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
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**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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**Date:** 13 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.

**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:** 1

**Date:** 19 April 2013

**Reviewer:** Dongbo Liu

**Major Compulsory Revisions**

1. Three to ten keywords representing the main content of the article should be provided.

   *Keywords have been added in the manuscript.*

2. The abbreviation should be defined at the first use, and later could be used directly in the text, for example, “streptzotocin”, “propidium iodide” and “malondialdehyde”, et al.

   *Corrections done in the manuscript*

3. Methods:
The model of some instruments used should be pointed out, for example, ELISA plate reader in the “cell viability assay” sub-section and dual beam spectrophotometer in the “Measurement of Lipid Peroxidation” sub-section. So did the manufacturer of Rat Insulin Elisa kit in the “Measurement of Insulin Secretion” sub-section.

   *Corrections done in the manuscript*
4. Results & Discussion:
It will be more explicit and clear if there are some sub-section titles in this section.

**Corrections done in the manuscript**

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?
Because the Cl and standard drug were dissolved in DMSO, did DMSO itself show any influence on RINm5F cell line?
It was mentioned that MDA levels decreased pointed to its anti-oxidant activity.
How to explain the conflicting results that MDA content increased in Phyllanthus emblica per se?

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.

The concentration of DMSO in the *C longa* extract and standard drug did not exceed 0.2% (which is an acceptable concentration for in vitro studies), which has no effect on the RINm5F cells.

The increase in the MDA content observed with *P. emblica* per se was a novel & interesting observation for which we have no explanation. A literature search too did not provide similar such findings by other researchers on this plant. Hence we thought it was important to report the same.

Paragraph 5: Phyllanthus emblica decreased MDA release at lower concentrations against the stress induced by STZ which was contrary to the effect exhibited by Phyllanthus emblica per se. The explanation is that it reflects the rasayana property of Phyllanthus emblica. Could you describe it in detail?

The protective effect observed with *P. emblica* on the STZ treated RIN cells can be explained on the basis of the rasayana effect of *P.emblica*. As per the definition, a rasayana plant is a plant that has the property of enhancing physiological conditions and restoring balance or equilibrium in pathological conditions. As *P. emblica* comes under the rasayana group of plants, this may help explain its protective effect in case of STZ induced damage.

However the increase in the MDA content in normal control cells was a novel & interesting observation for which we have no explanation. A literature search too did not provide similar such findings by other researchers on this plant. Hence we thought it was important to report the same.
Paragraph 7: In the last sentence, the data in the brackets (23.37±4.51, 9.58±2.48) could not found in Table 1.1. There is no Fig 2.1 in your figures; please mark it clearly (Figure parts should be denoted by lowercase letters, like a, b, c, d etc.).

The data given in the brackets are of Table 1.2. It is a typographical mistake which has been corrected in the manuscript. Figures are re-numbered and corrections are made accordingly in the manuscript.

Paragraph 10: The data of treatment “Cells+Tc100 μg/ml+ STZ” (14.05±1.80) in the text is not consist with that in the table 1.3 (14.5±1.80).

It was a Typographical error – corrections have been done in the manuscript.

Paragraph 11: How do the data in the bracket (54.28 ±6.65, 39.13±6.69) come from? Besides, it sounds farfetched that the explanation of “different experimental conditions” for the results of the Glibenclamide obtained in this study contrary to previous studies.

Typographical error - corrections have been done in manuscript. Explanation for the varying results seen with Glibenclamide has been modified in the manuscript.

5. Tables & Figures
In the figure 1, each part should be denoted by lowercase letters (a, b, c, etc.). In the table 1.3, the data of treatment “Cells+Tc 25 μg/ml+ STZ” (16.77±3.65) is significantly different from control “Cells + STZ” (24.69±2.19) with signs of “$$”. Are there any significant differences between the treatments “Cells + Tc 50 μg/ml + STZ” (14.16±1.69), “Cells + Tc 100 μg/ml+ STZ” (14.5±1.80) and the control “Cells + STZ” (24.69±2.19)?

The effect of *T. cordifolia* is significant at all the concentrations which were not highlighted in the manuscript. Corrections have been made in the manuscript.

In the ninth paragraph, it was pointed out that high concentrations of 25 and 50 mg/ml of Curcuma longa alone per se showed effect in the sub G0 (apoptotic) population as compared to the control RIN cells. However, in the table 1.2, there are no signs (*or $) indicating significant difference. Moreover, the data of the sub G0 cell population with STZ alone in the text (23.37±4.51) is not consist with that in the table 1.2 (23.37±4.03).

The effect is not statistically significant, hence there are no signs indicating statistical significance. Regarding data, it was a typographical error which has been corrected in table section of manuscript.
In the ninth paragraph, why compared the data of treatment of “cells + Cl 10 μg/ml” (10.94±3.78) with “cells+STZ” (23.37±4.51)? Should the data of 10.94±3.78 be a negligence of the data of treatment “cells+Cl 10 μg/ml+STZ” (12.74±3.07)?

We have not compared the data of cells + Cl 10 μg/ml” (10.94±3.78) with “cells+STZ” (23.37±4.51) instead we have compared the data of cells treated with STZ and Cells treated with STZ and Cl 10 μg/ml (12.74 ±3.07).

Was the treatment “Cells + STZ” and “Cells + STZ + Gb (1 μg/ml)” significantly different from the respective control? There is no sign indicating significant difference in table 1.4.

Yes the data of treatment “Cells + STZ” and “Cells + STZ + Gb (1 μg/ml)” was significantly different from the respective control. Statistics was applied and respective changes made in the document.

Minor Essential Revisions

1. Background
Paragraph 1: the sixth and seventh sentence: I would suggest these two sentences become one “The pancreatic β-cells are susceptible to oxidative stress leading to cell apoptosis and consequent insulin secretion reduction”.

Corrections done in the manuscript

Paragraph 3, 4: The statement of the experiment could be more concise, putting the emphasis on the aim of this study. For example, the explanation of RINm5F and STZ could be omitted. So did the mechanism of STZ to decrease the insulin secretion of this sentence “and activation of [poly (ADP-ribose) polymerase (PARP) leading to decrease in insulin secretion”.

Corrections done in the manuscript

2. Methods
The sub-section of “cell culture” could be omitted, because the specific operation was included in the sub-section of “assay procedure”. The colon after “Methodology”, “Measurement of Lipid Peroxidation”, “Measurement of Apoptosis”, “Measurement of Insulin Secretion”, “Statistical Analysis”, “Results & Discussion” should be deleted.

The subsection of “cell culture” can’t be omitted as it contains information regarding procurement and maintenance of the cell line whereas assay procedure describes the preparation of cells for the assay.
Discretionary Revisions

Results & Discussion
Paragraph 1: “Development diabetes mellitus” in the second sentence in the “Results & Discussion” section should be changed to “diabetes mellitus development”.

Corrections done in the manuscript

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'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: 1

Date: 23 March 2013

Reviewer: Uraiwan Panich

Reviewer's report:
The authors have evaluated the anti-diabetic effects of P. emblica, C. longa and T. cordifolia on RINm5F cells treated with STZ through their antioxidant and antiapoptotic actions. Nevertheless, the authors did not discuss the possible active ingredients in the test extracts responsible for the effects.

The authors have evaluated the anti-diabetic effects of P. emblica, C. longa and T. cordifolia on RINm5F cells treated with STZ through their antioxidant and antiapoptotic actions. Nevertheless, the authors did not discuss the possible active ingredients in the test extracts responsible for the effects.

Accepted. We have amended the manuscript discussion to include this point.

1. In “Methods”, the authors should explain how 3 medicinal plants were standardized or have data concerning fingerprint analysis of the herbal extracts (e.g., content and presence of putative active substances) in order to ensure a consistent quality of the plant extracts studied.

   We had procured the standardized extracts from Natural Remedy, Bangalore, as it is reputed Company which specializes in the production of standardized extracts for research and commercial use. The Certificate of analysis & HPTLC fingerprint of the extracts is available with us on file. The same has been mentioned in the manuscript.

2. In “Results”
   - Fig. 1: Author should put A or B to specify the graph when both are in the same figure. The authors mentioned that “As seen in Fig 1, P. emblica showed a dose-dependent increase MDA levels as compared to the control RIN cells (or cells without STZ) and C. longa showed a dose-dependent decrease in MDA as compared to control cells” Why were symbols indicating significant difference not shown in the graphs? Actually, as shown in the graphs, it seemed that P. emblica did not cause a significant change in MDA level in the control cells (non STZ-treated cells).
The Figures legends have been changed as suggested. The effect observed with the plant extracts is not statistically significant hence symbols of significance are not mentioned in the graphs.

- The authors should also discuss results concerning treatment of Gb with the cells why Gb caused an increase in MDA release.

Explanation has been added in the Discussion part of the manuscript

- Table caption: Should “$p<0.05; $$p<0.01; as compared to STZ untreated cells” be “$p<0.05; $$p<0.01; as compared to STZ treated cells”?

Correction done in the table section of the manuscript

- For apoptotic study, whereas P. emblica protected the RIN cells against STZ-induced damage, why did treatment of the cells with P. emblica alone increased number of apoptotic cells?

The protective effect observed with P. emblica on the STZ treated RIN cells can be explained on the basis of the rasayana effect of P. emblica. As per the definition, a rasayana plant is a plant that has the property of enhancing physiological conditions and restoring balance or equilibrium in pathological conditions. As P. emblica comes under the rasayana group of plants, this may help explain its protective effect in case of STZ induced damage.

However the increase in the number of apoptotic cells in normal control cells was a novel & interesting observation for which we have no explanation. A literature search too did not provide similar such findings by other researchers on this plant. Hence we thought it was important to report the same.

- Protective effect of P. emblica on STZ-induced cell damage was not dose-dependent.

The reason for the above query has been explained in the manuscript. The increase in MDA levels at higher doses of P. emblica per se or in presence of STZ may be due to the presence of polar substances in the hydro-alcoholic extracts of P. emblica, which may have interfered with the anti-oxidant effect thus affecting its degree of protection.

- Authors should re-check statistical significance of difference for %cells in Pre G1 between cells + Pe (25 and 50 μg/ml) and control cells.

The effect of Pe at 25μg/ml was not significant whereas its effect at 50μg/ml was significant (p<0.05). Corrections have been made in the manuscript.

- Authors mentioned that T. cordifolia demonstrated a dose-dependent decrease in the sub G0 population with maximum effect at 100μg/ml, although there was no symbol
indicating significant difference. The author should re-check statistical significance of difference for %cells in Pre G1 between cells + Tc (50 and 100 μg/ml) and control cells.

_T. cordifolia_ demonstrated a dose-dependent decrease in the sub G0 population and the effect was statistically significant at all the concentrations studied. These corrections have been incorporated in the manuscript.

- For insulin secretion study shown in Fig. 3, while there was no statistical difference between groups, the author can't really confirm nor conclude the effects of 3 plants studied on insulin secretion in the cells with and without STZ. While the author mentioned that Pe (10 μg/ml) showed an increase in insulin secretion on STZ-treated cells, there was no symbol showing statistical difference between Pe (10 μg/ml)-treated group and STZ-treated group. The authors should check the data since the bar height between 2 groups (Pe (10μg/ml)-treated group and STZ-treated group) doesn't look different. Also, the authors mentioned C. longa demonstrated a decrease in insulin secretion and that can't be concluded while the difference between groups was not statistically significant.

The results obtained in case of insulin secretion were not significant, hence was difficult to conclude. Also as the effect of Pe (10 μg/ml) on insulin secretion was minor (5.57 ± 0.415) as compared to Cells treated with STZ (5.25 ± 0.40) which was not clearly depicted in the figure. The effect of C. longa was also not statistically significant.

3. From the results of this study, it can't be concluded that prolonged administration of these medicinal plants may lead to increased insulin secretion and improved glycemic control.

Depending on the results of Flow Cytometry, the plants can abrogate apoptosis induced by STZ resulting in a cytogram with similar profile to control cells. Their anti-apoptotic action may help in preserving the residual β-cell mass which would probably help in maintaining insulin levels for a longer time. Although the effect on insulin secretion was not seen in this model, based on these observations, we concluded that prolonged administration of these medicinal plants may lead to increased insulin secretion and improved glycemic control.

4. In “Discussion”, the authors should discuss the possible active ingredients in the test extracts responsible for the pharmacological effects observed in this study.

Points about the possible active ingredients have been discussed in the manuscript.

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

**Quality of written English:** Needs some language corrections before being published
**Statistical review:** No, the manuscript does not need to be seen by a statistician.

**Declaration of competing interests:** I have not competing interest in this subject.

Given below please find our replies to the Editorial comments on the manuscript:

- Please include details in your manuscript on who identified the plants used in your study. Similarly, please confirm whether voucher specimens of the plant material have been deposited in a publicly available herbarium, and include this information in your manuscript. Please also be sure to include your deposition number for this, if you have one.

  The plants were identified based on a thorough literature search and were finalized after consulting an Ayurvedic Expert. We had procured the standardized extracts from Natural Remedy, Bangalore, as it is reputed Company which specializes in the production of standardized extracts for research and commercial use. The Certificate of analysis & HPTLC fingerprint of the extracts is available with us on file. The same has been mentioned in the manuscript. We have requested them to provide us with the herbarium details and the deposition number which we will forward to you as soon as we receive from them.

- Please remove your figure legends from your uploaded figure and instead include these in your manuscript, in accordance with our formatting guidelines. Further information on these guidelines can be found through the following link: [http://www.biomedcentral.com/bmccomplementalternmed/authors/instructions/researcharticle#preparing-figures](http://www.biomedcentral.com/bmccomplementalternmed/authors/instructions/researcharticle#preparing-figures)

  The figure legends have been removed from the uploaded figure and included in the manuscript, in accordance with the formatting guidelines.

I would be obliged if the said manuscript is reviewed 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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