Author’s response to reviews

Title: Anti-hyperalgesic properties of a flavanone derivative Poncirin in acute and chronic inflammatory pain models in mice

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Version: 3 Date: 06 Dec 2018

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Reviewer reports:

Joseph Stephan (Reviewer 1): The manuscript titled "Anti-hyperalgesic properties of a flavanone derivative Poncirin in acute and chronic inflammatory pain models in mice" is an interesting and comprehensive assessment of the effect of Poncirin in several models of inflammatory pain in mice. The paper is of potential interest and the results are clear. A few minor points below should be addressed:

Minor comments:

1. Comment.

Formalin model: the methods state that mice were observed for 60 minutes, divided into the first 10 minutes (early phase) and a late phase (10-30 min), which gives a total of 40 min total max:
correct numbers; also, related figure divides 1st phase into 0-5 minutes rather than 0-10; correct - Legend of figure 3 conflicts with text as to what figure 3c represents, make clearer please

Response: The reviewer is highly acknowledged for raising important point. The formalin-induced nociception is divided into two phases. The 1st phase comprises from 0-10 minutes while the second phase start from 10-30 minutes. However, before the administration of formalin, the animals were transferred to observation box for 30 min to get habituated to the environment. The figure 2 is changes as per recommendation of the reviewer in the revised manuscript. This is the common protocol followed for the formalin-induced assay.

The figure legend of figure 3 was corrected in the revised manuscript as per recommendation and figure 3c represent mechanical allodynia and the changes in the figure legend were highlighted.

2. Comment.

"There was no tolerance effect of poncirin on mechanical hyperalgesia and allodynia during interruption of dose on day 5, which was reestablished when the drug was administered on day 6 (Fig.4C & D).": the difference is very hard to see in the presented graphs, and doesn't seem very clear cut. The authors should temper their conclusion or its language. - The reference provided in the discussion for the use of poncirin in ancient times (ref 14) is inaccurate; cite original reference

Response: The reviewer is highly appreciated for the valuable suggestion. The dose was skipped at day to observe the tolerance effect i.e. whether the drug have persistent effect or the dose of the drug should be regularly to exhibit the analgesia. The data of the figure 4c and 4d was analyzed and the graph were plotted as shown in the revised manuscript. The skipping of the dose was described in the revised manuscript as per recommendation. The original paper was cited for the traditional uses of Poncirus trifoliate.


The data of the figure 4 was again analyzed and the results obtained are plotted as per reviewer suggestion in the revised manuscript.

1. Comment.

Figure 11: an effort to show larger fields should be made by the authors; also, was any attempt at quantification of the data conducted? It is unclear what the statistical analysis referred to in the figure 11 legend refers to, and where it is shown!

-In general, the materials section is very large, and I feel this has been done a bit at the expense of the results, which occupy a significantly smaller portion of the paper. Authors should attempt to compensate for this imbalance

Response: The reviewer is highly appreciated once again for the valuable suggestion. The H and E and the X-ray analysis related figure 11 was double blindly visualized by the histopathologist and radiologist respectively. The histopathologist categorized the images based on the infiltration of immune cells infiltration and synovial hyperplasia. The radiologist assigned different groups based on the calcification, edema. No other quantification was applied and the figure caption was modified in the revised manuscript as per recommendation. The methods and results section were arranged as per reviewer recommendation in the revised manuscript. Furthermore, the materials and results section was modified as per reviewer recommendation in the revised manuscript.

Typos and other textual corrections:

2. Comment.

Page 3 line 13: should be heme oxygenase

Response: The heme oxygenase spelling was corrected as per reviewer recommendation in the revised manuscript.

3. Comment.

Page line 45: remove "while" from beginning of sentence

Response: The changes were made in the revised manuscript as per reviewer recommendation.
4. Comment.
Page 9, line 45: renal and liver toxicity; "renal and hepatotoxicity" is incorrect
Response: The changes were made according to the reviewer recommendation in the revised manuscript.

5. Comment.
Page 12 line 12 and 15: should read poncirin doesn't
Response: The changes were made according to the recommendation of the reviewer in the revised manuscript.

6. Comment.
Page 12 line 23: in plasma
Response: The changes were made as per reviewer recombination in the revised manuscript.

7. Comment.
Page 13 line 36: with unbearable side effects
Response: The changes were made according to the reviewer recommendation in the revised manuscript.

8. Comment.
Page 13 line 40: and these medicinal plants have been used
Response: The changes were made and highlighted in the revised manuscript as per reviewer recommendation in the revised manuscript.