Reviewer’s report

Title: Evaluation of anticancer properties of a decoction containing Adenanthera pavonina L. and Thespesia populnea L.

Version: 0 Date: 29 Aug 2015

Reviewer: Andrea Cabarkapa

Reviewer’s report:

The authors presented a study about decoction composed of Adenanthera pavonina L. and Thespesia populnea L. that is currently being used in the treatment of cancer patients in Sri Lanka. Having in mind that cancer is a major health issue nowadays in entire world, the study about possible cytotoxic potential of decoction composed of Adenanthera pavonina L. and Thespesia populnea L that was addressed in this study is an interesting topic with the benefit to a broader scientific community.

However, paper requires some major revision before it can be acceptable for publication.

1. In the abstract the authors state that apoptosis was detected in the HEp-2 cancer cells but less toxicity was shown against a normal cellular system. In the Results there are no data on the effects of decoction on a normal cells, so this statement should be removed from the abstract.

2. The Introduction section is too broad with many irrelevant and too generalized information. I suggest removing the lines 51-63, 19-21, 29-34, 39-41 since they do not provide any relevant information, and rewrite the entire Introduction to be more concise, as in the work of Indeewari Kalhari Silva and Preethi Soysa. Evaluation of phytochemical composition and antioxidant capacity of a decoction containing Adenanthera pavonina L. and Thespesia populnea L. Pharmacogn Mag. 2011 Jul-Sep; 7(27): 193-199. Line 48-53 does not belong to Introduction section, transfer the entire sentence to line 9 in the section Plant material and preparation.

3. The plant extracts should be from authenticated sources or extract should be defined so that future extracts made can be compared to make sure that the composition is the same. Some reference on the material used for the preparation in this study should be given, if such material was used in some previous study and the preparation procedure in more details (again please see the example of Indeewari Kalhari Silva and Preethi Soysa. Evaluation of phytochemical composition and antioxidant capacity of a decoction containing Adenanthera pavonina L. and Thespesia populnea L. Pharmacogn Mag. 2011 Jul-Sep; 7(27): 193-199.) . Clean cheese cloth is not acceptable description, do you mean cotton cloth? Please provide the name of the producer in bracket. If it is an uninvestigated plant material used in the study, HPLC or similar analysis of components is necessary. HPLC profiles best define extracts chromatographically to complement research on previously unknown material.
4. Activities such as cytotoxicity that might reduce carcinogenesis, could be tested by several ways. The authors displayed results of MTT, SRB and LDH assay and Ethidium Bromide/Acridence Orange Staining. A variety of experiments can be used and the most basic is to compare the rate of proliferation of a cancer cell line in the presence and absence of the test substance, usually after a specified time. Please provide explanation why did you use incubation time for 24h and not the 72h as it is mostly done in such tests.

5. The authors showed that the cells treated with the decoction have dose dependent increase of LDH release, and dose-dependent growth inhibition in MTT and SRB assay. Different EC50 values were obtained in each test. The similar results was seen in the study by Slamenova et al 2004, (Cytotoxic and DNA-Damaging Effects of Diterpenoid Quinones from the Roots of Salvia officinalis L. on Colonic and Hepatic Human Cells Cultured in vitro. Basic & Clinical Pharmacology & Toxicology Volume 94, Issue 6) where the diterpenoid quinones (found also in T. populanea) decreased the viability of the cells proportionally to the concentration and to the time of treatment and cytotoxicity did not correlate in all cases with the increased level of apoptotic nuclei. In current study on the decoction of A. pavonina and T. populanea, the morphological changes characteristic to apoptosis were shown to some extent in treated cells with Ethidium Bromide/Acridence Orange Staining. However, the apoptotic index (AI) should be calculated to confirm that decoction-treated cell death was via apoptosis. AI is described as the percentage of apoptotic cells and apoptotic bodies within the overall population of cells and the statistical differences should be given between the control group and treated group. The percentage of apoptotic cells in the overall population (apoptotic index) using the AO/EB staining procedure, would then indicate that the morphological features of a hepatoma cell line in apoptosis were dose dependent, i.e., a stronger apoptosis signal was induced with higher concentrations of the respective extract. Provide these results if possible, or comment this in Discussion. Also please provide explanation why the two mentioned concentrations were selected for the apoptosis analyses.

6. Another issue is that the tests described above should also be used to determine whether the cytotoxic effect is merely cytostatic i.e. it stops cells growing or dividing, or cytocidal, where the cells are killed. A similar result may be given as if the cells were not alive or not proliferating. At high concentrations no cells remain at this time i.e. all cells have been killed, but if some viable cells remain, it is not possible to know if they are capable of revival and proliferation once the toxic substance is removed. To determine if the effect of decoction was really cytotoxic and not cytostatic, the treatment of cells with decoction should be followed by 24 hr post-incubation of cells in complete medium, to see if the cells would recover and proliferate in the absence of the test substance. Again, please see example of Slamenova et al 2004. I suggest the re-investigation of extract which did not display much direct cytotoxic activity, with some additional test such as BrdU assay to make sure that the cell proliferation indeed inhibited by mentioned decoction.
7. In the Results section, put indication of the Figure that presents the certain result for SBR and LDH assay (line 7 and line 19 page 10). There are no indications in the text in which Figure the results are depicted.

8. It is not clear whether the each concentration was tested in triplicate or entire experiment was done 3 times, please clarify...For MTT in the Results section it is stated triplicate was done, but in figure1 legend you stated four repetitions, which one is correct?

9. The results of LDH assay should be given as bars, so the control values could also be included in the chart and the result would be more easily interpreted.

10. All figures should use uniform way of labeling. X axis should be named Decoction concentration (unit in the brackets) on all charts, and Y axis should be the same in figure 1 and 3. On figure 1 it is written percentage, and on No3 you put % before cell viability.

11. Spelling and grammar are correct, pay attention to some missing dots in the text (examples line 26 page 4, line 46 page 6, line 21 page 8) and capital letters (line 53, page 6).

12. In Conclusion section line 36, avoid saying MARKED for the apoptotic activity, since only the morphology and not the amount of apoptotic cells in the treated samples was given in the results, otherwise calculate the apoptotic index to confirm this statement.

Are the methods appropriate and well described?
If not, please specify what is required in your comments to the authors.

Yes

Does the work include the necessary controls?
If not, please specify which controls are required in your comments to the authors.

Yes

Are the conclusions drawn adequately supported by the data shown?
If not, please explain in your comments to the authors.

No

Are you able to assess any statistics in the manuscript or would you recommend an additional statistical review?
If an additional statistical review is recommended, please specify what aspects require further assessment in your comments to the editors.

I recommend additional statistical review

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