Author’s response to reviews

Title: Anti-inflammatory, analgesic and acute toxicity effects of fermented soybean

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Author’s response to reviews:

Editorial comments:

Comment: There are varying statements regarding the anesthetic/euthanasia in this study, please ensure that all of the statements are the same.

Response: Anesthetic/euthanasia method was rewritten clearly throughout the manuscript (Line 160, page 7)

Comment: The Availability of data and materials section refers to the raw data used in your study and presenting tables and figures is not sufficient to state that all data is contained within the manuscript and additional files. Please only use this statement if you have indeed provided all raw data on which your study is based. We strongly encourage all authors to share their raw data, either by providing it in a supplementary file or depositing it in a public repository and providing the details on how to access it in this section. If you do not wish to share your data, please clearly state this in this section along with a justification. Data availability statements can take one of the following forms (or a combination of more than one if required for multiple datasets):
• The datasets generated and/or analysed during the current study are available in the [NAME] repository, [PERSISTENT WEB LINK TO DATASETS]

• The datasets used and/or analysed during the current study available from the corresponding author on reasonable request.

• All data generated or analysed during this study are included in this published article [and its supplementary information files].

• The datasets generated and/or analysed during the current study are not publicly available due [REASON WHY DATA ARE NOT PUBLIC] but are available from the corresponding author on reasonable request.

• The data that support the findings of this study are available from [third party name] but restrictions apply to the availability of these data, which were used under license for the current study, and so are not publicly available. Data are however available from the authors upon reasonable request and with permission of [third party name].

Please also note that if you include your raw data as a supplementary file you will need to provide, after the References, a section titled “Additional files” where you list the following information about each of your supplementary files: * File name (e.g. Additional file 1), * Title of data, * Description of data. All additional files will also need to have been cited in the main manuscript.

Response: Authors thanks for the guidance from editor. We have edited our statement to “The datasets used and/or analysed during the current study available from the corresponding author on reasonable request”

Comment: Please consider the list of authors as it currently stands with reference to our guidelines regarding qualification for authorship (http://www.biomedcentral.com/submissions/editorial-policies#authorship).

Currently, the contributions of the authors do not automatically qualify them for authorship. In the section “Authors’ contributions”, please provide further clarifications on their contributions, and see our guidelines for authorship below.
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Each author is expected to have made substantial contributions to the conception OR design of the work; OR the acquisition, analysis, OR interpretation of data; OR the creation of new software used in the work; OR have drafted the work or substantively revised it

AND to have approved the submitted version (and any substantially modified version that involves the author's contribution to the study);

AND to have agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, are appropriately investigated, resolved, and the resolution documented in the literature.

Acquisition of funding, collection of data or general supervision of the research group, alone, does not usually justify authorship.

If these guidelines are not met, we would request the following change of authorship form be filled out and sent to our editorial office - https://resource-cms.springernature.com/springer-cms/rest/v1/content/7454878/data/v5

Anyone who contributed towards the article who does not meet the criteria for authorship can be acknowledged in the ‘Acknowledgements’ section.

Response: Authors thanks for the suggestion by editor. We have rewrite to clearly indicate the contribution of authors in this manuscript.
Ah Abou Zeid (Reviewer 1):

Comments: The word (In vivo) in the title of the manuscript must be removed as in page 5 there is a title (in vitro antiinflammatory activity).

Response: Title was changed according to the suggestion

Comments: Some words need spaces between each others in different pages:
Page 2, line 37, page 4, lines 79 and 80, page 5, line 102.

Response: Authors apologize for this error caused by change of word file. We have carefully edited the words throughout the manuscript.

Comments: Page 4, line 101: (the) must be removed.
Response: “The” was removed as corrected by reviewer.

Comments: Page 5, line 101: (of ) must be removed.
Response: “of” was removed as corrected by reviewer

Comments: Page 5: from line 102 to line 106 are not clear.
Response: Method and results for NESTE quantification was improved in this section. We have clearly cited reference [14], which is the source of the method and results for this section.

Comments: Page 8: Method of Mice paw analgesic test is not clear.
Response: Method for mice paw analgesic test was rewritten.

Comments: Page 11: the first line (anti ) should be removed from the word (anti-analgesic), All the method is not clear.
Response: “anti-” was removed and all the method was revised.
Haiqiu Huang (Reviewer 2):

Comment: There are several issues with the study design and statistical analyses, and the results cannot support the claimed biological effects. This manuscript needs significant improvement and more data before it can be accepted.

Response: Authors have improved the writing of study design, remove the unsupported experiment and edit the statistical analysis based on the comments by reviewer 1, 2 and 3 throughout the manuscript.

Comments: Page 6, Line 126. LPS induction concentration used in the in-vitro assay was too high. At >1 µg/mL LPS, which is a much higher concentration of LPS than that in sepsis, the inflammatory pathways were perpetually activated, and such activation possesses little resemblance to any biological conditions. Considering the minor effects shown in this study (<<1 fold change), it may be hard to observe any effect in any in-vivo system.

Response: Authors thanks comment by reviewer and apologized for the typo error for the concentration used. As we are referring to the Huang et al. [16], which used 1µg/mL of LPS for induction of inflammation, we have corrected the concentration to 1µg/mL.

Comments: Page 8, Line 166. Since soybean is consumed as a food, and most peptides, phytochemicals, and lipids undergo extensive digestion and metabolism in the GI tract, the topical route of administration designed in this study may not generate any relevant results. The choice of this surrogate assay cannot support the conclusion of fermented soybean, as a food, to have an anti-inflammatory effect.

Response: Authors agree with the suggestion from reviewer 2 where this topical application of SBE and NESTE are not representable for the oral intake of these samples and thus have removed this section throughout the manuscript.
Comments: Page 9, Line 185. 0.2 and 1 g/kg feeding levels are relatively high. Are the choices of such feeding levels supported by any food consumption data? How much tempeh shall a person consume to achieve similar level used in the animal model?

Response: When converting from mice dosage to human, dosage is divided by 12.3 (based on body surface ratio of mice to human, by Nair and Jacob [26]. Thus, the dose per kg for human are 0.976g/60kg and 4.878g/60kg, respectively. As the extraction recovery for the tempeh extract was 25% [14], the amount of tempeh to achieve the tested dosage are 3.904g/60kg and 19.512/60kg of person. This concentration is below the recommended daily dosage proposed by Nakajima et al. [27].


Comments: Page 10, Line 210. Figure 2. The asterisks in the figure cannot symbolize the pairwise comparison results from the Duncan test. Without the pairwise comparison, the conclusion of the dose-dependent inhibition effect cannot be reached. Please update the figure to reflect the result of each comparison.

Response: Statistical analysis for Figure 2 was updated.

Comments: Page11, Line 233. Figure 3. No statistic results were shown in the figure.

Response: Statistic results were shown in Figure 3.
Comments: Page 12, Line 276. If the toxicity in question may arise from foodborne infection, using extract was not a representative sampling and testing method. Acute toxicity, in this case, may only result from chemical toxins or metabolites, rather than a bacterial source. Therefore, the author cannot claim the safety of foodborne infection using the described analysis methods.

Response: Authors thanks the valuable comments by reviewer and agree that acute toxicity was not answering the safety of foodborne infection. Thus, we have removed foodborne infection in the text and only retaining foodborne intoxication. (page 12, line 259).

Silvio Sosa, Ph.D. (Reviewer 3):

Comments: improvement from a linguistic point of view.

Response: Authors thanks the suggestion from reviewer and have improved the Language of the manuscript.

Comments: Abstract Methods: This part does not report any indication on the methods.

Response: Abstract Methods was improved based on suggestion by reviewer.

Comments: Abstract Results: Results should be re-written reporting the quantitative evaluation of the effects, including the effect of reference drugs/compounds.

Response: Results in the abstract was rewritten to include the quantitative effects from both sample and reference compounds/drugs.

Comments: Materials and methods should more clearly organized (for instance, reporting first materials, then in vitro studies followed by in vivo studies) and described, reporting also the solvents used to dissolve the administered substances.

Response: Section for materials and methods was reorganized as suggested by reviewer and the solvents used to dissolved the administered substances were stated in the section.
Comments: Results; Lines 209-2014: Authors evaluated SBE and NESTE effect on NO production by LPS-stimulated macrophages only at two concentrations, which cannot allow to estimate a concentration-dependent effect. Moreover, the effect had to be compared to a reference compound.

Response: Authors thanks and agree with the suggestion by reviewer. We have removed the word dosage dependent in the manuscript. In addition, we have included results for curcumin, which is the reference compound for the in vitro anti-inflammatory study.

Comments: Results; Lines 225-229: The effect of more than two doses of the testing substances had to be evaluated. Edema reduction (%) should be reported also in the text.

Response: Authors agree with the suggestion from reviewer 2 where this topical application of SBE and NESTE are not representable for the oral intake of these samples and thus have removed this section throughout the manuscript.

Comments: Results; Lines 232-238: As previously reported, no dose-effect can be observed from the obtained results.

Response: Authors thanks and agree with the suggestion by reviewer. We have removed the word dosage dependent in the manuscript and only reported the best effect was observed from the 1000mg/kg of NESTE. (Line 221-226, page 10)

Comments: Figure 2. The results should be reported in the same manner for all the three parameters (NO production is reported as % inhibition, whereas the cytokines are reported are levels in the culture medium).

Response: Figure 2 was improved and standardized based on suggestion by reviewer (to % of inhibition for NO, IL-1b and TNFa).

Comments: Table 1: Results should be reported both as edema (mean ± SE) and % edema inhibition.

Response: Authors agree with the suggestion from reviewer 2 where this topical application of SBE and NESTE are not representable for the oral intake of these samples and thus have removed this section (including Table 1, which is the result for the section) throughout the manuscript.