Author's response to reviews

Title: Growth arrest and apoptosis via caspase activation of dioscoreanone in human non-small cell lung cancer A549 cells

Authors:

Pintusorn Hansakul (hansakul@gmail.com)
Kalaya Aree (k_attanavanich@hotmail.com)
Sermkiat Tanuchit (stanuchit@gmail.com)
Arunporn Itharat (iarunporn@yahoo.com)

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

We have now carefully revised the manuscript entitled “Growth Arrest and Apoptosis via Caspase Activation of Dioscoreanone in Human Non-Small-Cell Lung Cancer A549 Cells” according to the reviewers’ comments. The points which needed further discussion are listed below. As you will see, the points raised by the reviewers are repeated (in bold letters) and are followed by a detailed reply and discussion by the authors. Additionally, if necessary, text passages that have been added to the revised manuscript are given (written in italics).

Reviewer: Supinya Tewtrakul

1. Please add the data of the positive control for cytotoxicity test in Table 1 as well as in the discussion part.

For the cytotoxicity test, we have added the data of pacritaxel, an anticancer drug, which was used as a positive control in Table 1. A text passage below explaining the data of paclitaxel has been added in the Results Section of the manuscript instead so that the data of paclitaxel can be compared clearly to those of DN as written in the previous paragraph.

Page 12: In contrast to DN, paclitaxel showed a very strong antiproliferative activity with ∼140-1270-fold higher GI_{50} and TGI values in all tested cell lines (except NCI-1688). However, paclitaxel appeared to exert selective growth inhibitory effects on NSCLC cancer cells but high cytotoxicity on human normal lung fibroblasts. Therefore, DN has potential as a promising anticancer agent because of its selective antiproliferative and cytotoxic activities without harming normal cells. Several studies described below were performed to provide insights into the molecular mechanism(s) underlying the anticancer activity of DN.

2. Figure 1-7 have poor quality.

We have increased the resolution of all seven figures.

3. Please indicate the statistical significance for Figure 2B and Figure 7 when compared to that of the control group (*p < 0.05 or **p < 0.01)

We have indicated the statistical significance (*p < 0.05) for Figure 2B and Figure 7 as compared to the control group.

4. The color for the control bar graph has poor quality
We have made the control bar graph filled with a dot-pattern style in place of the light or unclear color in the first version of our manuscript.

5. Why the control group for the figures 6 and 7 did not have a y-bar error?
For Figures 6A and 6B, the caspase-3 activity in untreated cells is taken as 1-fold so that there is no y-bar error, and the figures on the chart display the relative changes of such activity in the treated cells.
Similarly, for Figure 7, mRNA levels of Bax, Bcl-2 and Bax/Bcl-2 in control groups for each incubation time are taken as 1-fold. These levels in treated A549 cells versus untreated cells are expressed using relative numerical values.

Reviewer: Liron Berkovich

1. Add “Dioscoreanone” and “Dioscorea membranacea” to the manuscripts’ keywords.

We have added all these recommended keywords.

2. In Table 1 the term “amount of DM” should be revised to “DM concentrations” and concentration units should be indicated in the table.

This misuse of terminology has been corrected in the revised manuscript.

3. On page 12, 2\(^{nd}\) paragraph, of the manuscript the fact that DM’s growth inhibition had been evident starting from 24 and 48 hr treatments should be mentioned in the text. Also, Figure 2 itself, statistical significance of growth inhibition effects should be indicated as *p value

A sentence “Evidently, the growth inhibitory effect of DM started from 24 to 48 h-long treatments.” has been added in the revised version.

The wording at the end of the paragraph of the Results section in the topic The antiproliferative activity of DN in adenocarcinoma A549 cells has been changed to the sentences below.

“In contrast, only 13.6% of DN-treated parental cells underwent at least two rounds of cell division after 72 h, indicating that cell division greater than 85% of the A549 cells was hindered. Altogether, the results revealed DN-induced cell division arrest.”
4. I would suggest to delete the first sentence of page 13, 1st paragraph, and start it with “the effect of DN on cell cycle distribution...”

This sentence has been deleted as suggested.

5. Regarding figure 3 and 4; has its data been statistically analyzed? If not, it should undergo a statistical analysis and p values should be indicated in the figure in order to consider this data significant. In addition, at each case it should be noted how many such independent experiments were performed.

A statistical analysis and p values have been indicated in figures 3 and 4. A phrase “at least three independent experiments” has been added in a legend (a brief description) for all figures.

6. On the Discussion Section; published data regarding the biological availability of Dioscoreanone when consumed per os should be discussed in compliance with the DN’s concentrations examined in this study. If such data is not available, this should be addressed by the authors as well.

We have expanded the discussion on this point. The sentences below have been added.

Besides new insights on the anticancer mechanisms of DN, understanding its bioavailability when consumed per ounce (oz) also helps determine the amount of DN to be taken orally in compliance with the DN concentrations examined in this study. However, no such data are available, and in vivo oral bioavailability of DN needs to be further evaluated.

In addition, the revised manuscript has been proofread and edited by a native English editor.

Sincerely yours,
Pintusorn Hansakul
Please address all correspondence to:
Pintusorn Hansakul, Ph.D.
Preclinical Science, Department of Biochemistry, Faculty of Medicine
Thammasat University (Rangsit campus)
Khlong Luang, Pathum Thani, 12120, Thailand
Tel: (662) 926-9744 (office), Fax (662) 926-9710
Mobile phone: 66-81-906-4374
E-mail: hansakul@gmail.com