Author’s response to reviews

Title: Acteoside inhibits inflammatory response via JAK/STAT signaling pathway in osteoarthritic rats

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Author’s response to reviews:

Dear Editor, Dear reviewers,

Thank you for your letter. We thank your and reviewers for the time and effort that you have put into reviewing the previous version of the manuscript. Your suggestions have enabled us to improve our work. Based on the instructions provided in your letter, we uploaded the file of the revised manuscript. Accordingly, we have uploaded a copy of the original manuscript with all the changes highlighted by using the track changes mode in MS Word.

Appended to this letter is our point-by-point response to the comments raised by the reviewers.

Point -to-point response to comments:

BMC Complementary and Alternative Medicine operates a policy of open peer review, which means that you will be able to see the names of the reviewers who provided the reports via the online peer review system. We encourage you to also view the reports there, via the action links on the left-hand side of the page, to see the names of the reviewers.

Reviewer reports:

Salmon Adebayo, PhD (Reviewer 1): Inconsistent font sizes and styles used for references, Figures and legends
Response: Thanks for the comments! We are sorry for the mistake due to the format process using Endnote. We have corrected the font sizes and styles issue accordingly.

Mokrish Ajat, Ph.D. (Reviewer 2): Comments to author:
1. The title should be changed to 'Acteoside inhibits inflammatory response via JAK-STAT signaling pathway in osteoarthritic rats'.
Response: Thanks for the comments! We have changed the title accordingly.

2. The author did not mention about how the ACT extract was prepared.
Response: Thanks for the reviewer’s suggestions. We have provided the information of ACT extraction in methods section according to the reviewer’s comments.

3. The author should include positive control groups in this experiment so the result can be benchmarked with a known response effect.
Response: Thanks for your suggestions and we have supplemented the experiment of acetaminophen (ACE) as a positive control (revised Figure 2, Figure 3 and Figure 4). The concentration of ACE was determined according to the previous reports as follows.
Reference:

4. Although the result is significant, the usage of words such as 'drastically reduced' and 'remarkably enhanced' should be avoided as the results was not too significant.
Response: Thanks for the reviewer’s suggestions! We have revised the wording accordingly.

5. Should include diagram on how and where ACT inhibit IL-1β in JAK/STAT pathway.
Response: Thanks for the reviewer’s suggestions. We have provided a diagram on how and where ACT inhibit IL-1β in JAK/STAT pathway according to the reviewer’s comments (Figure 7).

6. JAK-STAT or JAK/STAT? Not consistent.
Response: Thanks for the reviewer’s suggestions! We have revised the format as JAK/STAT to make it consistent.

Spelling/Grammar error
Line 229: (Our confirmed)
Line 137: (10-week old, n = 30)
Response: Thanks for the reviewer’s suggestions! We have made the correction accordingly.

Diagram/Graphs/Images
Fig 1B: The cartilage cytoplasm cannot be viewed clearly
Response: Thanks for the reviewer’s suggestions. Toluidine blue staining for rat articular chondrocytes identification, as showed in Fig 1B, the dark blue indicated the nucleuses and other color area indicated the cytoplasm.

References
50% of the references are outdated (more than 5 years)
Response: Thanks for the reviewer’s suggestions! We have tried to update with the newest references. We used some outdated references with original discoveries.

Naveenkumar Chandrashekar, Ph.D (Reviewer 3): The manuscript "Acteoside inhibits inflammatory response via JAK-STAT signaling pathway in osteoarthritis rats" by Qiao and colleagues investigates the therapeutic effects of acteoside, a major herbal component on inflammatory cytokines correlates
with JAK/STAT signalling axis and joint protection in surgery-induced osteoarthritis in rats.

The main idea of manuscript is interesting, study is well designed, results are presented straight, but I have some concerns regarding suitability of the manuscript for publication. The author's response to the following concerns is essential and may use the suggestions below in revising their manuscript.

Major issues

1. The important concern is centred on animal experiments.
   (1) The rationale for the in vivo dose of acteoside is not clearly stated. On what basis dose selection of acteoside in rats was chosen, justification is required.
   (2) The concentration of acteoside (100mg/kg body weight) tested appear to be high. Authors should discuss regarding potential side effects of such high concentrations on different organs.
   Response: Thanks for the reviewer’s suggestions. According to the literature, ACT is administered orally and injected at doses ranging from 2 mg/kg to 200 mg/kg in animal models. In order to validate the pharmacological effects of ACT, 100 mg/kg was selected for the experiment.
   Reference:
   (Nakamura, Tomonori, et al. "Acteoside as the analgesic principle of cedron (Lippia triphylla), a Peruvian medicinal plant." Chemical and pharmaceutical bulletin 45.3 (1997): 499-504.)
   (Bergonzi, M. C., et al. "Antihyperalgesic activity of verbascoside in the chronic constriction injury of the sciatic nerve (CCI) and intra-articular injection of sodium monoiodoacetate (MIA) models of neuropatic pain." Planta Medica 75.09 (2009): PA45.)
   (3) The authors mentioned that the treatment of acteoside was started after 4 weeks of surgery. Why the resting period of 4 weeks was given for rats after surgery?
   Response: Thanks for the reviewer’s suggestions. In fact, after four weeks of rest, significant inflammation was observed in the joints. In addition, we refer to the experimental design reported in the literature. (Pan, Tianlong, et al. "Alpha-Mangostin protects rat articular chondrocytes against IL-1β-induced inflammation and slows the progression of osteoarthritis in a rat model." International immunopharmacology 52 (2017): 34-43.).

2. The other important concern is on histological studies.
   (1) Knowledge about the histological pattern of the tissue is important for diagnostic reasons and the understanding of the development of osteoarthritis. There is a lack of data relating the microscopic appearance of cartilage to its histological properties. In addition, there is a lack of data relating to the substantial changes in articular cartilage composition during the treatment and development of osteoarthritis.
   Response: Thanks for the reviewer’s suggestions. We are sorry that at the end of the experiment, all rats were killed by cervical dislocation and ribs were removed. All articular cartilage was isolated and cut into small pieces, followed by digestion. We are sorry that we can't replenish this part of the experiment. However, we will continue to conduct more comprehensive animal experiments with a positive control and histological studies in the future.

Minor issues

1. The methods make no statement or unclear whether the cell line has been authenticated for the study or not?
   Response: Thanks for the reviewer’s suggestions. The primary chondrocytes was used in our experiment and we described the cells in the Figure 1 of Results section.
2. In the methods section, it is unclear about the solubility of acteoside used? Which vehicle is used to solubilize the acteoside?
Response: Thanks for the reviewer’s suggestions. We have provided the information of ACT in methods section according to the reviewer’s comments as follows:
ACT (purity ≥98%), and dimethyl sulfoxide (DMSO) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). ACT was dissolved in DMSO as a 100 mg/ml stock solution and stored at 4 °C. Further dilution was done in cell culture medium.

3. In the results section, the figures should be made clearer using the appropriate graphing software and the image quality for some of the figures is poor.
Response: Thanks for the reviewer’s suggestions! Yes, the figures in PDF file showed a low resolution. Please review the attached figures directly. In addition, if necessary, we can provide high resolution original pictures. Or if there's a picture with low resolution, we will make further correction according to your suggestions.

4. The Figure legends should include detailed information on the number of technical and biological replicates used.
Response: Thanks for the reviewer’s suggestions! We have revised the figure legends and included those information.

5. Did the authors check the Gaussian distribution of the data before applying ANOVA statistics?
Response: Thanks for the reviewer’s suggestions! Yes, all data were checked by Gaussian distribution and all data fitted the normal distribution.

6. Manuscript needs English revision and needs to be rectified by a native English speaker.
Response: Thanks for the reviewer’s suggestions! We have an English native speaker to help us proofreading the whole manuscript.

We would like also to thank you for allowing us to resubmit a revised copy of the manuscript. We look forward to hearing from you regarding our submission. We would be glad to respond to any further questions and comments that you may have.

Sincerely,
Liu Ming