Author's response to reviews

Title: Protective activity of Panduratin A against Thioacetamide-induced oxidative damage: demonstration with in vitro experiments using WRL-68 liver cell line

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Author's response to reviews: see over
08/10/2013

Dr. Victor Kuete
Mr. James Prozenko
BMC Complementary and Alternative Medicine Editorial Team


Dear Dr. Kuete:

We thank you for the email dated 11.09.2013 concerning the review of the above manuscript (MS). We are submitting a revised, annotated and clean copy of the MS. We thank the expert reviewers for reading the original manuscript thoroughly and providing their constructive critiques, which were beneficial and significantly improved the MS. We diligently worked to incorporate each of the suggestions by the reviewers.

In the revision, we addressed all of the reviewer’s concerns and carried out the required modifications. We responded each criticism point by point below and indicated the changes made in the MS by highlighting the relevant text with yellow color wherever possible.

Thank you for your considerations on this MS.

Sincerely Yours,

Mehmet Bilgen
Reviewer 1:

We thank Prof. Prasenjit Manna for carefully reading the manuscript (MS) and providing constructive critiques that helped improving the MS significantly. Below we provided our point-by-point responses to the critiques and highlighted the changes made in the revised MS by yellow color.

Major Comments:

“The authors should describe how they calculated the DPPH radical scavenging and ferric reducing power of the tested compounds. There is no information about the blank. The authors need to include appropriate solvent blank in the study.”

Thank you. As suggested, we added the calculation and required information in subsections 2.2 and 2.3.

“In the “Assessment of MDA, CAT, SOD, and GPx” section there is no information about the incubation time with TAA. In addition, the information about the protease and phosphatase inhibitors in the cell lysing buffer is also missing.”

Incubation time was added. Information about the protease and phosphatase inhibitors was provided. Please see the corresponding subsubsection.

“In the “Statistical Analysis” section, the authors did not describe the “n” value of each experiment. The authors should include this. The authors should also mention this in all the figure legends.”

The n value is now described and included in the figure legends.

“There is one discrepancy in the FRAP unit between the text (nmol FeII/mg) and the Figure 3 (nmol FeII/µg). The authors should correct this.”

Corrected according to the applied methodology in the study.

“Figure 2 legends; there is no information about the significant symbol as well as statistics.”

This information is now added.
“In Figure 3, there is no information about the concentration of the compounds, only unit was given (no scale) in the X-axis.”

The X-axis represents the types of the compounds tested and the concentration is now clarified on the axis according to the applied procedure in the study.

“Figure 4, there is some formatting error in placing significant symbol.”

This error is now fixed.

“Oxidative stress plays an important role in the pathogenesis of TAA induced hepatotoxicity. In the “Discussion” section, the authors mention that TAA triggered ROS production but there is no information about it. The authors may include the effect of TAA and PA on the intracellular ROS production.”

Thank you. This issue is now clarified. Please see the Discussion section.
Reviewer 2:

We thank Prof. Agata Stanek for carefully reading the manuscript (MS) and providing constructive critiques that helped improving the MS significantly. Below we provided our point-by-point responses to his/her critiques and highlighted the changes made in the revised MS by yellow color.

Minor Essential Revisions:

Abstract:

“MDA concentrations (levels), SOD, CAT and GPx activities
The activities of SOD, CAT and GPx were significantly elevated....”

Done as suggested.

Materials and Methods

“Materials and methods-Assessment of MDA, CAT, SOD and GPx should be activities of antioxidants enzymes (CAT, SOD and GPx).”

Done as suggested.

Results

“MDA and the antioxidant enzymes CAT, SOD and GPx assays.”

Done as suggested.

“Authors should correct the unit of MDA [nmol/mg protein]”

Corrected according to the applied procedure in the study.

“Instead of “the increased level of antioxidant enzymes” it will be better “increased the activities of antioxidant enzymes”

Done as suggested.
Reviewer 3:

We thank Prof. Kawther El-Gendy for carefully reading the manuscript (MS) and providing constructive critiques that helped improving the MS significantly. Below we provided our point-by-point responses to her critiques and highlighted the changes made in the revised MS by yellow color.

Minor comments:

1- Key word:
   • WRL-68 live cell line must be changed to WRL-68 liver cell line
     Changed.

2. Materials and Methods

2.1. Isolation of the compound Panduratin A from Boesenbergia rotunda extract
   • In the tenth line of this paragraph remove word "solvent".
     Removed as suggested.

2.2. DPPH scavenging activity of Panduratin A
   • In the third line "Dimethyl sulfoxide" should be changed to "dimethyl sulfoxide"
     Changed as suggested

   • In the fourth line omit "solvent"
     Removed as suggested.

2.4. In vitro Experiments

"Effects of Panduratin A in TAA-cytotoxicity" should be "Effect of Panduratin A in TAA-cytotoxicity"

    Done as suggested.

2.5. Statistical Analysis
   • Remove "The radical scavenging activity was quantified using the equation
% of fundamental scavenging activity = (Abs blank – Abs sample) X100/Abs blank” to the end of paragraph titled "2.2. DPPH scavenging activity of Panduratin A" in Materials and Methods Section.

Done as suggested

- Also, Remove "The percentage of cell viability was calculated by the formula, "% Cell Viability = (Absorbance of the TAA-treated cells / Absorbance of sterile distilled water-treated cells) x100" to the end of paragraph titled "Determination of the IC50 dose of thioacetamide” in Materials and Methods Section.

Done as suggested

3. Results

3.3. FRAP value of PA

- In the middle of this paragraph "Further, Silymarin the results revealed significant decrease in the FRAP value of Silymarin..." changed to "Further, the results revealed significant decrease in the FRAP value of Silymarin", remove "Silymarin'.

Changed as suggested

MDA and the antioxidant enzyme CAT, SOD and GPx assays

- In the second line add word "protein" after mg.

Added as suggested.

- Fig(3): the legend in the vertical axis “FRAP Value mmol FeII/µg” µg of what??

Clarified and added as suggested.

4. Discussion

- Add a paragraph about "the isolation of Panduration A", because there is no any discussion and interpretation on it (Fig 1)

Added as suggested.
• The numbers of the all figures in the Discussion Section is not right and should be corrected according to the numbers mentioned in Results Section.
  Corrected as suggested.

• In the third paragraph of Discussion Section: remove (fig. 2).
  Removed as suggested

7. References

Omit reference number 35 from References list.
  Removed as suggested.