Author’s response to reviews

Title: The synergism of Clinacanthus nutans Lindau extracts with gemcitabine: downregulation of anti-apoptotic markers in squamous pancreatic ductal adenocarcinoma

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Authors’ response to reviewers’ comments on the paper

“The synergism of Clinacanthus nutans Lindau extracts with gemcitabine: downregulation of anti-apoptotic markers in squamous pancreatic ductal adenocarcinoma”

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Technical Comment (1):

Please reformat the manuscript body according to our submission guidelines: https://bmccomplementalternmed.biomedcentral.com/submission-guidelines/preparing-your-manuscript/research-article
1. Declaration section - Please note that all manuscripts must contain all the following sections (respecting the same order) under the heading 'Declarations'. The Declarations should follow the Abbreviations section, and be before the References.

- Ethics approval and consent to participate
- Consent for publication
- Availability of data and material
- Competing interests
- Funding
- Authors' contributions
- Acknowledgements

Authors’ Response:

Thank you for the comment. The authors had revised the Declaration section according to the submission guideline. (Line 534-571)

Technical Comment (2):

2. Rename 'Materials and Methods' to 'Methods'.

Authors’ Response:

Thank you for the comment. The authors had revised the title to ‘Methods’ (Line 137)

Technical Comment (3):

3. In the 'Funding' statement, please declare the role of the funding body in the design of the study and collection, analysis, and interpretation of data and in writing the manuscript.
Authors’ Response:

Thank you for the comment. The authors had revised the statement under Funding. “The project was funded by International Medical University (BP-I-01-2018(13)) and Universiti Putra Malaysia (UPM) for research consumables and laboratory facilities. Both funding bodies did not involve in the design of the study, and the collection, analysis, interpretation of data, as well as in writing the manuscript.” (Line 552-555)

Reviewer reports:

Reviewer #3’s Comment: It may be important to add the previously reported studies evaluating the chemical composition of extracts from C mutant plant using mass spectrometry in order to further explore or discuss which substances could contribute to the different effects among LP, LN, SP and SN which the authors examined in this study.

Authors’ Response:

The authors wish to thank the reviewer#3 for the comments. We have revised the discussion to include new findings as Supplementary Table 4, and to expand the discussion in this context (Line 392 to 404).

Previous study (Khoo LW et al. Phytochemistry Letters 14 (2015) 123–133) published extensive metabolite profiling using ultra performance liquid-chromatography-tandem mass spectrometry (UPLC-MS/MS) and 1H NMR on the leaves and stem extracts of C. nutans. The left and stem extracts prepared using oven drying and soaking methods were adopted due to the folk’s practice and also a favourable preliminary screening as reported in our previous study (CW Mai et al. Front Pharmacol (2016) 7). We expanded our study to investigate the folk’s claim in C. nutans as potential anticancer remedy. The extracts were prepared from the previous study. Since the characterisation has been reported in previous study, therefore this manuscript will not include the same data.

The authors agreed to the reviewer#3 to correlate the chemical compositions correlation to its biological activity. Based on the previous study (Khoo LW et al. Phytochemistry Letters 14
betulin, lupeol, beta-sitosterol were the major compounds in SN extracts. However, all these 3 compounds were not active as anticancer agent (Table S4). Compounds identified in other extracts but absent in stem extracts were isovitexin, vitexin, rutin, chlorogenic acid and gallic acid were also tested. However none of these compounds were active against pancreatic cancer. Thus, research is still ongoing to isolate compounds that were not yet reported in Khoo LW et al. Phytochemistry Letters 14 (2015) 123–133. Future studies will focus in identifying compounds which were not isolated from previous studies or other literature. We are also exploring other phytochemistry methods, with the aim to isolate new compounds that have were missed in previous study.

Reviewer #3’s Comment: The difference among three pancreatic ductal carcinoma cell lines used in this study in terms of synergistic effects of gemcitabine and SN extracts should be further discussed, especially SW1990/BxPC3 versus AsPC1 cells. In addition, the mechanisms of this synergistic effects should be more discussed.

Authors’ Response:

The authors wish to thank the reviewer#3 for the comments. We agreed that further explanation of the difference and its mechanism may enhance the readers’ understanding. Our previous study (Er JL et al Apoptosis. 2018, 23 (5–6), pp 343–355) classified AsPC1 cells are progenitor pancreatic cancer cells, while SW1990 and BxPC3 are squamous pancreatic cancer cells. We have expanded the discussion to support the difference observed could be due to the transcription factors in endodermal development and differentiation. TGF-β and MYC were usually overexpressed among the squamous carcinoma, but not progenitor carcinoma. [Reference 45] In view of the upregulation of TGF-β among squamous carcinoma, gemcitabine treatment may down-regulate TGF-β in pancreatic tumor and resulting a better treatment outcomes achieved among the squamous carcinoma. The underlying mechanism behind gemcitabine inhibitory effect on TGF-β could be driven by its modulatory effect on STAT3 phosphorylation [Reference 46] Alternative explanation to the observed stronger synergism against squamous carcinoma may be attributed to MYC inhibitor in SN extracts (eg: fisetin [Reference 47] ) may downregulate MYC to enhance the cytotoxicity effect of gemcitabine. Kim N et al elucidated fisetin enhanced cytotoxicity effect of gemcitabine in squamous carcinoma via inhibition of MYC signalling [Reference 47] (Line 414-436)
End of review.

Yours faithfully,

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