Author’s response to reviews

Title: The genotoxicity of an aqueous extract of Gyejibokryeong-hwan

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Author’s response to reviews:

Ms. No.: BCAM-D-17-00399

Title: "The genotoxicity of an aqueous extract of Gyejibokryeong-hwan

Authors: Meeyoung Lee; Changseob Seo; Hyekyung Ha; Jiyoung Kim; Hyeunkyoo Shin

Dear Suengmok

Thank you very much for your editorial decision letter, which also included the reviews of our manuscript by referees. We have made the changes as suggested by the reviewers. The changes are marked in blue in the revised text. We have made during the revision in a point-by-point response to each of the comments.

We hope the revisions made the responses provided are satisfactory, and our manuscript is now acceptable for publication in the BMC Complementary and Alternative Medicine.

Please, let us know if further revisions are needed.

Once again, thank you for all your help. We look forward to hearing from you.

Sincerely yours
Editor 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:

Wissam Faour, Ph.D. (Reviewer 2): Dear Editor, BMC CAM,

The authors presented data about the genotoxic effect of a traditional korean herb widely used in china. the study is of low quality and the treatment doses used by authors do not rule out the toxic effect of the herb at such high concentrations even if there are no obvious genotoxic effect. In addition genotoxic effect is preferably be judged first on gametes cells, liver and heart cells as well as kidney cells. Also, the authors need to assess the toxic effect on the Liver, heart and kidney using clinical and histopathological tests to document the potential effect at the cellular levels then move a step forward to check its effect on DNA. the authors didn't explain the rational of using such high concentrations of the extract to treat the animals

- We appreciate your comment. We strongly agree with as commented by reviewer, the present study was conducted in accordance with the guidelines established by the FDA Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies (21 CFR Part 58). We added these comments in Methods section (Page 6, line 9). Also, Gyejibokryeong-hwan is a traditional Korean herbal prescription consists of 5 species of medicinal herb. Sometimes herbal medicine’s daily dosage over 2000 mg/kg (Maximum dosage of OECD guide line), therefore we administrated the 5000 mg/kg of Gyejibokryeong-hwan.

Please, let us know if further revisions are needed.

Svetap Aydın, Ph.D. (Reviewer 3): Manuscript number: BCAM-D-17-00399

Title: The genotoxicity of an aqueous extract of Gyejibokryeong-hwan

This study showed the genotoxic evaluation of aqueous extract of Gyejibokryeong-hwan by in vitro chromosomal abberation and Ames tests also in vivo micronucleus test. It needs major revisions below:
Comment to author:

Abstract

Page 2, Line 2: Background information related to the study is not adequate. It needs some explanation.
- We appreciate your comment. As commented by reviewer, we added an appropriate explanation.

Page 2, Line 4: Delete "Methods" which was mistakenly written.
- We appreciate your comment. As commented by reviewer, we deleted “Methods”.

Page 2, Line 16: Conclusion part in the abstract should be rewritten. Can aqueous extract of Gyejibokryeong-hwan be genotoxic according to the expected levels in the blood? In vitro condition, the treatment doses (4 mg/mL) in the cells may be high, therefore this must be taken into account.
- We appreciate your comment. We strongly agreed with as commented by reviewer. Because the present study was conducted in accordance with OECD guidelines for the Testing of Chemicals Section 4 Health Effects Test No. 473 about In vitro Mammalian Chromosome Aberration Test (21 July 1997), we used the highest concentration given the guidelines. We added these comments in the page 6, line 14.

Page 2, Line 16: Delete "Under the current test conditions," , write "In conclusion,...".
- We appreciate your comment. As commented by reviewer, we changed to “Under the current test conditions” to “In conclusion”.

Background

Page 3, Line 1: Background is not satisfying; this part needs to be revised. The information on the purpose of the study was not adequately addressed.
- We appreciate your comment. As commented by reviewer, we mentioned the additional information on the purpose of the study.
Page 3, Line 9: General information related to the purpose of this work is not enough. Information is needed to reveal its purpose. The relationship with the selected references substance must be revealed.

- We appreciate your comment. As commented by reviewer, we mentioned the additional information on the relationship in page 3, line 16.

Page 3, Line 14: The sentence "The aim of a genotoxic assay is to detect carcinogens and other mutagens (Demma et al., 2009)." should be checked, which might be miswritten.

- We appreciate your comment. As commented by reviewer, we corrected to the reference number.

Page 3, Line 15: revised as "…the present study was aimed to determine the acute toxicity and genotoxic properties of an aqueous extract of Gyejibokryeong-hwan (GJBRHE)."

- We appreciate your comment. As commented by reviewer, we changed to “the present study was aimed to determine the acute toxicity and genotoxic properties of an aqueous extract of Gyejibokryeong-hwan (GJBRHE)”.

Methods

Page 4, Line 4: Revised as "To obtain the aqueous extract of GJBRHE,…".

- We appreciate your comment. As commented by reviewer, we changed to "To obtain the aqueous extract of GJBRHE".

Page 4, Line 21: Give temperature during sonication for 10 min.

- We appreciate your comment. As commented by reviewer, we added the temperature during sonication.

Page 5, Line 14: Give references for HPLC analysis.
- We appreciate your comment. As commented by reviewer, we added as comments ‘amygdalin, albiflorin, paeoniflorin for P. persica, coumarin, cinnamic acid, cinnaldehyde for C. cassia, paeonol for P. suffruticosa’

Page 6, Line 1: "Based on this study, a dose …[?]."

- We appreciate your comment. As commented by reviewer, we changed to "Based on this study" and the present study was conducted in accordance with the guidelines established by the FDA Good Laboratory Practice (GLP) for Nonclinical Laboratory Studies (21 CFR Part 58). According to the guideline, the dose finding test is carried out before this test, and the result is the dose finding test.

Page 6, Line 6: Did not the mixture GJBRHE become a solution? If not, why was it not solved instead suspended in distilled water? It should be solved other solvent such as olive oil.

- We appreciate your comment. For Ames test, GJBRHE was used as a solution and distilled water was used as a solvent. After the solubility test of GJBRHE, distilled water was used as a solvent.

Page 6, Line 6: Delete "The used experimental methods were based on the report of Maron and Ames [14], with minor modifications. The Ames test was conducted as described previously [13]." Start with Ames test was used to determine……Then give some detail for the procedure.

- We appreciate your comment. As commented by reviewer, we added these comments in Page 6, line 10.

Page 6, Line 5 and Line 11: In Ames, CA, and MN tests, the experimental part should be detailed.

- We appreciate your comment. As commented by reviewer, we added these comments in Page 6, line 10 and 20 and Page 7, line 8.

Statistical Analysis:
Page 6, Line 22: The Statistical Analysis part should be rewritten. Statistical evaluation used for each test should be clearly written, instead giving references. Delete "were performed as described previously [13, 19, 20] and".

- We appreciate your comment. As commented by reviewer, we added the statistical analysis method in Page 8, line 13. And we deleted "were performed as described previously [13, 19, 20] and".

Results

Acute oral toxicity

Page 8, Line 2: Checked as "During the experimental period, no clinical symptoms or …."

- We appreciate your comment. As commented by reviewer, we changed to "During the experimental period, no clinical symptoms or".

Page 8, Line 4: revised as "The changes is body weight are shown in Fig.2." "….between the treated rats.."

- We appreciate your comment. As commented by reviewer, we changed to "The changes in body weight are shown in Fig.2." and "between the treated rats".


- We appreciate your comment. As commented by reviewer, we clarified the mean of observations by adding “pathological”.

Page 8, Line 10: add "when" before "compared".

- We appreciate your comment. As commented by reviewer, we added ‘when’.

Page 8; Line 16: add "the frequency of" before "metaphases.".

- We appreciate your comment. As commented by reviewer, we added ‘the frequency of’.
Page 8, Line 17: Placed "for 6 h" after "S9 fraction".
- We appreciate your comment. As commented by reviewer, we added ‘S9 fraction’.

Page 8, Line 18: Placed "for 6 h" after "S9 fraction".
- We appreciate your comment. As commented by reviewer, we added ‘S9 fraction’.

Page 8, Line 19: Delete "these seems that the methods used in this study were valid." write (Table 3).
- We appreciate your comment. As commented by reviewer, we changed to "these seems that the methods used in this study were valid" to ‘(Table 3)’.

Page 8, Line 22-23: The first 2 sentences should be mentioned under "Acute oral toxicity" part.
- We appreciate your comment. The first 2 sentences are results in “in vivo MN (micronucleus) test”. For in vivo MN test, GJBRHE was administered once daily to mice by oral gavage for 2 days. Therefore, general observation including clinical signs, mortality and bodyweights were also recorded.

Page 9, Line 8: delete "indicating that the present study was performed under good laboratory conditions."
- We appreciate your comment. As commented by reviewer, we deleted "indicating that the present study was performed under good laboratory conditions”.

Discussion

Page 9, Line 23: Safety doses would be mentioned.
- We appreciate your comment. As commented by reviewer, we edited the sentence in page 11, line 19.

Page 10, Line 3: revise as "…but genotoxicity was detected.."
We appreciate your comment. As commented by reviewer, we changed to "but genotoxicity was detected".

Page 10, Line 7-9: This part would be rewritten. The sentences "The in vitro chromosome aberration test is used to identify structural chromosomal aberrations [15]." should be placed in Page 10, Line 5 before the sentence "In the in vitro chromosome aberration assay, there were significant…"

- We appreciate your comment. As commented by reviewer, we rearranged the sentence "The in vitro chromosome aberration test is used to identify structural chromosomal aberrations [15]" and changed ‘In the in vitro chromosome aberration assay, there were’ to ‘In present study, there were’.

Page 10, Line 11: revise as "The Ames test was developed by Ames and coworkers in the early 1970s to assess the mutagenic potential and to estimate carcinogenic potential of environmental mixtures [23]."

- We appreciate your comment. As commented by reviewer, we changed to "The Ames test was developed by Ames and coworkers in the early 1970s to assess the mutagenic potential and to estimate carcinogenic potential of environmental mixtures [23]".

Page 10; line 18-19: There is too much information for Ames test principle. It needs to be summarized/shortened. The sentence "Ames tests in four histidine auxotroph strains of S. typhimurium and one tryptophan auxotroph strain of E. coli were investigated. These four strains have been shown to be sensitive to mutagenic activity [25]" would be deleted.

- We appreciate your comment. As commented by reviewer, we deleted "Ames tests in four histidine auxotroph strains of S. typhimurium and one tryptophan auxotroph strain of E. coli were investigated. These four strains have been shown to be sensitive to mutagenic activity [25]".

Page 11, Line 4-5: "no abnormal signs in the general appearance and body weight of mouse were observed in any of the GJBRHE treated groups." This acute toxicity data should be mentioned after first sentence in Page 9, Line 23.

- We appreciate your comment. As previously mentioned, this sentence is also related to the MN test.
Page 11, Line 8: Revise as "MN assay has revealed the accumulated genotoxic damage during the lifetime of the cells [30]. Delete "therefore MN can be a target of carcinogenesis". Therefore, GJBRHE is associated with a low risk of carcinogenesis.

- We appreciate your comment. As commented by reviewer, we changed to "MN assay has revealed the accumulated genotoxic damage during the lifetime of the cells [30]” and deleted “because MN can be a target of carcinogenesis. Therefore, GJBRHE is associated with a low risk of carcinogenesis.”

Page 11, Line 10: Revise as "In our study, we conclude that GJBRH may be associated with a low risk of carcinogenesis." and placed to conclusion part.

- We appreciate your comment. As commented by reviewer, we rearranged “In our study, we conclude that GJBRH may be associated with a low risk of carcinogenesis” in Conclusions part..

Conclusion

Page 11, Line 13: Revise as "GJBRHE did not cause detectable genotoxic effects in the bacterial mutation test or the in vivo MN assay, however genotoxic effect was detected in the in vitro chromosomal aberration assay."

"Our results suggest that GJBRHE is genotoxic" This statement is very ambitious. Authors should come to the conclusion in a more realistic way. Because the treatment dose in this study are high to conclude GJBRHE is genotoxic. The conclusion part should be revised.

- We appreciate your comment. As commented by reviewer, we changed to ""GJBRHE did not cause detectable genotoxic effects in the bacterial mutation test or the in vivo MN assay, however genotoxic effect was detected in the in vitro chromosomal aberration assay." In addition, we added “Thus, further detailed experiments would be needed to clarify the compound responsible for inducing this genotoxicity of GJBRHE and to determine its mechanism.”

References

The references should be checked.
- We appreciate your comment. As commented by reviewer, we corrected the reference style for BMC cam format.

Page 15; Line 1: The references [15] should be checked in reference and text part..

- We appreciate your comment. Repetition of references [15] was mistakes and we corrected it by deleting the next reference [15], one of them.

Table 2, the amount of mg/kg would be clarified under table.

- We appreciate your comment. As commented by reviewer, we added the clarified comments under table 2.