Author's response to reviews

Title: Annona muricata Leaves Induced Apoptosis in A549 Cells through Mitochondrial-Mediated Pathway and Involvement of NF-kB

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Author's response to reviews: see over
26th July 2014

Dear Dr. Lyndy McGaw,

Thank you for the very helpful reviewers’ comments on our manuscript. We have carefully modified the manuscript to fully address all comments. Please find below our point to point reply to the comments of reviewers. We hope that these changes meet both yours and the reviewers’ expectations. Please do not hesitate to contact us for further clarifications.

Thank you in advance for your kind consideration.

Sincerely Yours,

Associate Professor Dr. Habsah Abdul Kadir
(Corresponding Author)
Comment # 1 (Major)

In figure 4A-D, the max value of Y-axis (number) is inconsistent. The authors should unify this value or these data would be misleading. Furthermore, the image resolution is too low, the symbols are barely recognized even zoom to 200%. They should be replaced with better images.

Reply:
The resolution of the Figure 4 was modified accordingly.

Regarding the Y-axis number in the Figure 4, the flowcytometric analysis of cell cycle was carried out with 10,000 cells per sample. Therefore, the results in 4A to 4D show the consistent number of events (10,000 cells). The software analysis of the flow cytometer (BD FACSCanto™ II) is ModFit LT software (Verity Software House, Inc., Topsham, ME). Based on the peaks of the graphs, the software automatically adjusted the most suitable Y-axis number. The results were orginally taken from the software without any modifications, similar to other published reports below, where different Y-axis number were selected based on the peaks of the graphs.


Comment # 2 (Major)

It is very confusing in figure 7B. Compared with the data (figure 9) in the reference [19] which the authors mentioned in methods section, the patterns in figure 7B seem not to be typical images for western blot analysis. It is much better to represent as original images if the authors use negative effect in the data.

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Reply:
The figures of the western blot analysis are original data from the gel doc device (Fusion FX7, Vilber Lourmat, Eberhardzell, Germany) without any further negative effects. In fact, the images with white bands and black backgrounds were chosen based on the option of the device to take in this shape same as the following published manuscript. Regarding the reference [19], we referenced it only regarding the methods of the assay and the images were taken based on the options of the gel doc device. Since we did not take the images with white backgrounds and black bands, we are afraid that the requested images are not available in the device.

Stefanie Jeruschke, Anja Katrin Bu¨ scher, Jun Oh, Moin Ahson Saleem, Peter Friedrich Hoyer, Stefanie Weber, Perihan Nalbant, 2013, Protective Effects of the mTOR Inhibitor Everolimus on Cytoskeletal Injury in Human Podocytes Are Mediated by RhoA Signaling, 8(2), e55980. (This study used the same gel doc device, Fusion FX7)


Comment # 3 (Major)
In figure 8, there are only representative bar charts to illustrate the data. The authors should provide original data (images of gel electrophoresis) such as the data in figure 3, 4, 5, and 6. Moreover, the statement of figure 8 in result section is missed. The authors should mention figure 8 in the paragraph “AMEAE induced upregulation of Bax and downregulation of Bcl-2 at the gene expression level”.

Reply:
“Figure 8” was mentioned in the respective paragraph.

Thank you for your kind advice. Please note that the RT-PCR data shown in the Figure 8 was derived from Quantitative PCR (Q-PCR) by using a real time PCR machine (Applied Biosystems StepOnePlus™ system), not through semi-quantitative RT-PCR which data derived from gel electrophoresis (where gel pictures can be shown). The data of real time PCR machine were presented as fold change (1/2^(-ΔΔCT)) normalized against a housekeeping gene (GAPDH), consistent with the graph in Figure 8.
Similar to other published reports below, where Q-PCR data were presented as fold increase or decrease by graph, we decided not to show the raw data Table in the manuscript (however the raw data has been attached to the report as a supplementary excel file).


Comment #3 (Minor)

On page 14, line 1–2, the authors mentioned that “AMEAE treatment at different time periods (24, 48 and 72 h) resulted …” However, labels on X-axis of figure 3E are “control”, “24”, “47”, and “72”, respectively. The authors should confirm this.

Reply:
The test was carried out at different time periods of 24, 48 and 72 h, which was corrected in the Figure 3E, accordingly.

Reviewer #2:

Comment #1 (Minor)

Extraction procedures described in Methods should be given more details, such as extract time. Whether solvent affect the results? Why the author did not set a solvent control group?

Reply:
It has been done accordingly. Extraction procedure was described with more details in the revised manuscript (section: Plant material and extraction procedures).
Regarding the solvent effect in our study, all measures have been taken to completely remove the solvent from all extracts. The procedure started with the filtrate being fully evaporated using a rotary evaporator until dryness. In addition, this method of extraction is a technique used in numerous published studies with no indication of solvent effect. Therefore, a solvent control group was deemed unnecessary in this study. Belayachi L, Aceves-Luquero C, Merghoub N, et al. Retama monosperma n-hexane extract induces cell cycle arrest and extrinsic pathway-dependent apoptosis in Jurkat cells. BMC complementary and alternative medicine. 2014;14(1):38.

Comment # 2 (Minor)

What does the 100 in Table 1 mean? Which should be explain under the table.

Reply:

100 represents the IC50 values greater than 100 µg/mL, which was explained under the table, as the respected reviewer requested.