Reviewer's report

Title: Beta-sitosterol induces G1 arrest and causes depolarization of mitochondrial membrane potential in breast carcinoma MDA-MB-231 cells

Version: 1 Date: 2 July 2013

Reviewer: suresh kumar

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Minor essential revisions

I have reviewed the submitted research article entitled “Beta-sitosterol induces G1 arrest and causes depolarization of mitochondrial membrane potential in breast carcinoma MDA-MB-231 cells” submitted by Shanthi Sri Vundrua, Raosaheb K. Kalea,b, Rana P. Singh. The study primarily deals with B-Sitosterol, a phytosterol and its efficacy on induction of apoptosis and growth inhibition using three different cancer cell lines. The study was well planned and executed, and represented.

However it is felt to improve certain points and those are minor corrections to be considered for modifications. With the suggested modifications, this may be considered for publication.

Comments:

2.4 Annexin V Apoptosis Assay

“To quantify ST-induced apoptotic death of MDA-MD-231 cells, annexin V/PI staining was performed followed by flow cytometry, as described earlier”

Comment: It is MDA-MB not MDA-MD

Check on page no 10

To quantify the apoptosis, ST treatment for 48 hrs, 72 hrs at 90UM dose, whereas at 60Um dose for 72 hrs treatment shows similar amount of increase in apoptosis- Is it because the ST has been metabolized after 48 hrs?

Comments: Author may need to explain or mention in discussion

3.5 Sentence incomplete

To investigate this possibility cells were stained with FITC-Annexin V (green fluorescence) and the non-vital dye propidium iodide (red fluorescence) that allows (bivariate analysis), the discrimination of intact live cells (FITC-PI-), early apoptotic (FITC+PI-) and late apoptotic or necrotic cells (FITC+PI+) (Fig. 5A).

Comments: What is the observation? This sentence seems incomplete.
From Fig 3, p21 expression is increased at 90uM when compared with 60uM, however cyclin D1 doesn’t show any significant reduction at 60uM concentration of ST treatment. And with same treatment ie. ST treatment for 48 hrs,72hrs at 90uM and 72hrs of 60uM dose doesn’t increase apoptosis. Author may look in to critically and represent datas that are more convincing. Author may repeat the experiment or may add qRT-PCR result to support the results.

And in section 3.6 Mitochondrial Membrane potential –it shows 60uM dose shows significant effect on apoptosis.

Comments: For reviewer it seems there is discordance with molecular expression data and flow cytometry based apoptosis data, and mitochondrial membrane potential. Author may try to corroborate these events with expression data on p21, cyclin D and ERK expression with appropriate dose for concordant results.

Fig 5C:
Comments: Reviewer could not get convinced that Fig 5 C, the Bax Expression hasn’t shown significant increase.

Under section 3.5 Effect of #-Sitosterol on Apoptotic Cell Death in MDA-MB-231 Cells

Western blot analysis was done to assess Bax and Bcl-2 protein level expression.- This may be written in a such way a new reader may understand with little introduction.

Comments: This may be written as “To investigate the anti apoptotic and Pro-apoptotic gene expression, western blotting was performed against Bax and Bcl 2 proteins.

In discussion-first para
Thus, finding effective and nontoxic agents that inhibit cancer initiation, promotion and progression to locally invasive carcinoma and metastatic disease are desired.

Comments: this may be modified in better way.

English part to be corrected
3.5. The morphology of MDA-MB-231 cells ascompared to A431 and A549 cells after 48 h of ST treatment suggests that they may be going under apoptosis”.

Comments: This may be written as “treatment suggests that cells may undergo apoptosis or apoptosis process” or this sentence may be reframed in better way.

IN Page no: 14 under discussion : Usage of ‘AND’ and avoid long sentence.
The Bcl-2 family includes both anti-apoptotic (e.g., Bcl-2 itself) and pro-apoptotic (e.g., Bax) proteins, and the levels, interaction, ratio, and translocation of different members in this family determine the overall pro-apoptotic or anti-apoptotic fate of the cell following an agent’s treatment [35].

Bax protein is a pro-apoptotic member of this family and increased expression of this protein often associated with the increased apoptosis, and increased expression of Bcl-2 has been associated with the inhibition of apoptosis and cell survival mechanisms —

Comments: “And’ may be properly used- Multiple usage of “And”, long sentence may be avoided, sentence may be reframed.

Level of interest: An article whose findings are important to those with closely related research interests

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests:

i declare that i have no competing interests.