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.

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Reviewer: Andy Göbel

Reviewer’s report:

Second report on the manuscript “Modulation of Heat Shock Proteins and Apoptosis by Flueggea leucopyrus (Willd) decoction: possible mechanisms mediating cytotoxicity to three breast cancer phenotypes”

Major Compulsory Revisions (in comparison to previous suggestions)

1. “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.”

The author didn’t respond to this sufficiently. Again, it is not clear, why experiments with paclitaxel as a positive control were performed in Fig. 1 but not in all the other figures. The rationale for using paclitaxel in this study should be mentioned and explained. As the author want to link a reduced HSP70/90 expression with apoptosis by the decoction, one would expect, that the authors used paclitaxel since it has been shown already, that this substance also reduces HSP70/90. Otherwise it doesn’t make sense to include paclitaxel at all. In addition, to support the hypothesis that a reduced HSP70/90 expression is linked with apoptosis, the author should have included a more suitable positive control that is already known for its inhibitory effect on HSPs thereby exerting anti-tumor effects in cancer cells. If not further explained, using paclitaxel as a positive control in a study about linking HSP70/90 and apoptosis in cancer cells still lacks a reasonable rationale.

2. “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.”
The author also didn´t respond to this point sufficiently. As they were able to show a loss of cell survival and an activation of caspases as well as a positive AO/EB staining 24 h after the treatment with concentrations up to 100 µg/ml, it is still confusing that they exceeded these concentrations and the incubation time for DNA fragmentation assay. Apoptosis always results in the fragmentation of DNA. Hence, the used concentrations for the caspase activation assay should be high enough to achieve an obvious effect. Moreover, the authors show gene expression of HSP70 and HSP90 upon the treatment with 10 µg/ml and 20 µg/ml, but not for the concentrations that were used for the DNA fragmentation assay. It is not predictable if the HSP70/90 expression follows the same pattern 48h after treatment with these high concentrations as shown for lower concentrations in a shorter incubation time. Nonetheless an incubation time of 48h would be acceptable upon using the same conditions as in the other experiments.

3. In line with the suggestions of Reviewer 1 and evaluating the results of this study at a glance, the author should be more careful with their conclusion, that a reduced expression of HSP70/90 by the decoction induces apoptosis in the breast cancer cell lines. It should be further discussed, that it may not only be one bioactive compound of the decoction but rather a synergistic effect of different compounds that are exerting these effects. One possible scenario could be that one component reduces HSP70/90 activity in the cancer cells thereby facilitating an increased pro-apoptotic effect of any other – so far unknown – component. Nonetheless, the discussion of the contradictory results in SKBR-3 cells is acceptable.

4. Finally, comparable to paclitaxel, it is not clear, why MCF-10A cells as a non-cancerous breast cell line was just included in figure 1 but in none of the other experiments.

Minor Essential Revisions

1. Line 233 contains a mistake: “…has significant cytotoxic 24h incubation= 28.6 µg/ml)

2. No reference mentioned in line 269: “In breast cancer cells, overexpression of HSP90 and HSP70 are reported to correlate with poor prognosis.”

3. If not indicated so far, please mention that the isolation and identification of the bioactive compound(s) of the decoction is necessary

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.