Author’s response to reviews

Title: Anti-proliferative and Cytotoxic activities of Allium autumnale P. H. Davis (Amaryllidaceae) on Human Breast Cancer Cell Lines MCF-7 and MDA-MB-231

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Author’s response to reviews:

Dear editor,

Thank you for your response regarding our manuscript “Anti-proliferative and Cytotoxic activities of Allium autumnale P. H. Davis (Amaryllidaceae) on Human Breast Cancer Cell Lines MCF-7 and MDA-MB-231”.

We are grateful for the opportunity to submit a revised manuscript. We have addressed all of the revisions in a point by point fashion below and in the submitted manuscript.

1- Please change the text under Ethics and Consent to Publish to: 'not applicable'

Response: The statement 'not applicable' has now been added on line 399 in the submitted manuscript.

2- Please revise your Authors’ contribution section to confirm all authors read and approved the final manuscript.

Response: The statement "All authors read and approved the final manuscript." was added to the lines 384-385 in the revised manuscript.

3- Please add a list of figure legends to the end of your manuscript.

Response: All the figure legends have now been added on the lines 407-444 as follows in the revised manuscript "List of Figure Legends

Figure 1: AAB extract causes significant anti-proliferative effect on MCF-7 and MDA-MB-231 cells. Concentration-effect curves of AAB extract treatment 625–10000 μg/mL for 24h(A), 48h(B), and 72 h(C) in MCF-7 and MDA-MB-231 cells. Normalized cell number was calculated by using measurements from six independent MTT assays. Data is represented as mean ±S.E.M.
Statistical significance: #, p<0.05 vs control according to ANOVA followed by Newman-Keuls post hoc analysis.

*, p<0.05 and **, p<0.01 MCF-7 vs MDA-MB-231 according to Student’s t-test.

Figure 2: AAS extract causes significant anti-proliferative effect on MCF-7 and MDA-MB-231 cells. Concentration-effect curves of AAS extract treatment 625–10000 µg/mL for 24h(A), 48h(B), and 72 h(C) in MCF-7 and MDA-MB-231 cells. Normalized cell number was calculated by using measurements from six independent MTT assays. Data is represented as mean ±S.E.M. Statistical significance: #, p<0.05 vs control according to ANOVA followed by Newman-Keuls post hoc analysis. *, p<0.05 and **, p<0.01 MCF-7 vs MDA-MB-231 according to Student’s t-test.

Figure 3: AAB extract significantly reduces viability of MCF-7 and MDA-MB-231 cells. The % viability of breast cancer cells was determined by viability assay for 24h (A), 48h (B) and 72h (C). Data represents mean ±S.E.M. of six independent experiments. Statistical significance: #, p<0.05 vs control according to ANOVA followed by Newman-Keuls post hoc analysis. *, p<0.05 and **, p<0.01 MCF-7 vs MDA-MB-231 according to Student’s t-test.

Figure 4: AAS extract significantly reduces viability of MCF-7 and MDA-MB-231 cells. The % viability of breast cancer cells was determined by viability assay for 24h (A), 48h (B) and 72h (C). Data represents mean ±S.E.M. of six independent experiments. Statistical significance: #, p<0.05 vs control according to ANOVA followed by Newman-Keuls post hoc analysis. *, p<0.05 and **, p<0.01 MCF-7 vs MDA-MB-231 according to Student’s t-test.

Figure 5: AAS extract induces membrane blebbing on MDA-MB-231 cells. Light microscope images (20x) show non treated (left panel) and 2500µg/mL AAS extract treated (right panel). Arrows indicated plasma membrane blebbing after incubations with Allium autumnale stem extract. Scale Bars=50µm.

Figure 6: Effects of AAB and AAS on lateral motility of MCF-7 and MDA-MB-231 cells from 24h incubation. Bar graph showing lateral motility data obtained for MCF-7(A) and MDA-MB-231(B) for 24h incubation. “x” represents no significant difference compared to the control experiments. Representative inverse light microscope images of AAB and AAS incubation of MCF-7 (C) cells and MDA-MB-231(D) which caused no significant effect on lateral motility. Scale bars 50µm.

Figure 7: GC-MS chromatogram of compounds from ethanol extract in (A) Allium autumnale bulb and and (B) Allium autumnale stem. The numbered peaks correspond to the numbers and molecules in Table 1.

We thank you and all the reviewers for all their valuable input.

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