Author's response to reviews

Title: Cytotoxicity and apoptotic activities of alpha-, gamma- and delta-tocotrienol isomers on human cancer cells

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Author's response to reviews: see over
Dear Editor in Chief,

Revisions on MS: 2570316611431701 - *In vitro* antiproliferative and apoptotic activities of alpha-, gamma- and delta-tocotrienol isomers on human cancer cells

Thank you for handling this manuscript. I would also like to thank the reviewers for the constructive comments. On behalf of all authors, I would like to state our responses on reviewers’ comments as arranged in the following table. Please note that all corrections are highlighted in the revised manuscript.

<table>
<thead>
<tr>
<th>No.</th>
<th>Reviewer’s Comments</th>
<th>Authors’ Responses</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Propose to change the title: “in vitro antiproliferative” to “cytotoxicity”, as the method used is to determine the cell viability of the cultures</td>
<td>Agreed. The title has been changed from “in vitro antiproliferative” to “cytotoxicity”. “Cytotoxic” or “cytotoxicity” has been used to replace antiproliferative at relevant sessions as well.</td>
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<tr>
<td>2.</td>
<td>As this is not a “cytostatic assay”, thus it is recommended not to use “GI”, instead, “IC” should be used</td>
<td>Agreed. All “GI” has been changed to “IC”.</td>
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<tr>
<td>3.</td>
<td>Line 25 page 3: should add “the” after at…concentrations ranging…</td>
<td>Has been done accordingly.</td>
</tr>
<tr>
<td>4.</td>
<td>Line 1 page 4: should add “the” after at…concentrations ranging…</td>
<td>Has been done accordingly.</td>
</tr>
<tr>
<td>5.</td>
<td>Line 19 page 5: kinetic study (data not shown)- the data can be incorporated as appendix for understanding</td>
<td>Agreed. The data for kinetic study has been added as Additional file 1 into Additional Files section for better understanding of readers.</td>
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<tr>
<td>6.</td>
<td>Line 19 page 10: data not shown- recommended to add in as this is essential for reader to have further understanding the finding</td>
<td>Agreed. FDA&amp;PI results have been incorporated in Figure 2 for better understanding of readers.</td>
</tr>
<tr>
<td>7.</td>
<td>All the findings for the standard control, e.g.: vinblastine, should be incorporated in the results as comparison, it is essential for reader to have comprehensive understanding of the paper</td>
<td>Basically, vinblastine is a commercial drug which has been studied extensively and therefore was recruited in the study as positive control. We have published some data using vinblastine in previous studies [20 and 21] on A549 and U87MG cells including the cell viability, cellular morphologies and comet profiles. Therefore, republishing similar data is prohibited, but we have cited these references at relevant sections for comparison purpose where reader could refer to. Besides, the manuscript is rather long with 11 figures and 5 tables; we don’t want to place additional figures showing negative results of vinblastine for caspase-8 assay and others. Vinblastine is just a control for assays and not our focus for the elucidation of its mechanism. In fact, the apoptotic pathways for vinblastine have been elucidated before and are of no value to be published again. We have stated the vinblastine observations in the text of relevant Results</td>
</tr>
</tbody>
</table>

Reviewer 1: Yang Mooi Lim
sections and/or cited previous vinblastine reference [36] to serve as comparisons to tocotrienols which we think are more appropriate. We have however included the MMP results of vinblastine because to our knowledge, it is new and valuable for publication as vinblastine effect on MMP has not been published before.

8. Line 7 & 8 page 28: change “have been” to “is”

“Have been” changed to “are” (plural).

**Reviewer 2: Nik Mohd Afizan Nik Abd. Rahman**

1. No presented data so please transfer sentences start line 19 (page 10) till line 2 (page 11) into “Discussion” part

We would like to follow the suggestion of Reviewer 1 by including FDA&PI results in Figure 2 to enhance understanding of readers. Therefore, no transfer of sentences has been made.

2. Please indicate how many wells or replications have been performed for cell cycle analysis

Triplicates of each sample were used in the cell cycle analysis. This statement has been added into the text under Cell cycle phase evaluation by flow cytometry section.

3. MMP analysis was conducted for 3 h and 24 h but there is no data included for 24 h in “Figure 8”

For tocotrienols case, the data for 3 h and 24 h are comparable (no significant difference), so, only representative results for 3 h are shown. Whereas for vinblastine, representative results for both 3 h and 24 h have been added in Figure 8. Results for vinblastine indicate that vinblastine does not significantly cause MMP disruption at 3 h and even up till 24 h. Majority of cells are fluoresced red at both concentrations and treatment periods tested.

4. Combining Figure 2 and Table 3 into a single data, so that readers can analyze and count the data quickly and easily

Figure 2 and Table 3 do not provide exactly the same information. Table 3 depicts the percentage analysis of an average of at least 200 cells under different stages of apoptosis, whereas Figure 2 just exhibits some examples of cellular morphologies under different stages of apoptosis (but not all). Moreover, FDA&PI images have been included in Figure 2 as recommended by Reviewer 1. Hence, it is not appropriate to combine Figure 2 and Table 3 into a single data.

**Reviewer 3: Beow Chin Yiap**

1. To include positive control in all the results

Basically, vinblastine is a commercial drug which has been studied extensively and therefore was recruited in the study as positive control. We have published some data using vinblastine in previous studies [20 and 21] on A549 and U87MG cells including the cell viability, cellular morphologies and comet profiles. Therefore, republishing similar data is prohibited, but we have cited these references at relevant sections for comparison purpose where reader could refer to.
Besides, the manuscript is rather long with 11 figures and 5 tables; we don’t want to place additional figures showing negative results of vinblastine for caspase-8 assay and others. Vinblastine is just a control for assays and not our focus for the elucidation of its mechanism. In fact, the apoptotic pathways for vinblastine have been elucidated before and are of no value to be published again. We have stated the vinblastine observations in the text of relevant Results sections and/or cited previous vinblastine reference [36] to serve as comparisons to tocotrienols which we think are more appropriate. We have however included the MMP results of vinblastine because to our knowledge, it is new and valuable for publication as vinblastine effect on MMP has not been published before.

| 2. | To include a flow-chart or diagram on the proposed mechanism of action | Agree. A schematic diagram on the proposed apoptotic mechanisms of action has been added as Additional file 2 into Additional Files section. |

In addition, the revised manuscript and files have been checked and are conformed to the journal style and format.

We hope to get your favorable decision on this revised manuscript.

Thank you very much.

Yours sincerely,

HS Loh (Corresponding author)