Author's response to reviews

Title: Ethyl acetate extract of Wedelia chinensis inhibits tert-butyl hydroperoxide-induced damage in PC12 cells and D-galactose-induced neuronal cell loss in mice

Authors:

Wea-Lung Lin (wll@csmu.edu.tw)
Shao-Ming Wang (tu76721@yahoo.com.tw)
Ying-Jui Ho (yjho@csmu.edu.tw)
Hsing-Chun Kuo (guscsi@gmail.com)
Yean-Jang Lee (leeyj@cc.ncue.edu.tw)
Tsui-Hwa Tseng (tht@csmu.edu.tw)

Version: 3
Date: 3 October 2014

Author's response to reviews: see over
Dear Editor:

We have completed the revision of our manuscript MS: 2028313444132600 (Title: Ethyl acetate extract of *Wedelia chinensis* inhibits tert-butyl hydroperoxide-induced damage in PC12 cells and D-galactose-induced neuronal cell loss in mice)). Here listed below is the response for the precious comment. Thank you very much for your kind assistance.

Sincerely

Tsui-Hwa Tseng

Response

Reviewer 1

**Reviewer’s report:**

It is good work.

Here is discrepancy in the reference citation, reference citation each of 2012 and 2013 is not enough. Some of the references have been repeatedly reported. Updated references should be inserted in all sections.

Response:

The references are updated (Reference 1, 6, 23, 31). The repeated references have been deleted and the References section has been corrected.

Updated references:

The authors investigated the neuroprotective effects on a traditional Chinese medicine (Wedelia Chinensis) using both PC12 cells and an animal model. The data suggest that the extract of this herb and its major components can block cytochrome C release and improve BCL2 family. In addition, antioxidant effects were also noticed. In general, the results are interesting and may encourage scientists to further explore this herb for clinical applications. Some issues need to be considered by the authors.

1. The flow cytometry data (figure) need to be presented.
   **Response:**
   The flow cytometry data has been added in the upper panel of Figure 4B. The figure legend on page 26 has been rewritten.

2. The authors may want to tell the readers how many hours the cells or animals need to be treated with the herb to get neuroprotective effect. The current data only said 24 hours pre-treatment. What if 12 or 6 h pre-treatment?
   **Response:**
   We added description about our preliminary assay in the revised manuscript (p.14, line 270-273). In our preliminary study, it showed pretreatment of EAW for 6 h didn’t exhibited apparent reduction effect on t-BHP-induced intracellular ROS. But pretreatment of EAW for 24 h it showed reducing effect significantly.

3. In figure 3, tBHP treatment is 30 min to damage the cells, but why the same treatment needs to be 3 hours in figure 4. In addition, is the dose of tBHP reasonable for humans?
   **Response:**
   There is a mistake in Figure legend. According to description in Methods section, t-BHP was treated for 3 h and DCFH-DA was treated for 30 min. The figure legend of Fig. 3 has been corrected.

Oxidative stress is one of the general mechanisms in neurotoxicity. T-BHP is commonly used as a model substance for evaluation of mechanisms of cellular
alterations resulting from oxidative stress in cell and tissue. According to cited references and the following references (1-2), the used concentration is 100 \( \mu \text{M} \)-1 mM. The dose of t-BHP in human needs further evaluation.

References:

4. What is a reasonable dose of this herb for humans? It is not clear whether the experimental dose way beyond the dose for human consumption.

Response:
The authors thank the reviewer’s comment. For addressing this issue, we added a statement in the revised manuscript (p.18), as: “One might be interested in knowing the dosage of EAW when applying in the human. By using the body surface area normalization method [1], the effective dose, 10 and 25 mg/kg, of EAW in preventing neuronal loss in the cortex in mice can be translated to the human equivalent dose, 0.81 and 2.03 mg/kg. However, further study is needed to evaluate the effective amount of *W. chinensis* when used in human for improving the health or for a therapeutic purpose.”