Author's response to reviews

Title: Anti Hyperglycemic and Renal Protective Properties of Terminalia chebula Seed Powder Chloroform Extract in Streptozotocin Induced Diabetic Rats

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Author's response to reviews: see over
Reviewer's report
Anti Hyperglycemic and Renal Protective Activities of Terminalia chebula Seed Powder
Title:
Chloroform Extract in Streptozotocin Induced Diabetic Rats
1 10 June 2005 Version: Date:
T Dunning Reviewer:
Reviewer's report:
General
There are a number of typographical errors, inconsistencies, missing words and structural issues that require attention.
We have made changes according to the referee comments.
Specific comments
Title
The title is appropriate and reflects the contents of the paper. However, “properties” may be a more appropriate term than “activities”.
We agree with referee. We changed it to “properties”.
Abstract
The abstract requires some revision to improve the flow of the information and to more accurately reflect the information reported in the text.
The aims/hypothesis need to be stated.
The methods require clarification for example how blood glucose levels were determined is not clear.
Made the changes accordingly.
The phases of the study require clarification. “Acute” usually refers to a hospital admission and “chronic” to incurable diseases. “Short” or “immediate” and “long term” effects may be more appropriate.
We agree with the referee comments. So we changed the terms a) acute study as Short-term study b) chronic study as Long-term study.
Were baseline urine samples collected and compared with the 8 week samples? Is 8 weeks sufficient time to show a change?
Yes. We observed the change. So we have given the results.
The method describes rats but results section discusses “diabetic rabbits”—clarify what happened to the rats.
We rectified the error.
In the main text T chebula was compared with glibenclamide and glipizide.
Introduction
The introduction is very brief. More discussion about diabetes, its complications, conventional management and other herbs with hypoglycaemic properties such as Momoridica charantia, Opuntia streptocantha, Trigonella foenum graceum, should be included. Discuss issues concerning the standardisation and purity of herbal therapies and other safety issues would also enhance the paper.
We made the necessary changes.
The information detailing the indications for use of T chebula could be presented in a table.
We thought the given information is enough.
The sentence “It has also claimed to be useful in diabetes” is vague. Clarify what “useful” means in this context.
Made the necessary changes in sentence
The aims/hypothesis need to be stated. It is not clear whether the aim was to establish hypoglycaemic effect and/or compare T chebula with conventional oral hypoglycaemic
agents, or whether it was a dose finding trial. Clearly stating the aims of the study and outcomes measured would help clarify these issues.

**We made the changes according to the referee comments**

**Materials and methods**

An appropriate method appears to have been used although the aims/hypothesis was not clearly stated. The study aims are very important in determining the most appropriate method to use.

**We made the changes according to the referee comments**

The information could be reorganised to make this section easier to read. For example indicate the study consisted of two parts: a) acute and b) chronic. These terms need to be defined for the purpose of the paper. “Acute” often refers to care in hospital or curable conditions and chronic to incurable diseases such as diabetes whereas the context here appears to be the immediate effect of the T chebula extract after a fast (acute) and the longer term effect (after 28 days) of treatment with T chebula extract.

**We agree with the referee comments. So we changed the terms a) acute study as Short term study b) chronic study as Long term study.**

2.1 Details about how the plant material was verified could be included, for example was a plant classification system such as the International Code of Botanical Nomenclature used?

2.2 Suggesting products were a gift could represent a conflict of interest. It is preferable to use phrases such as “supplied by”.

**We have made the changes according to the referee comments.**

**Clarify whether “Dr Reddy’s laboratories” is the correct name.**

**Yes it is the correct name.**

**Specify what the “other chemicals” were and what they were used for.**

**We have withdrawn the sentence.**

2.3 Clarify that there were eight groups with five rats in each group (n=40).

**We have withdrawn the sentence.**

Clarify the composition of the rat diet.

**Clarify what is meant by “when the condition of diabetes was stabilised”. Usually “stabilised” refers to controlling or normalising the blood glucose levels, however here it appears to refer to ensuring the rats actually had diabetes and significant hyperglycaemia.**

**We have withdrawn the sentence.**

2.3.1 Note previous comments about terminology.

The section would be easier to follow if the study groups and treatment mode were listed or presented in a table. Currently it is not clear whether all the rats in groups 1–3 were given incremental doses of T chebula commencing at 100 mg/kg and increasing to 200 then 300 mg/kg or whether group 1 had 100 mg/kg, group 2, 200 mg/kg and group 3, 300 mg/kg.

**We have made the changes according to the referee comment in description to make it more understandable.**

**Clarify what “appropriate volumes of vehicle” means.**

**We have withdrawn the sentence.**

Indicate the glibenclamide group served as a comparison medicine and indicate why it was used.

**It is a long acting agent. So for comparison with extract activity it is chosen.**

**Why was a 16 hour fast necessary? For example glucose tolerance tests are often performed after 12 hour fasts Was the extract administered orally or intravenously after the fast?**
We agree with referee comment. It is sufficient. But due to experiment delay we mentioned it. However we observed that there is no such difference in 12 h fasting or 16 h fasting.

The method of analysing the blood glucose levels is not clear. Several glucose assay methods involve glucose oxidase reactions including blood glucose meters and testing strips using a visual colour comparison method. In addition, the yellow springs analyser (YSI) is frequently used in laboratory work. The reference cited for this method is very old, 1969, which could mean outdated technology was used.

We have given the recent reference also. It is not outdated technology.

2.3.2 Note earlier suggestions about terminology “chronic”, “standard” and presentation of the material.

Clarify the dose intervals for the T chebula, and glibenclamide. Glibenclamide is a long acting agent; therefore a daily dose would be indicated. Is the duration of action of T chebula established?

In short term study only one dose was administered. In long term study one dose for every 24 hours. We studied the extract action up to 12 hours only.

Indicate how 28 days was chosen as the end point of the chronic study.

Indicate why blood glucose was only estimated weekly. This could be too infrequent and not capture meaningful data. Continuous blood glucose monitoring indicates wide fluctuations in blood glucose levels over 24 hours that are not captured by capillary blood glucose monitoring four times a day (recommended for many people with diabetes).

The study is sufficient. It is acceptable. In short term study we have estimated the blood glucose levels at 0.5, 1, 2, 4, 6, 8, 12 h. This reflects the action of the extract

2.3.3 Clarify whether blood glucose monitoring continued for 8 weeks (32 days) or whether this aspect of the study only measured kidney function.

This aspect is measured for kidney function. We monitored glucose levels also.

Clarify what “standard diagnostic kits were”. Was the albumin creatinine ratio estimated? Was microalbumin measured in urine or was it albumin? Microalbuminuria precedes albumin and is an early marker of renal disease. Were kidney pathology, morphology and blood flow examined? In the early stages of diabetic nephropathy the kidney is enlarged, but reduces to some extent when treatment is initiated and the blood glucose normalises. It may be premature to claim renal protective activity for the T chebula without more information about the mechanisms of action. These issues need to be discussed in the discussion.

We had given references for albumin, creatinine. We observed changes in renal activities after eight weeks. We reported that. No pathological studies, morphological studies done.

The abstract and results discuss urine tests where blood samples are discussed in this section.

Clarify the discrepancy.

We removed the term blood samoles and made changes accordingly. Rectified the typographical error.

Results and discussion

In paragraph 1, sentence 7 a dose of 150 mg/kg is reported but this dose is not described in the method.

We removed the dose 150 mg/kg and made changes accordingly. Rectified the typographical error.

Note suggested terminology.
More discussion about the clinical relevance of the study is needed and whether rat data can be applied to humans.
Clarify why such an old reference, 1986, is quoted to support the need for “more appropriate therapies” given the enormous advances in oral hypoglycaemic agents and insulin in recent years.
Discuss how herbal medicines might be used in place of or with these agents and the safety issues that arise when the herbs are used with conventional medicine. 
We added the discussion about the role of herbal medicines.
Are the researchers appropriately state the need to identify the “active principles” responsible for the hypoglycaemic effects and their mechanisms of action.
Yes. The term is appropriate.
Clarify what earlier results the study substantiated.
Note comments about the effects on renal function.
We have added the comments about renal function.
Include conclusions and discuss the limitations of the study.
References
Note references 11, 12 and 13 are very old and more up-to-date references exist and should be used.
Tables and figures
The tables and figures appropriately summarise the results.
Table 1 is difficult to interpret. Clarify that n=5 means five rats per group (also table 2).
We have made the necessary changes. Yes, n=5 means five rats per group.
Clarify that row two refers to time in hours.
We have added hours. It refers to hours
Clarify diabetic controls refers to blood glucose level.
Write out “figure” in full, figures 1 and 2.
Only figure 1 is there. It repeated as figure 2. We made the changes accordingly.

Major Compulsory Revisions (that the author must respond to before a decision on publication can be reached)

Minor Essential Revisions (such as missing labels on figures, or the wrong use of a term, which the author can be trusted to correct)

Discretionary Revisions (which the author can choose to ignore)