Author’s response to reviews

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

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Author’s response to reviews:

Dear editor,

We are so sorry for our late response. Our manuscript entitled “Investigation of the anti-cataractogenesis mechanisms of curcumin: in vivo and in vitro studies” (ID: BOPH-D-17-00163R1) has been further revised according to the reviewers’ comments. We appreciated so much for their comments. Those comments are of high guidance value for our research and for the quality improvement of our manuscript. We have responded to their comments point-by-point below and revised the manuscript accordingly using track changes mode in MS word. Now we re-submit this revised manuscript and hope it can be considered for publication in BMC Ophthalmology.

Point-by-point response to editor and reviewers’ comments

Editor Comments:

The sentences are similar to the previously published papers. Please, write your own words.

Response: Thanks very much for your kind suggestion. We have rewrite some sentences referenced to the previously published papers and checked the similarity index using www.turnitin.com. As you know, most of the methods are regular molecular biology experiments and some fixed usage could not be avoided. We really hope it is qualified for the prescribed limit.
Reviewer reports:

Moonsun Jung (Reviewer 1): Dear Authors

Thank you for your response.

This paper became more reasonable and easy to read, through a revision. I appreciate for your efforts trying to accept my recommendations. But there are still weak points in the paper to accept. I recommend you few additional modifications.

Comment 1. There are many in vivo and vitro experiments demonstrated that curcumin has anti-oxidative effect and suppresses apoptosis, which conduces toward reducing the development of cataract. It is difficult to what is special this research to compare other researches about the mechanism of curcumin only through these experiments. You replied me you did in vivo and in vitro experiments, and add other experiment about superoxide anion. I think it is better than before but not sufficient to differentiate the meaning of the paper. Please add your special interpretation of experiment to the discussion. It is not enough to say that the mechanism of cataract prevention by curcumin.

Response: Thanks very much for your kind suggestion. Yes, we admitted that the results related with the anti-oxidative effect are of little progress compared with previous studies. However, few studies reported the anti-apoptosis of curcumin in cataract. We have added some discussion about our special interpretation according to your kind suggestion. Thanks very much again.

Comment 2. Methods

In cell proliferation analysis

What did you use cell line? After that paragraph, in cell apoptosis, you used HLEBs cells. Of course I can suppose you used HLEBs cells in proliferation analysis, but you'd better describe it first.

Response: Thanks very much for your kind suggestion. We have added “HLEB-3” in this sentence.

3. There is no reference in intracellular O2- concentration detection. Is that your own experiment? If not, please add reference.
Response: Thanks very much for your kind suggestion. The methods for intracellular O2-concentration detection was followed previous references and we have added the reference in the revised manuscript.

Comment 4. Results

Figure 1, in these photos, it is difficult to observe the degree of the cataract. Although you explained that you have enlarged the photo and modified it in the box, I couldn't see it in the revision. It was not changed. If you want to explain the degree of the cataract with photo, you'd better show the enlarged photo of the lens, or you'd better get rid of it.

Response: Thanks very much for your kind suggestion. We have checked our original data and unfortunately there were no better photos. Therefore, we have to get rid of it according to your kind suggestion.

Comment 5. You'd better unify the terminology.

Cell proliferation in methods and results vs cell viability in figure 3B. What is more suitable word? For reader's understanding, please unify the word.

Response: Thanks very much for your kind suggestion. We have modified “cell proliferation” to “cell viability” in the manuscript for better unify the terminology.

6. Figure 4

There is no explanation for bcl-2 in figure 4, please add it. C-met & slug was increased but not significant. How should I interpret this? Why did you do test for m-RNA of the caspase 3, bax, bcl-s, cox-3, c-met and slug? Some of these changed and some of these didn't change. For reader's understanding, please add the interpretation of these results in discussion. I think it is important for the anti-cataract effect of curcumin.

Response: Thanks very much for your kind suggestion. Discussion about the changes of “bcl-2, C-met, slug” has been added in the revised manuscript.

Hyuk Jin Choi, M.D., Ph.D. (Reviewer 2): Reviewer's comments
The authors correctly answered all questions and introduced the requested changes. I think this improved the manuscript considerably. However, there are a couple of minor issues that have arisen.

1. Statistical analyses

I respect a statistical expert's opinion on using t-test in the analysis of small sample size. However, I still do not think t-test is suitable for the analysis as the data from small sample size usually do not follow a normal distribution. Non-parametric analysis such as Mann-Whitney U test would be better than parametric analysis.

Response: Thanks a lot for your kind suggestion. We really learn a lot from this communication. As you suggested, data should follow a normal distribution if t-test is used. Therefore, we preformed normality test using SPSS. We attached some results as examples here:

Normality test for ELISA results (n=6 for each group)

<table>
<thead>
<tr>
<th></th>
<th>Kolmogorov-Smirnova</th>
<th>Shapiro-Wilk</th>
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<td>df</td>
</tr>
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<td>Control1</td>
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</tr>
<tr>
<td>Model1</td>
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<td>6</td>
</tr>
<tr>
<td>Curcumin1</td>
<td>.164</td>
<td>6</td>
</tr>
<tr>
<td>Control2</td>
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<td>6</td>
</tr>
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<td>6</td>
</tr>
<tr>
<td>Curcumin3</td>
<td>.213</td>
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</tbody>
</table>
Control4  .312  6  .069  .869  6  .223
Model4  .160  6  .200  .975  6  .925
Curcumin4  .273  6  .182  .828  6  .104
Control5  .286  6  .137  .833  6  .114
Model5  .190  6  .200  .939  6  .647
Curcumin5  .139  6  .200  .990  6  .990

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*. 这是真实显著水平的下限。

Where control 1, model 1 and curcumin1 represent the protein level of 8-OHdG, control 2, model 2 and curcumin2 represent the protein level of CAT, control 3, model 3 and curcumin3 represent the protein level of SOD, control 4, model 4 and curcumin4 represent the protein level of MDA, and control 5, model 5 and curcumin5 represent the protein level of GSH-Px.

Since the sample size is below 50, the Shapiro-Wilk test should be used. From the above results, we could find that all the significance is larger than 0.05, suggesting the data follow normal distribution.

Normality test for in vitro RT-PCR results (n=3 for each group)

<table>
<thead>
<tr>
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<tr>
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<tr>
<td>Control1</td>
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<tr>
<td>Model1</td>
<td>.360</td>
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<td>3</td>
</tr>
<tr>
<td>Model2</td>
<td>.368</td>
<td>3</td>
</tr>
</tbody>
</table>
Where control 1, model 1 and curcumin1 represent the mRNA level of caspase 3, control 2, model 2 and curcumin 2 represent the mRNA level of bax, control 3, model 3 and curcumin 3 represent the mRNA level of cox-2, control 4, model 4 and curcumin 4 represent the mRNA level of c-met, control 5, model 5 and curcumin 5 represent the mRNA level of bcl-2, and control 6, model 6 and curcumin 6 represent the mRNA level of slug.

Since the sample size is below 50, the Shapiro-Wilk test should be used. From the above results, we could find that all the significance is larger than 0.05, suggesting the data follow normal distribution.

According the above results, we believe student’s t test is appropriate for the statistical methods of this study. However, we also respect your opinion and will consider the choice of statistical methods with more caution in our later studies.

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2. Figure 3 (Revised) and description of the results

I think it's better that the description (Cell proliferation and apoptosis analysis) follows the order shown in the Figure 3 A-D.

Response: Thanks very much for your kind suggestion. We have modified the sequence of the description of the results. We really appreciated for your kind suggestion a lot.