Author’s response to reviews

Title: Investigation of the anti-cataractogenic mechanisms of curcumin through in vivo and in vitro studies

Authors:

Tao Wang (taowang@163.com)
Jing Cao (caojing11984@163.com)
Meng Wang (wangmeng11984@163.com)

Version: 1 Date: 19 Jul 2017

Author’s response to reviews:

Dear Dr. Young Joo Shin

On behalf of my co-authors, we thank you very much for giving us an opportunity to revise our manuscript entitled “Investigation of the anti-cataractogenesis mechanisms of curcumin: in vivo and in vitro studies”. (ID: BOPH-D-17-00163). We appreciate the editor and reviewers very much for their positive and constructive comments and suggestions. We have studied reviewer’s comments carefully and have tried our best to make revisions in track changes mode in MS word. Attached please find the revised version, which we would like to submit for your kind consideration.

We would like to express our great appreciation to you and reviewers for comments on our paper. Looking forward to hearing from you.

Thank you and best regards.

Yours sincerely,

Tao Wang; Jing Cao; Meng Wang

Point-by-point response to the comments:
Response to Reviewer 1

Comment 1 Dear Authors

It was an interesting experimental research.

Curcumin is a well-known substance for anti-oxidant. Also, it contributes to anti-cancer effect, neuroprotection, and calcium regulation.

The research was intended to verify the mechanism of curcumin that prevent cataract caused by oxidative stress. Selenite reduces numerous anti-oxidation substances such as CAT, SOD, MDA, GSH-Px and increases MDA and apoptosis which eventually generate cataract. Authors have shown the curcumin's influence on neutralizing the effect of selenite. Also, in vivo and in vitro experiments demonstrated that curcumin has anti-oxidative effect and suppresses apoptosis, which conduces toward reducing the development of cataract. However, it is difficult to judge the mechanism of curcumin only through these experiments.

Moreover, these contents were previously proved by other research papers. The paper is similar to 11th reference and the research 'Effect of curcumin on selenite-induced cataractogenesis in Wistar rat pups' done by Manikandan R (Curr Eye Res. 2010 Feb;35:122). Also, other paper (Cell Physiol Biochem. 2017;41:661) had previously reported the curcumin's effect on decreasing apoptosis was related with mitochondrial dysfunction in detail. In other report, curcumin has the effect of cellular protection by regulating intracellular calcium release through
transmitter. I thought that these reports described the effect of curcumin more specifically. So I believe that this report failed to differentiate its content with other previous researches. Authors' notification about the mechanism of curcumin represses apoptosis through the change of mRNA expression such as caspase 3, Bax, Cox-2, Bcl-2 etc. is alike to the result of precedent studies. The paper also needs additional explanations about c-met and slug which had no significance in conclusion.

In order to issue the paper, it must need its own creative experiment or the significant explanations about the meanings of these reports.

Response: Thanks very much for your fair suggestion. We have reviewed the references listed in the comments. We found that reference 11 and Manikandan R’s study only carried out in vivo studies and only antioxidant potential of curcumin was researched. In our study, in addition of in vivo study, we also carried out in vitro studies to investigate the anti-apoptosis effect and its molecular mechanism of curcumin. Besides, we added some new results and further discussion in the revised manuscript. We agreed that as a potential anticataract agent, curcumin has been one of the central areas of anticataract research. However, we believe any further research is warrant to promote its clinical use in anticataract therapy. We really appreciate your kind suggestion and will continue to investigate its clinical use in our further studies.

Comment 2 <Text errors>

Page 6. In the paragraph about Real time PCR, 'table 1' is mislabeled as 'table 2'.

Page 8. As shown in Figure 3B-D, H2O2 caused an increase in the number of cell death in HLEBs cells after treatment for 24 hours. (11.7% vs. 27.2%) The percentage value of 11.7% should be changed to 12.8% to match the percentage value provided in Figure 3.

Response: Thanks very much for your reminding. We are so sorry for this error and have revised it in the manuscript.

(Reviewer 2): Reviewer's comments

Manuscript BOPH-D-17-00163: "Investigation of the anti-cataractogenesis mechanisms of curcumin: in vivo and in vitro studies" by Tao Wang, Jing Cao and Meng Wang
The authors investigated the anti-cataractogenic activity of curcumin using in vivo and in vitro experiments. They concluded that curcumin might attenuate selenite-induced cataract by reducing intracellular production of reactive oxygen species and protecting cells from oxidative damage.

This manuscript is quite interesting and informative but overall English expression should be polished. Moreover, there are some issues that need improvement and clarification.

1. Abstract
No mention about HSP70 and GSH-Px in the Methods, which just can be found in the Results.
Response: Thanks very much for your kind suggestion. We have added the HSP70 and GSH-Px in the Methods section in the revised manuscript.

2. Selenite-induced cataract model
I think this model is already used in studies elsewhere. If so, kindly provide us with relevant reference.
Response: Thanks very much for your kind suggestion. Yes, this model is already used in studies elsewhere. We constructed the model by referencing previous studies. The references were given in Discussion, as follow:

“The in vivo cataract model was established by injecting with selenium in the eye. This is the classical method for creating cataract model (15, 16) which is widely used in studying the pathogenesis of senile cataract and the effect of anti- cataract drugs (17).”

We also add the references in methods section in the revised manuscript.

3. Statistical analyses
I'm not sure parametric methods (t-test) is suitable to analyze the data in this study. Considering the number of subjects (6 rats in each group) or experiments (3 times) is relatively small, non-parametric tests are considered to be more suitable to determine the statistical significance.
If non-parametric tests are more suitable, overall results can be changed according to the proper statistical analyses.

Response: Thanks very much for your kind suggestion. We have checked several references that carried out in vivo studies and found that majority of them employed t-test to analyze data. For example, “MicroRNA-378 enhances inhibitory effect of curcumin on glioblastoma” “Curcumin Inhibits Heat-Induced Apoptosis by Suppressing NADPH Oxidase 2 and Activating the Akt/mTOR Signaling Pathway in Bronchial Epithelial Cells” “Curcumin induces G2/M cell cycle arrest and apoptosis of head and neck squamous cell carcinoma in vitro and in vivo through ATM/Chk2/p53-dependent pathway” and so on.

Besides, we have consulted a colleague who is a statistical expert and he said that t-test is suitable for analysis of small sample size (n < 30) and the data should follow normal distribution. Therefore, we believe t-test is suitable for our data analysis.

4. Figure 1

From these photos, I'm not sure the opacity (B) was developed in the lens (corneal opacity also can be seen similarly). And anti-cataractogenic effect of curcumin (C) is doubtful. For the readers' understanding, I recommend the authors present more magnified images just showing the lens, iris and cornea.

Response: Thanks very much for your kind suggestion. This is really a critical problem. We reviewed the photos but it is such a pity that all photos were taken at this distance. However, we further boxed the opacity area in the revised Figure 1 and hope it is helpful for the readers' understanding.

5. Description in the Methods and Figure 2

For readers' smooth understanding, how about modifying the order of molecules described in the Methods (page 6 Biochemical examinations) and presented in the Figure 2 according to their properties?

For example, HSP70 (heat shock protein), 8-OHdG, MDA (oxidative stress markers), CAT, SOD and GSH-Px (free radical scavengers)

Response: Thanks very much for your kind suggestion. HSP70 was determined by RT-PCR, therefore, we described it in RT-PCR section on page 7. The other molecules in methods and Figure 2 were described in the order according to your suggestion.
6. Figure 3B-D

The actual value presented in Figure 3B (12.8%) is different from that in the text (11.7%, page 9, line 3 (11.7% vs. 27.2%).

Moreover, the authors showed a result from just one experiment. We can't say that cell apoptosis is prevented by curcumin unless it is clearly proved by statistics.

Response: Thanks very much for your kind suggestion. 11.7% in the text was a writing mistake, and we have revised it to the right value of 12.8%.

7. HSP70

As the authors described in the Discussion, HSP70 might protect lens against a oxidative damage (maybe anti-cataractogenic effect). However, curcumin significantly suppressed HSP70 which was upregulated by selenite injection. The authors would better explain possible cause or clinical significance of this unexpected result.

Response: Thanks very much for your comments. We hypothesized that this is because HSP70 is a stress induced protein and curcumin could decrease the oxidative stress caused by accumulated free radicals. We have added this discussion in the revised manuscript.

Editorial Policies

Please read the following information and revise your manuscript as necessary. If your manuscript does not adhere to our editorial requirements, this may cause a delay while this is addressed. Failure to adhere to our policies may result in rejection of your manuscript.

In accordance with BioMed Central editorial policies and formatting guidelines, all manuscript submissions to BMC Ophthalmology must contain a Declarations section which includes the mandatory sub-sections listed below. Please refer to the journal's Submission Guidelines webpage for information regarding the criteria for each sub-section (https://bmcophthalmol.biomedcentral.com/).
Where a mandatory Declarations section is not relevant to your study design or article type, please write "Not applicable" in these sections.

For the 'Availability of data and materials' section, please provide information about where the data supporting your findings can be found. We encourage authors to deposit their datasets in publicly available repositories (where available and appropriate), or to be presented within the manuscript and/or additional supporting files. Please note that identifying/confidential patient data should not be shared. Authors who do not wish to share their data must confirm this under this sub-heading and also provide their reasons. For further guidance on how to format this section, please refer to BioMed Central's editorial policies page (see links below).

Declarations

- Ethics approval and consent to participate

- Consent to publish

- Availability of data and materials

- Competing interests

- Funding

- Authors' Contributions

- Acknowledgements

Further information about our editorial policies can be found at the following links:
Ethical approval and consent:

http://www.biomedcentral.com/about/editorialpolicies#Ethics

Availability of data and materials section:

http://www.biomedcentral.com/submissions/editorial-policies#availability+of+data+and+materials

Response: Thanks very much for your kind suggestion. All these sections were confirmed to be provided in the revised manuscript.