Author’s response to reviews

Title: Centella asiatica Juice effects on DNA damage, Apoptosis and Gene Expression in Hepatocellular carcinoma (HCC)

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Version: 6 Date: 9 September 2013

Author’s response to reviews:

Dear Editor,

I am so thankful for giving valued comments and suggestions, which improve our paper.

Comments
Referee 1
Reviewer’s report

Abstract

1. Please write the full name of words like RT-PCR and MTT: Checked and Done
2. Please write full name of words like HCC for the first time: Done
3. page 4: full name of DMSO: Done
4. page 5: full name of TAE: Done
5. page 6: Leica Epifluorescence microscope (Company name?):
   Leica Epifluorescence microscope equipped with an excitation filter of 515–560 nm (Bellows Falls, VT, USA)
6. Please delete that was in sentence: One Step RT-PCR that was based on RT-PCR Premix:
   Done
7. How the result is showing a significant difference. Please include statistical analysis and p value: Checked and Done

8. Page 8:
   The juice had a significant chemoprevention activity (Please mention p value)
The cytotoxicity started at a concentration dose of juice as low as 0.1% when HepG2 cells were incubated up to 72 hr (Fig 2), which showed a chemopreventive activity against the cells.

9. The juice significantly increased DNA damage (Please show significant result)
The findings indicated that the juice significantly increased DNA damage in treated HepG2 cells at the concentrations of 0.1 and 1 % compared to respective controls (p<0.05) (Fig 4),

10. Please rewrite the sentence of : The Centella asiatica compounds can cause apoptosis [20, 21], protect against neurotoxicity as well as inhibit promotion [22] and invasion [23] of tumors [24, 25].
Centella asiatica compounds can cause apoptosis [20, 21], which protect the cells against neurotoxicity. Additionally, this event inhibits promotion [22] and invasion [23] of cells in different types of tumors [24, 25].

Reviewer’s report 2

Chemicals
1. What is the application of Trypsin in chemicals? It is used for detaching of cells
2. In cell culture part, please clarify the normal cell culture like how many cells were seeded in each flask and what was the frequency of changing the medium. Done (Every three days)
Then explain the cell treatment with juice.
3. Please mention the dose of penicillin/streptomycin: Done
4. The sterilization process of plant extraction should be mentioned in this part
Herbal extract sterilization will be done by using both high temperature and pressure.
5. In treatment part, it has been written that the suitable number of cells were treated, please mention the exact number of cells: Done
6. The MTT assay
In the process of reading the toxicity percentage, the wavelength should be mentioned: Done
7. In this part, you treat cell with juice in the 96 well plate, so what is the reason of treatment in the flasks which has been mentioned in the first part of method: Giving enough time to the cells for being exposed to the juice and then determining the cytotoxic effect
8. Flow Cytometry
Is propidium iodide (PI) the detector of flow cytometer? Please clarify it in more detail:
Yes, it is a light sensitive
9. If we consider PI as the dye, the cells should be permabilize for dye entrance. If yes, please clarify the penetration method. Triton X-100 is used to permeabilize

10. Comet assay
Mention the name of manufacturer and model of fluorescence microscope: Done

11. The number of cells which have been mentioned by the end of this part should be shifted to the beginning: Checked

12. Results:
Why gene concentration did not measure for higher concentration of juice like 10%: Because of decreasing rate of cytotoxicity.

13. Discussion
This part did not discuss figures properly. Meaning, it should explain why cytotoxicity percentage did not follow specific rule for example, concentration 0.01 is higher than 0.001 and 0.1 while it should be less than 0.0 and so on: It is following dose-concentration manner

14. Figures
In the manuscript has been mentioned the time is detective factor in cytotoxicity. Does it mean, cytotoxicity decreases during the time? Because cytotoxicity at the 24hr is higher than cytotoxicity at 48hr and so on. Toxicity effect is within a specific time duration

15. Could you justify the changes in G1?
SubG1 is contributing to show apoptosis process