Author's response to reviews

Title: PASS-predicted Vitex negundo activity: antioxidant and antiproliferative properties on human hepatoma cells-an in vitro study

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Version: 2 Date: 7 November 2013

Author's response to reviews: see over
Dear Editorial Board,

First, we would like to admire reviewers and editorial efforts for the comments. We highly appreciated their time and attention to evaluate our work. Below, are the comments of our respective responses.

Best Regards,

Prof. Dr. Normadiah M. Kassim

**Report 1:**

1. In **Abstract Background**, efficiently used in this study to reveal new multitalented actions for the isolated components of VN extract. (Please use full form when writing for first time).

   The word *Vitex negundo* (VN) is added as shown in page 2.

2. In **Abstract Results**: PASS study of the isolated and identified compounds of VN leave (leaves or leaf) showed that the most

   The word leave is replaced by leaves in the whole MS.

3. There are some minor grammatical mistakes.

   The MS was sent to Professional editing office service in University of Malaya, as shown in the revised MS

4. Follow the journals format for writing tables and figures.

   The revised MS was rewritten according to the journal style.

5. Site more recent references and adhere to the journals style for references

   Done, as shown in the revised MS.
1. **Abstract (background):** the last sentence should be rephrased. *for the isolated components of VN extract-* **for VN constituents**

   The sentence was rephrased to: *Vitex negundo* (VN) constituents – page 2.

2. **Abstract (Results):** BHT stands for what? **The word must appear before the abbreviation in the abstract**

   *butylated hydroxyl toluene* (BHT) was added in the revised MS- Page 2.

3. **Page 3:** Furthermore, the activation of caspase-3 enzyme suggesting that the observed cytotoxicity was mediated via intrinsic apoptosis pathway. 

   **In addition,** the activation of caspase-3 enzyme suggesting that the observed cytotoxicity was mediated via an intrinsic apoptosis pathway.

   The words were added as shown in page 3.

4. **Background:**

   **Page 5:** [9]. The ethanol extract of VN seeds showed analgesic. **VN seeds extract showed analgesic**

   VN seeds extract showed analgesic……… (As shown in page 5).

5. **Page 6:** normal embryonic liver cells (WRL68) and HepG2 cell lines based. **WRL68 and HepG2cell**………………

   The sentence was changed. As shown in page 6.

6. **Methodology:** Page 6: **Reference 18. This reference is not relevant to PASS study. Either you remove it or add a new reference.**

   Reference 18 was removed. As shown in page 6.
7. Page 7: the author measured radical scavenging activity and FRAP assays. It is necessary to measure the total phenolic content (TPC) and/or the total flavonoid content (TFC) to determine the antioxidant activity of this extract. It would be more informative if the author would add TPC and TFC of VN.

The TPC and TFC of VN extract are already tested and determined in our previous published data [1]. However, we have mentioned that in the discussion part of the MS as shown in page 14.

8. Page 8: *In vitro* VN antioxidant in WRL68 cell lines DMEM. Please add this sentence before the abbreviation: Dulbecco’s Modified Eagle Medium (DMEM).

The sentence was changed. As shown in page 8.

9. In page 9: **Cell culture** Cells were cultured in the Dulbecco’s Modified Eagle Medium (DMEM), Cells were cultured in the (DMEM),

The sentence was changed. As shown in page 9.

10. Could the author explain the rationale of using WRL68 and HepG2 cells?

For cell culture experiments, two types of cells were used, the normal embryonic liver cells (WRL 68) and hepatocarcinoma cells (HepG2). HepG2-cells are perpetual adherent, epithelial in morphology and routinely used for toxicology studies [2].

11. Page 9: 72 hrs., For 4 hours. **Please standardize the word hours in all MS.**

The word hours was used in the whole MS.

12. Could the author explain the rationale of using H_2O_2?

Many evidences showed that H_2O_2 can activate signalling pathways to stimulate reactive oxidative stress production in the cells. H_2O_2 activates NADPH oxidase, resulting in the production of O2.-, and, consequently, oxidant injury [3].
13. **Results and Discussion**: Page 12: In order to accelerate the search for potent natural product. In order to accelerate the research for potent natural product

   The word search was replaced by research. As shown in page 12

14. Table 1 to Table 5. Please change the type of table in the whole MS (using the borders to separate the cells)

   All the tables are changed according to the style of the Journal. As shown at the end of the revised MS.

15. Table 2: It is preferable to use numbers to indicate the isolated compounds rather than put the compounds names in the table.

   The table was modified per request. As shown in page 29 in the revised MS.

16. Page 14: BDE plays a central role in determining antioxidant efficacy, and is one of the most important physical parameters used for evaluating antioxidant activity in chemical compounds that are used as inhibitors of free radical reactions. (Add reference).

   Reference number [28] was added. As shown in page 14.

17. Page 14: Hence, VN has a wide variety of hydroxyl groups and therefore, exerts a wide variety of BDEs, forming synergistic system (antioxidant and co-antioxidant).

   The sentence was modified per request. As shown in page 15.

   Hence, VN has a wide variety of hydroxyl groups and therefore, exerts a wide variety of BDEs, forming a synergistic system between antioxidant and co-antioxidant.

18. Page 17: The estimated IC50 values of VN extract (concentration causing death of 50% of HepG2 cells) were 66.46 µg/ml, 57.36 µg/ml and 65.12 µg/ml.

   The sentence was modified as per request. As shown in page 17.
19. **Conclusions:** It would save unnecessary waste of chemicals as well as time.

The sentence was replaced by: It would save unnecessary wastage of chemicals and time by avoiding random plant selection methods. As shown in page 22.

20. **Page 21:** Thus, this study strongly suggests that the ethanolic extract of VN which involved broad spectrum of antioxidant

The sentence was replaced by: This study strongly suggests that VN extract significantly enhanced antioxidant activity and proposed a tumour preventive action against HepG2 cell lines at a dose and time-dependent manner but with lower toxicity toward WRL68 cells. As shown in page 22.

**References**

1. Kadir FA, Kassim NM, Abdulla MA, Yehye WA: **Hepatoprotective Role of Ethanolic Extract of Vitex negundo in Thioacetamide-Induced Liver Fibrosis in Male Rats. Evidence-Based Complementary and Alternative Medicine 2013, 2013.**
2. Wilkening S, Stahl F, Bader A: **Comparison of primary human hepatocytes and hepatoma cell line Hepg2 with regard to their biotransformation properties. Drug Metab Dispos 2003, 31(8):1035-1042.**

**Editorial comments:**

1. After reading through your manuscript, we feel that the quality of written English needs to be improved before the manuscript can be considered further.

   As per request, the MS was sent to Professional editing office service in University of Malaya as shown in the revised MS and it was rewritten again according to the journal style.

2. A simple amendment was done in Figure 8 regarding the axes. Kindly refer to the uploaded file.

Thank you in advance your cooperation,

Corresponding author,

Prof. Dr. Normadiah M. Kassim