Reviewer's report

Title: Inhibitory Effect of Curcuma purpurascens Bl. Rhizome on HT-29 Colon Cancer Cells through Mitochondrial-Dependent Apoptosis Pathway

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Reviewer: Shih-Shun Chen

Reviewer's report:

Major Revision

Comments for 1988872007142947

The manuscript by Rouhollahi et al. provided evidence that dichloromethane extract of Curcuma purpurascens Bl. rhizome (DECPR) could induce apoptosis of human colon adenocarcinoma HT-29 cell line. Their findings suggest an important role for the mitochondria-regulated death pathway in the apoptosis induction by DECPR. Despite interesting, the data look preliminary and not always in support of the authors conclusions.

Major points

1. Figure 2: The experiment was not properly designed. First, why did the authors use of 10% Tween 20 as a control in the examination of the cytotoxic effect of DECPR on rat normal tissues? As authors describe in the manuscript that DECPR from an air-dried and powered rhizomes was extracted using n-hexane followed by dichloromethane, and the extracts were dissolved in DMSO for further experiments. Therefore, to evaluate the cytotoxicity of DECPR-treated rats, DMSO should be used as a vehicle. Secondary, it is not clear why did the authors decide to perform acute toxicity assay instead of anti-tumor study in rats? The in vitro data showed that DECPR could induce apoptosis while there are no apoptosis and tumor growth inhibition related results from the in vivo experiments. Third, as the authors mentioned, the DECPR has less toxic effect on rat liver and kidney. Is there any immunological effects observed in the author used rat model?

2. The authors failed to show the effect of solvent controls hexane and dichloromethane to human cancer and normal cells in vitro experiments.

3. Is DECPR specific in inducting apoptosis in human colon adenocarcinoma HT-29 cell line? Please demonstrate with other cancer cell lines.

4. The rationale for selection of human colon adenocarcinoma HT-29 cell line for mechanism studies is missing.

5. It is not very convincing that the in vitro experiments were performed at different time points: ex. 48 h for MTT and LDH release assays, 24 h for cytoskeletal arrangement, ROS generation, caspase activities, and quantity of Bax, Bcl-2 and Bcl-xl mRNA levels.

6. What is the author interpretation for cytoskeletal arrangement assay? Are
there other potential targets for DECPR?

7. Figure 4A: Nuclear condensation or morphological change does not reflect the DNA fragmentation. In situ Tunel or DNA fragmentation assay is better.

8. The figure 6A images did not clearly show changes in the distribution of cytochrome c and the mitochondrial membrane potential. How did they normalize the data? It is hard to say that DECPR causes the release of mitochondrial cytochrome c into the cytosol and the reduction in mitochondrial membrane potential. Levels of ROS and Ca++ in the cytosol, which were determined by flow cytometry. Furthermore, the effect of caspase activation on DECPR-induced the expression of mitochondria death related proteins should be addressed.

9. Figure 7: To prove the role of caspase in DECPR induced apoptosis, caspase-3, -7, and -9 inhibitors should be used.

10. Figure 8: the authors should perform Western blotting for detecting the levels of Bcl-2, Bcl-XL, and Bax proteins at the same time point in the experiments.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.