Reviewer's report

Title: Modulation of Heat Shock Proteins and Apoptosis by Flueggea leucopyrus (Willd) decoction: possible mechanisms mediating cytotoxicity to three breast cancer phenotypes.

Version: 2
Date: 27 January 2015
Reviewer: R Narayanan

Reviewer's report:

Comments:
The manuscript from Anuka et al., aims at deciphering the molecular mechanisms underlying flueggea leucopyrus induced apoptosis in breast cancer cells and the possible role of HSP in mediating the anticancer effect. Overall the study is not well organized and needs more supportive experiments to prove the hypothesis. Also, several controls are missing; some of them are listed below. Though the role of F. leucopyrus and its anticancer activity is well established in other cancer cells, the author had tried to explore the possible mechanisms. Hence by considering the importance to develop the new drug targets for breast cancer, I would like to appreciate the attention however, these issues needs to be addressed strictly.

Major compulsory revisions:
1) It would be good if the author had shown the bioactive constituents in the decoction.
2) There is no clear explanation for choosing 10 & 20 µg concentrations for HSP70 & 90 expression studies. How do you arrive at this concentration?
3) MCF-10A cells should also be included for Apoptosis and HSP expression studies.
4) To confirm apoptosis, Caspase-3/7 should be performed.
5) For DNA fragmentation analysis, there is no explanation for exposing the cells for 48 h while in other experiments, it is 24 h. In addition, no clear explanations for using high concentrations (400 and 600 µg) of decoction. Also, there was no clear fragmentation observed. Support this with another set of data.
6) Author should come up with a confirmatory experiment to show the relationship between HSP expression and Apoptosis. Results of SKBR cells contradict this hypothesis between HSP expression and Apoptosis. Also, there are no clear explanations for this. Authors need to include MCF-10A cells in their confirmatory experiments.
7) Author should show the statistical values in the results.
8) In places, the English also requires strong editing and thorough proof reading.

Discretionary revisions:
1) At line No. 166, is it crossing cycle number or threshold cycle number – clarify it.
2) Line 285-286 states “same intensities of nuclear staining were maintained”, explain how this was measured?

Minor essential revisions:
1) Authors need to state why there is a lack of uniformity in the number of experiments performed.
2) Paclitaxel should also be included for Apoptosis and HSP expression studies.
3) Line 316 is not complete.
4) Support AO/EB with some form of quantification.
5) Recent reference relating HSP and breast cancer may be included.