any stocks or shares in an organisation that may in any way gain or lose financially from the publication of this manuscript, either now or in the future?

3. Do you hold or are you currently applying for any patents relating to the content of the manuscript?

4. Have you received reimbursements, fees, funding, or salary from an organization that holds or has applied for patents relating to the content of the manuscript?

5. Do you have any other financial competing interests?
6. Do you have any non-financial competing interests in relation to this paper?

If you can answer no to all of the above, write 'I declare that I have no competing interests' below. If your reply is yes to any, please give details below.

None

I agree to the open peer review policy of the journal. I understand that my name will be included on my report to the authors and, if the manuscript is accepted for publication, my named report including any attachments I upload will be posted on the website along with the authors' responses. I agree for my report to be made available under an Open Access Creative Commons CC-BY license (http://creativecommons.org/licenses/by/4.0/). I understand that any comments which I do not wish to be included in my named report can be included as confidential comments to the editors, which will not be published.

I agree to the open peer review policy of the journal