Author's response to reviews

Title: Do plants mediate their anti-diabetic effects through anti-oxidant and anti-apoptotic actions? An in vitro assay of 3 Indian Medicinal Plants

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Author's response to reviews: see over
Author’s response to reviews

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Version: 2
Date: 25 July 2013

**Author's response to reviews:** see over
The Biomed Central Editorial Team

Object: MS: 310030225897608

Do plants mediate their anti-diabetic effects through anti-oxidant and anti-apoptotic actions? An in vitro assay of 3 Indian Medicinal Plants

Thank you for consideration of our manuscript for publication in your journal. We have reviewed the above manuscript according to your reviewers’ comments. The answers to the comments are given in a point-by-point response to the concerns raised and the corrections are highlighted in the manuscript for your reference.

Reviewer's report
Title: Do plants mediate their anti-diabetic effects through anti-oxidant and anti-apoptotic actions? An in vitro assay of 3 Indian Medicinal Plants
Version: 2
Date: 25 July 2013
Reviewer: Dongbo Liu

Reviewer's report:
- Discretionary Revisions (which are recommendations for improvement but which the author can choose to ignore)
  None
- Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

--Paragraph 4: It was mentioned that Phyllanthus emblica and Curcuma longa per se showed a dose dependent increase and decrease in MDA levels, as compared to the control RIN cells. Is the difference statistically significant?
-- The effect in case of Phyllanthus emblica was statistically significant which has been mentioned in the manuscript as well as in the table representing the effect of P.emblica on MDA levels. In case of C. longa the decreasing effect was not statistically significant.
This question is not well answered. It is replied that the effect of Phyllanthus emblica was statistically significant while the C. longa was not. However, it seems not to be the case from figure 1A and figure 1C.

We apologize for the error on our part in our reply. There was no statistically significant effect of Phyllanthus emblica per se on the MDA levels. This has been correctly mentioned in the manuscript. On re-checking the statistics in case of Curcuma longa, a significant decrease in MDA levels was observed which we had not previously mentioned in our manuscript. We have made the necessary changes in the manuscript based on the reviewer’s comment.

The author should focus on the statistical significance of difference for some comparison. If there was no statistical difference, it is not significant for the level of decrease or increase. Also, it could not conclude the effect of these 3 plants. For example, (1) the anti-oxidant potential of these 3 plants per se could not be deduced according to their MDA release variation;

Yes it is accepted that the anti-oxidant potential of these 3 plants per se could not be deduced based on the MDA levels obtained. In our study we have discussed regarding the anti-oxidant activity exhibited by Curcuma longa based on the results obtained. On applying the statistics to this data, a significant effect was observed which was earlier not mentioned in the manuscript. The corrected results have been incorporated in the manuscript.

In case of Phyllanthus emblica and Tinospora cordifolia no significant effect was observed of the plant extracts per se in reducing the MDA levels, hence their anti-oxidant potential per se cannot be deduced on the results obtained. This observation has been corrected in the manuscript in para 3 on page 10. However in the presence of STZ, all the 3 plant extracts exhibited a significant decrease in MDA levels and hence based on these observations we had deduced the anti-oxidant potential of the plants.
(2) sub-section “effect on apoptosis”, paragraph 2: “subsequently increasing number of cells in G0/G1 population marked as M2 (45.28 ±5.61) as against the STZ treated RIN cells (39.13 ±6.69)”, it showed no statistical difference between “45.28 ±5.61” and “39.13 ±6.69”. Thus this “increase” is not significant;

In case of the apoptosis data, the increase in number of cells in G0/G1 population of RIN cells treated with Phyllanthus emblica in the presence of STZ, marked as M2 (45.28 ±5.61) as against the STZ treated RIN cells alone (39.13 ±6.69)” was not statistically significant. However the decrease in Pre G0/G1 population marked as M1 (11.77 ±2.31) as compared to STZ treated RIN cells (25.48 ± 3.77) was statistically significant. It is the effect on the M1 phase that is important to demonstrate the anti-apoptotic potential of a drug/plant and this is what we observed in our study.

(3) sub-section “effect on apoptosis”, paragraph 3: “...except at higher concentrations viz., 25 and 50mg/ml”, however, there is no statistical difference at concentration of 25 and 50mg/ml which means no effect as the other concentration;

Yes, we agree that the apoptotic effect seen with Curcuma longa per se, an observation made by the researchers, was not statistically significant. We have modified the results accordingly in the Manuscript.

(4) sub-section “effect on apoptosis”, paragraph 4: Though it is seemed that the data of M2 (48.64 ± 1.3) is larger than the data of “40.01 ± 9.17”, it is not statistically significant. It could not conclude that “increasing the cell in G0/G1 population ...”;

Yes, we agree that the data is not statistically significant. However we wished to explain that due to a decrease in apoptosis (M1) seen with Tinospora cordifolia in the presence of STZ, the cells then moved to the M2 phase indicating a protective effect.

(5) it is not significant to conclude “increase” insulin secretion with no statically significant difference.

We agree that the effect on insulin does not achieve statistical significance. However these are clinical observations made by the researchers which could reach statistical
significance if the sample size was suitably increased. We wished to point out trends seen during the experiment, which could be taken forward by others.

Results & Discussion, sub-section “effect on apoptosis”, paragraph 3: Please re-check the data of the sub G0 (apoptotic) (10.94 ± 3.78) and G0/G1 cell population (51.75 ± 5.09) at 10μg/ml Cl combined with STZ treatment.

**Apologies for the typographical error. Corrections done in the manuscript.**

Results & Discussion, sub-section “effect on apoptosis”, paragraph 4: It is mentioned that Tc did not affect the number of cells in sub G0 population. However, it is not consistent with the data showed in the table 1.3 that there is significant difference between treatment of “Cells + Tc 100 μg/ml (24.69 ± 2.19)” and “cells (5.87 ± 1.70)”.

**We apologize for the error in reporting of the statistics applied with respect to Tinospora cordifolia. The necessary changes have been made in the manuscript & Table 1.3.**

Results & Discussion, sub-section “effect on apoptosis”, paragraph 5: Why compared the data “53.22 ± 11.81” with “40.24 ± 6.71” to explain the protection of Glibenclamide against the apoptotic damage induced by STZ?

**Yes, it was an error. We wished to highlight the protective effect of Glibenclamide on the M1 phase (19.91 ± 5.05 vs. 28.5 ± 8.85) in the presence of STZ and the M2 data was given to demonstrate that the cells then moved to the M2 phase indicating a protective effect of the drug. The same has been modified in the Manuscript.**

In the adding discussion of the possible active ingredients, alkaloids and glycosides in the Tc has been reported to play an anti-diabetic role. How about these two constituent contained in Pe?

**There is no reported literature proving the anti-diabetic role of alkaloids and Glycosides with respect to Phyllanthus emblica.**
• Major Compulsory Revisions (which the author must respond to before a decision on publication can be reached)
None.

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

Declaration of competing interests: I declare that I have no competing interests.
Reviewer 2

Title: Do plants mediate their anti-diabetic effects through anti-oxidant and anti-apoptotic actions? An in vitro assay of 3 Indian Medicinal Plants

Version: 2 Date: 25 July 2013

Reviewer: Uraiwan Panich

Reviewer’s report:
The authors have responded adequately to my comments.

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

Quality of written English: Acceptable

Statistical review: No, the manuscript does not need to be seen by a statistician.

I would be obliged if the said manuscript is reviewed once again and accepted for publication. I hope we have made satisfactory replies to the concerns raised.

Looking forward to a positive reply,

Warm regards

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