Author's response to reviews

Title: Effect of Hydroalcoholic Leaves Extract of Indigofera Spicata Forssk. on Blood Glucose Level of Normal, Glucose Loaded and Diabetic Rodents

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Author's response to reviews: see over
Dear Dr. Priyankar Dey

Thanks for your critical and essential revision of the manuscript. It is really my great pleasure to have such comments and suggestions that could improve the quality of the manuscript.

Considering comments, on the behalf of all the authors I have forwarded the following revisions and answers

Here is the general Information on the revision of the manuscript

- I have tried to improve my language as much as possible
- Of course the title is too long. if you agree, it could be “Effect of Hydroalcoholic Leaves Extract of Indigofera Spicata Forssk. on Blood Glucose Level Of Normal, Glucose Loaded And Diabetic Rodents” and please decide on it.
- The abstract is also a bit longer but it is not beyond the maximum limit, while I have omitted the phrase “on medicinal plants” on the second statement of the background section just to reduce the number of words consequently, the total number of words becomes 288, including the key words.
- Due to the incorporation of new references in the introduction part the references are also reordered.(Line: 130-142)

Key:

- Your comments
- Answers/explanations
- References for the answers
- The modified statements existing in the manuscript text
• Introduction, Line 99: what does DM mean?

Answer:

Thanks for reminding, Just it is to mean ‘Diabetes mellitus’
As a result it is corrected in the text, line 100

‘The total number of people with diabetes is estimated to rise from 285 million in 2010 to 439 million or more, that is predicted to be ≥7.7% from the world’s adult population in 2030. Regions with greatest potential are Asia and Africa, where diabetes mellitus rates could rise to two to three-folds than the present rate [4, 5].’

• Introduction line 101: the prediction of increase in the rate of diabetes according to the reference 4 & 5 dates back to 10-13 years. Please use current references while mentioning such likelihoods.

Answer:

Sure, our expectation was just if they are predictions of the current and the future incidence of DM taking such references is possible even if they were published a decade ago.
Whatever this section is well modified with references published accordingly. Line: 94-101

• The authors should specify in the introduction part which type of diabetes mellitus they have investigated. Type I or Type II.

Answer:

Type I and it is stated from line:130-142

‘Currently like streptozotocin, alloxan-induced diabetes is one of the widely used model to induce Type I diabetes mellitus and study hypoglycemic activity in animal models. Though, alloxan has multiphasic effect on the blood glucose level in its early course of action, permanent diabetic hyperglycemia could be induced within 24-48 h after administration. And this is due to the selective pancreatic beta cell toxicity of alloxan. Surprisingly, the non-beta cells and other endocrine and non-endocrine islet cell types
• Streptozotocin is more reliable than Alloxan in the induction of diabetes in rodents. Literature search will show that alloxan possess various drawbacks as a diabetogenic agent because of the ability of animals to reverse the diabetes condition (to normal) when induced when alloxan. Thus, some of the findings might be due to the reversal and not the actual treatment in the intervention trial.

Answer:

Really, compared to streptozotocin, alloxan has limitations e.g. instability in aqueous solution, narrow dose range, effectiveness, etc.

But we have done our study with alloxan due to the following reasons

• At the time of the study we don’t have streptozotocin and it is not available in Ethiopian markets.

• And from the scientific point of view

I. Currently, many researchers are utilizing alloxan as a diabetogenic agent and its hyperglycemic effect is evident to be permanent after 48hrs.

Reference:


II. Autoreversal of alloxan induced DM in animals is possible when low doses of alloxan (90-140mg/kg) are used or the induced DM is mild

Reference:


But to avoid these drawbacks in our study

- we use the commonly recommended high dose, 150mg/kg
- the animals selected for the study were having a confirmed fasting blood glucose level of > 200mg/dl after 72 hrs alloxan IP injection.
- Genetic expression which are responsible for the reversal of such biochemical changes have slow onset of action since they mainly mediated by Nuclear receptors. Accordingly, Risk of recovery is minimal because in all the three models blood glucose level is determined within 12hrs of post exposure of the treatment.

III. There was proper randomization of the animals and the effect of the extract is expressed in comparison with the control groups. If there is reversal of the diabetogenic action of alloxan in extract treated groups, there will be also the some degree of reversal in the control groups. Indeed, if I am not mistaken as long as the condition is similar and controlled the net effect will not affect the result of this study.

• Please elaborate the rationale behind the usage of 80% hydro-methanol extract for the treatment.

Answer: Hydromethanolic solvents (especially 80% methanol) will be more efficient in extracting almost all the necessary bioconstituents of the plant material which is owing to their expanded polarity range. By virtue of the cosolubility, many compounds, which are insoluble individually in pure state in MeOH can be extracted quite easily with hydroalcoholic solvents. In addition, secondary plant metabolites (alkaloids, flavonoids, saponins, tannins and steriods) which do have recognized hypoglycemic activity could be readily extracted by methanol. In addition to dissolving lots of compounds, methanol is commonly recommended to be used as a solvent in evaluating the possible
pharmacologic activity of crude extracts because it is relatively inexpensive, and easily evaporated.

Reference:


• The authors have performed the preliminary phytochemical analysis, however proper standardization of the extract using chromatographic methods is highly encouraged. The basic bioactive constituents in the extract remained completely unknown.

Answer:

Yes, bioactive constituents are even better to be isolated and structurally elucidated using sophisticated instrumental methods (e.g. chromatographic, absorption and mass spectroscopy, NMR,…) but in resource limited conditions like us it is inaccessible and still recommendable to do at least preliminary phytochemical screening by observing the possible color changes and/or precipitate formations during chemical interactions. Even fractionating with solvents of different polarity is important to purify and explain more on the bioactive constituent.
Reference:

a. Aiyegoro OA and Okoh AI. Preliminary phytochemical screening and In vitro antioxidant activities of the aqueous extract of Helichrysum longifolium DC. BMC Complement Altern Med. 2010, 10:21


• The authors have evaluated the anti-hyperglycaemic activities viz. anti-diabetic activities only by means of glucose level measurement. However, type I diabetes (alloxan induced) affects various other biophysical and chemical parameters of the body. Therefore, other parameters such as normalization of serum insulin level, hepatic and muscular glycogen level, serum and tissue antioxidative enzymes and malondialdehyde levels needs to be evaluated.

Answer:

• Sure, evaluating other biochemical parameters is also essential and recommendable to make the study more qualified and advanced. But as it is explained before to do such additional tests, more expense is needed which is unaffordable personally and even at institution level in our set up. As a result this was our main limitation to perform all the aforementioned tests and the possible anti-hyperlipidaemic effect of the extract.

Thanks in Advance for your consideration and time!!

I am waiting your feedback soon after.