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: 13 November 2014
Reviewer: Andy Göbel

Reviewer's report:

Major Compulsory Revisions

1. Fig. 1.1 shows the cell survival of different breast cancer cell lines after exposure to different concentrations of F. leucopyrus decoction. It is confusing, especially for the MDA-MB-231 cells, that there is a dose-depending decrease of cell survival with up to 100 µg/ml, but with higher concentrations, cell survival increases again. This observation is not discussed or explained by the authors. Here, it’s the question, if it’s really necessary to include such high (probably unphysiologic) concentrations since there are already strong effects with low concentrations. Furthermore, the figure does not contain any standard deviations or information about significant changes of cell survival by the treatments.

2. It should be mentioned in general, how many independent experiments were performed to achieve the results in Fig. 1.1, 2.1 and 3.1 and the standard deviations. Performing one experiment in triplicates is not the same as performing at least three individual and independent experiments what should be the basis for the statistical analysis. Just one experiment in triplicates is not acceptable for a proven significance.

3. In Fig. 1.2 it is obvious that already low concentrations of Paclitaxel reduce the tumor cell survival. The possible advantage of using F. leucopyrus decoction rather than established anti-cancer agents should be discussed since quite higher concentrations have to be used to achieve comparable results.

4. It’s not mentioned by the authors, why Fig. 1 contains Paclitaxel as a positive control, but none of all the other experiments. The treatment with Paclitaxel should at least be included in the assessment of DNA-fragmentation or Acridine orange/Ethidium bromide staining to compare the effectiveness of the decoction treatment.

5. Fig. 5 doesn’t contain any legend which should be included since it is not explained which lane contains which treatment. Additionally, also this figure should contain at least one positive control (e.g. Paclitaxel). Furthermore the concentrations that were used for this experiment should be comparable to those that were used for Acridine orange/Ethidium bromide and HSP70/HSP90 staining. A DNA fragmentation with 400 µg/ml of decoction and more could also be a non-specific effect by overloading the cells with the isolated plant proteins. This is of importance to possibly link decreased HSP70/90 expression and
induced loss of membrane integrity with induction of apoptosis by 20 µg/ml or 40 µg/ml decoction.

6. The author’s discussion may include, that the used concentrations of the decoction are possibly not achievably under physiological conditions and that the results warrant further investigations by using appropriate animal models.

Minor Essential Revisions

1. The passage “Evaluation of cytotoxicity…” in “Methods” contains a mistake in the concentration unit (“µg/µl” should be changed to “µg/ml)

2. The manuscript could hugely benefit from assessing the activation of caspases 3/7 (for example by Western Blot analysis of cleaved caspases or cleaved poly-ADP-ribose-polymerase) to further underline the anti-tumor effects elicited by F. leucopyrus decoction

3. The immunofluorescence analysis could benefit from adding a staining with an isotype control antibody and could be supported by additional Western Blot analysis of HSP70 and HSP90 proteins isolated from treated breast cancer cells.

Discretionary Revisions

1. The manuscript could benefit from mentioning the sequence of all the primers that were used to assess gene expressions.

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.