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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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)

Response:

The reviewer is highly appreciated for the valuable suggestion. In the current study four different inflammatory pain models were studied because these inducers produce pain via a different mechanism. Thus, we employed to find out whether poncirin is effective in different types of pain models. Furthermore, most literature reported multiple anti-nociceptive assays to investigate the potential therapeutic effect of new chemical entity.


The reviewer is highly appreciated for pointing out the biphasic pain response associated with the Carrageenan administration. However, bunch of studies also reported that the Carrageenan as acute model of inflammatory pain.


2. Preventive protocols have been used for the test item poncirin. For better mimics that in the clinical setting, the intervention protocol will be more appropriate. What is the reason that the prevention protocol was used rather than intervention?

Response:
The pretreatment (preventive treatment) protocol was followed and the drug was administered prior to the inducer in order to avoid any damage to the animals expected to be caused by the inducer. Accumulative reports suggest that pretreatment produced significant results as compared to the co-treatment. A study reported in 2009 in which effects of aqueous extract of ziziphus maritiana (ZM) leaf on serum and liver cholesterol and triglycerides were studied in chronic alcoholic administered rats. It was found that pretreatment produce significant inhibition of the cholesterol and triglycerides as compared to the co-treatment.


Furthermore, in most of the animals studies pretreatment was initiated compared to the co- or post-treatment.


3. 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.

Response:

Male albino mice were used in the current study. The actual weight of the mice was 25-30g and was corrected in the revised manuscript as per reviewer recommendation. The xy figure of the age of the each treated group animals were included as part of the result as per reviewer recommendation in the revised manuscript.

4. Dose volume of the vehicle control should be stated
Response:

The volume of the vehicle control was same as with that of other treated groups and the dose of the vehicle control was added in the revised manuscript as per reviewer recommendation.

5. 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.

Response:

The reviewer is highly acknowledged for the valuable comment. The mechanical hyperalgesia and mechanical allodynia responses both baseline and the onward readings are different in different manuscript. As for the reviewer question is concerned that the Cho H et. article have used rats rather than the mice, in this manuscript just the method was followed. The baseline and onward reading were performed in a double blindly to rule out any biasness. Furthermore, the baseline threshold are different in different manuscript. In some mice studies the allodynic and hyperalgesic threshold is even higher than the rats, while in several rat anti-nociceptive studies the threshold is lowered than what is reported for the mice.


6. ".....cardiac puncture following anesthesia with xylazine + ketamine (16 mg + 60 mg)". I will assume this should be 16mg/kg + 60mg/kg. Please double check.

Response:

The reviewer is highly appreciated for the valuable correction and the mg/kg was added into the revised manuscript as per reviewer recommendation.

7. At what time point/s Authors collected the serum samples for the renal and liver toxicity test?

Response:

The reviewer is highly appreciated for the valuable suggestion and the sampling was done at the end of the experiment i.e day 6. The changes were made in the revised manuscript as recommendation.

8. Under Method section, Statistical analysis. Did Authors use SPSS for the statistical analysis or SPSS and Sigma Plot?

Response:

The data was analyzed using SPSS, however, graphs were drawn using Sigmaplot software. The statement was corrected in the revised manuscript as per reviewer recommendation.

9. Table 2: Should include dexamethasone result in Table 2 if available.

Future direction: a positive control that alters (mild/moderate) the liver/kidney functions should be included.

Response:

The reviewer is highly appreciated for the valuable suggestion. The result of the positive control treated with the dexamethasone were added into the table 2 as per reviewer recommendation in the revised manuscript.