Reviewer’s report

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

Version: 4 Date: 27 Mar 2019

Reviewer: Reviewer 2

Reviewer's report:

PEER REVIEWER ASSESSMENTS:

OBJECTIVE - Full research articles: is there a clear objective that addresses one or several testable research questions? (Brief or other article types: is there a clear objective?)

Yes - there is a clear objective

DESIGN - Is the current approach (including controls and analysis protocols) appropriate for the objective?

Not sure - key details are missing from the manuscript

EXECUTION - Are the experiments and analyses performed with sufficient technical rigor to allow confidence in the results?

Not sure - key details are missing from the manuscript

STATISTICS - Is the use of statistics in the manuscript appropriate?

Yes - appropriate statistical analyses have been used in the study

INTERPRETATION - Is the current interpretation/discussion of the results reasonable and not overstated?

Yes - the author's interpretation is reasonable
OVERALL MANUSCRIPT POTENTIAL - Has the author addressed your concerns sufficiently for you to now recommend the work as a technically sound contribution? If not, can further revisions be made to make the work technically sound?

Probably - with minor revisions

PEER REVIEWER COMMENTS:

GENERAL COMMENTS:

1. The Authors have provided a thorough in vivo examinations of four types of inflammation mice pain model. What are the differences between these four inflammation models and/or how this strengthens Author's conclusion should be clearly stated (e.g. mimics the acute or chronic state of inflammatory pain). For example, Carrageenan induced two phases of edema with the first phase been the low-intensity edema.

Ref: Mouse paw edema. A new model for inflammation? (PMID:3690058)

This reviewer is asking Authors to clearly state the key differences between four types of inflammation mice pain model and based upon Authors' results to suggest the possible underlying mechanism/s of poncirin.

2. Method section, animals: What strain of mice? Also, typical body weight for mice at the age of 3-4 weeks should be ~20-25g. Please double check. If available xy fig for the animal body weights should be included as part of the result.

Authors did not provide the strain of mice. If the source is unknown, this need to be specifically stated.

3. Major concern: The behavioural readings (e.g. mechanical hyperalgesia and allodynia baselines) are much higher compared to other published data using mice. For example, Authors cited Cho H et al 2012 (ref #22), their von Frey and Randall Selitto baseline readings (Supplementary Fig6) are similar to this manuscript (~300g cf ~400g). However, Cho H et al use SD rats (SFig6) not mice. Based on my knowledge and experience the behavioural baselines between rats and mice are distinctly different.
Thanks for providing six references. This reviewer, however, would like to point out that for references 1-5 they all use male SD rats not mice and so in fact supported this reviewer's original comment. The Authors should carefully select the supporting references for their rebuttal.

For further reference, this reviewer would like to recommend Authors to provide a summary or in dot-points that support their claim. For examples:

1. Diana K. Schoeniger-Skinner et al., Interleukin-6 mediates low-threshold mechanical allodynia induced by intrathecal HIV-1 envelope glycoprotein gp120. Brain, Behavior, and Immunity 21 (2007) 660-667
   * Adult male SD rats (300-450g).
   * Using manual von Frey
   * Their von Frey baseline readings before gp120-induced mechanic allodynia are ~10g.

   * Adult male SD rat (200-350g)
   * Manual von Frey
   * Formalin-induced inflammatory pain
   * Fig 1: pre-drug (formalin treated) baseline ~5g. Morphine treatment reverted von Frey reading back to ~13 g.

   * Male SD rats (120 -150g)
   * Manual von Frey
   * Pre-SNL surgery vF baseline ~15g (Fig 1A).
   * Their vF value of 5.2 = 15.8g (under their animals and surgery section).
Reference #6 provided by the Authors is a paper that supported Authors' behavioural data.

* Male ICR mice (30-35g)

* Using manual von Frey and Randall Selitto with baseline readings of ~8g and ~400g respectively.

After google scholar search using e.g. ICR mice, Randall Selitto as keywords, I can only found the following paper that might support Authors' claim.

Effects of Astaxanthin from Litopenaeus Vannamei on Carrageenan-Induced Edema and Pain Behavior in Mice. DOI: 10.3390/molecules21030382

* Male ICR mice, eight weeks old, weighting 30-35 g,

* Baseline Randall Selitto value of ~350g (Figure 3). However, according to section "4.6. Behavioural Assessment of Mechanical Nociceptive Threshold Test", "The maximum force applied was limited to 200 g to avoid any tissue damage".

REQUESTED REVISIONS:

The behavioural data (especially, Randall Selitto and hot-plate data) are very different to the majority of published data. I still have my reservation on how a mice hindpaw can withhold >400g of force and last ~27 sec on a 55oC hot-plate.

Authors should carefully select their supporting reference for their rebuttal.

**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.

No

**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:

Acceptable

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