Reviewer's report

Title: The flavonoid beverage Haelan 951 induces growth arrest and apoptosis in pancreatic carcinoma cell lines in vitro

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Reviewer: Prem Prakash Tripathi

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Present manuscript entitled “The flavonoid beverage Haelan 951 induces growth arrest and apoptosis in pancreatic carcinoma cell lines in vitro” from Heike Weber examines effect of Haelan in inhibition of proliferation and induction of apoptosis in pancreatic cancer cell lines. Previous research from author has demonstrated that beneficial role of the bioflavonoid in pancreatic cells. Present study addressed an important point, though some points (there may be others as well) should be strengthened.

1) Cell proliferation was measure using WST-1 which is colorimetric assays that analyze the number of viable cells and surviving cells more accurately than telling about proliferating cells. Classically, BrdU have been used to measure DNA synthesis based cell proliferation. In addition, PCNA and Ki67 have good coverage of every phase of the cell except G0 so author can reconfirm their result using these markers.

2) Author used Haelan as a promising agent for treatment of human pancreatic cancer. It would be interesting to know if concentration used in this study is comparable with human does?

3) Annexin-V do not tells about dynamics of apoptosis and a single endpoint measurements, while TUNEL assay is considered as a gold standard for measuring apoptosis over the years. Moreover, TUNEL assay also give information about phase of cell cycle where apoptosis is occurring. So checking quantification with TUNEL assay will make the work more impactful. Moreover, author did not show any data about what is happening at protein level? Upregulation of cleaved caspase-3 by western blot after treatment will give further important information.

4) Fig 2A- Why effect of 2% and 4% Haelan on proliferation of Capan-1 cells was opposite? i.e. why proliferation was increased rather than decreased at lower concentration?

5) Fig 4- Why Haelan induced apoptosis by Annexin-V was not observed on Capan-1 cells? It will be interesting for reader to know how Haelan modulate PD and BAPTA on Capan-1 cells which is not shown.

Level of interest: An article of importance in its field

Quality of written English: Acceptable
Statistical review: Yes, but I do not feel adequately qualified to assess the statistics.

Declaration of competing interests: 'I declare that I have no competing interests'