Author's response to reviews

Title: Evaluation of the effects of 80% methanolic leaf extract of Caylusea abyssinica (fresen.) Fisch. & Mey. on glucose handling in normal, glucose loaded and diabetic rodents

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Author's response to reviews: see over
Dear Editor,
Thank you very much for giving us the opportunity to recast our manuscript. We also appreciate the referees for providing constructive comments, which enable the MS to assume the current form. We have addressed shown below and hope that the MS is in an acceptable form for publication.

Kind regards.
Ephrem Engidawork (PhD)

Response to comments of Referee-1

- Results are arranged as same order as material and method
- FBGL corrected to reflect only fasting conditions and abbreviations avoided in subtitles. It is also corrected in the materials and method.
- The Authors name of the plant was included in the plant material.
- Statistical significance: checked and double checked. p values not shown in Tables included.
- Hypo (hyper)glyceamic vs. hypo(hyper)glycemic: the later used consistently throughout the text.
- Updated reference was given for the statement ‘However, little is known…about the mechanism of action.’ but we decided to maintain the reference cited for the practice as it is one of the few and trusted references we have in folklore medicine in the country.
- The aim of the study was placed in clear terms at the end of the introduction
- The Su for liter corrected as “L” throughout the text
- Table 1 was corrected as per the comment.
- STZ induction just corrected by adding only for animal models
- CA200 and glibenclamide similarity in their mechanism is stated (as enhancing insulin release or insulin like effect).
- C. abyssinica might contain agents which may delay and impede hypoglycemic…. Specifically the agents are listed as simple sugars, high molecular weight proteins or some alkaloids.
• ‘Antidiabetic’= added in the key word list.
• Standard pellet diet= rewritten as ‘pellet diet’. It is not important in the development or treatment of the animals.
• Preliminary photochemistry screening is corrected by presenting it next to the extraction.
• The absent metabolites of the phytochemicals of the plant is removed.
• Compounds having phenolic groups are written as suggested by the referee.

Referee-2
• Abstract= revised as commented
• Introduction of references = updated as commented
• Aim (objective) included at the end of the last paragraph
• Family and the part of the plant used included
• How many animals were per cage? = 6 - 9 rodents per cage and this was shown in the materials and method section
• Induction of diabetes with STZ: well diabetes was induced in mice not in rats. Seventy were used for induction and fifty developed experimental diabetes. Two died and all the others survived until the end of the experiment. Mice have been shown to survive until one week following STZ administration. Included in the results section. It is usually multiple administrations that’s associated with death of animals. One could refer to works done by He-Lin et al., 2010;
• Why males? There is a huge difference b/n Male and female rodents which can affect the study. For example, females have estrous cycle which leads to a relative hormonal fluctuation unlike males. Besides, females are less sensitive to insulin or glucose. And also, females face severe diabetes which leads to reduced survival from STZ induction relative to males. This is indicated in the manuscript.
• It is indicated in the methods that after 72 h of induction that the animals were used for the study.
• Mode of blood sample collection was from tail vein of the animals using aseptic techniques (disposable syringes and clean areas to avoid any contamination). BGL was determined using commercial kits in triplicate and the average was taken. This is also mentioned in the material and methods.
• Statistics???
  ➢ We think that mean ± SEM is very clear for any reader that is familiar with statistics. What’s done was also very clear. We took blood for each animal before and after (at different time points) administration of either the vehicle or test substances. Glucose level was then read for each animal and the average was taken for a given group. The number of the animals was presented as (n=6 or 9) in the Tables. For each animal BGL was determined once at each time point. The text is modified to convey that message.
  ➢ About 2-way ANOVA: as far as the analysis is concerned, it is one way ANOVA that is usually applied. Two way ANOVA is meant for complex data sets requiring two or more outcome variables to be assessed at the same time within groups. We don’t have such variables the only one is BGL which can easily be compared using one way ANOVA by comparing the initial BGL with the other time levels of a group or the negative group with the treated groups. We don’t see the necessity of using two way ANOVA. F values are less specific than p-values because they only tell us if the means of the groups are different or not in the decision of either rejecting or accept the null hypothesis. But the level of significance tells us how significant the mean difference is and it is significant when the F value is significant as well. P values are provided whenever there is a significant difference. Normally, P values are not provided if the result is not significant.
• Results: we think there are no as such mistakes in the way narration was made in this section. There are two approaches we used in describing the results. We did “within group” analysis to show if there is any effect with a given treatment across time using a specific control for each group. This shows you how good your treatment is compared to a vehicle. In addition, we did “between group” analysis to show how good the extract is compared with the standard as well as which dose is associated with a good effect. We think this could be the source of confusion. We also think it is not appropriate to use numerical values in the text, as they are already shown in the Tables. We indicated the percent changes with p values when it was appropriate.
• MS looks a thesis: we partially agree with the referee and did a significant revision on the MS, particularly, the discussion.
The need for molecular studies: one of the plagues for the development of traditional medicines has been the lack of scientific data backing the traditional claim. What we attempted to do is to provide the basic evidence for safety and efficacy in animal model of diabetes. We evaluated whether the plant has any effect on normal glucose levels (that gives an idea whether the plant suffers a problem similar to conventional agents like sulfonylureas), in the setting of hyperglycemia caused by diabetic inducing agents (to see whether it has any antidiabetic effect) and glucose itself (to see the effect of the plant on normal glucose handling mechanisms). Thus, these are the major markers for such studies. Once this is published, we continue doing further studies as appropriate.

Conclusion part= rewritten as per the comment