Reviewer's report

Title: Modulation of apoptosis in human hepatocellular carcinoma (HepG2 cells) by a standardized herbal decoction with anti-hepatocarcinogenic effects.

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Reviewer: AR Khuda-Bukhsh

Reviewer's report:

The paper deals with the evaluation of apoptotic potentials of a standard herbal decoction in Hep G2 cell line. Although the paper is of some interest, there are too many concerns to be taken care of by the authors in order to meet the standard of a BMC journal. I am pointing out a few glaring ones, that the authors should take care of before the paper can be reconsidered for publication.

Major points

1. HepG2 should normally be grown without the presence of CO2, but the authors have grown the cells in the 5% CO2.

2. In the sets of experiments, the author haven’t shown any cytotoxicity assay to check the viability of the cells in the presence of the drug, the assays like MTT assay, trypan blue assay or LDH activity assay should have been done to make the findings more authentic and scientifically sound.

3. The LD50 value of the drug hasn’t been determined or mentioned in the article, without which the actual drug activity couldn’t be understood properly.

4. The activity of the drug on the normal cells hasn’t also been shown in the sets of experiments, so the author should include that part in this article. At least, author should include the cytotoxic assay on the PBMC (peripheral blood mononuclear cells), as one arm of the control.

5. The authors have shown the gene and protein level expressions of Bcl-2 and BAX, but the ratio of BAX/Bcl-2 should be included in a tabular form. Then the actual difference of the ratio of them would be understandable to the readers. (The altered ratio of BAX/Bcl-2 is responsible for the change in the membrane potential of mitochondria which in turn releases the cytochrome c, responsible for the downstream activity of the caspases).

6. In figure 3 [A], the resolution and the magnification of the cells are not clear, so, if the author could change those 4 sets (Control, 1200 µg/ml, 1500 µg/ml, 1800 µg/ml) of figures, then the morphological changes may be more clear.

7. For RT-PCR and Western blot analyses, the authors have added the results of 12, 24 and 48 hrs, but for the other experiments like detection of morphological changes related to apoptosis and DNA fragmentation analysis, they have shown the concentration-dependent figures only, not the time-dependent ones, which are more important. The author should maintain a uniform time point for performing the experiments and for presentation of results.
8. The author should add the FACS analysis with Annexin-V (FITC conjugated) to find out the actual percentage of apoptosis (both late and early apoptosis) to make the result more understandable, complete and convincing.

9. In Discussion part, the authors have mentioned many cross-talks of cell signaling events. But if the authors want to cover-up that part, more signal proteins will have to be studied for accurate linking up of events of cell-signaling, otherwise the present study of only three signal proteins and commenting too much makes the study more speculative than scientifically acceptable and convincing.

10. In fluorescent microscopic study (0-1800 µg/ml) concentration range was chosen, whereas in RT-PCR, western blotting, caspase assay (0-1200 µg/ml) a different concentration range was used. Make a proper dose selection to all experiments.

11. Chromatographic separation study could also have been included to spell out the active principles of each component or the actual ingredients present in the decoction could have been mentioned from existing literature to make the paper more informative.

12. Did you check the extrinsic pathway of the caspase activities as well? If yes, provide evidence.

13. The references should again be checked for accuracy of citation and style.

Minor points:
1. In page 3, “Apoptosis can occur….or a mitochondrial (intrinsic a) pathway. The “intrinsic a” should be corrected.

2. Write the full form of abbreviations at the first mention, e.g. MTT assay, TAE buffer, etc.

3. Primer sequences can be presented in a table.

4. SHIMATZU, Japan) should be SHIMADZU

5. Glyceraldehydes-3-phosphate …should be …glyceraldehydes-3-phosphate

6. Fluorescent microscopy…should be””fluorescence microscopy..

7. Fig. 1 is showing only the graphical representation of m-RNA level expression, the PCR bands should better be incorporated in the figure as well.

8. Fig. 2a. Photograph of histochemical staining not very clear, better photograph should be more convincing.

9. Fig. 3a, the photograph of morphological features of the cells presented some impression that the control cells were not properly maintained. As they had their cell outlines not in the proper shape.

10. In Fig. 3c, no fragmented DNA is visible in the photograph.

11. In Fig. 3d, it is difficult to conclude whether the cells are undergoing apoptosis or necrosis.

12. The statistical part be consulted with a statistician whether both forms of the
post-hoc tests are necessary.

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

Quality of written English: Acceptable

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

Declaration of competing interests:

No, I have no financial conflict of interest to declare.