Author's response to reviews

Title: Evaluation of toxicity after one-month treatment with Bauhinia forficata decoction in streptozotocin-induced diabetic rats

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PDF covering letter
To the Referee

Araraquara - SP
Brazil.


This is to certify that I have read with care the enclosed revised manuscript entitled "Evaluation of toxicity after one-month treatment with Bauhinia forficata decoction in streptozotocin-induced diabetic rats", submitted by the authors M. T. Pepato et al., in order to review the standard of English used. In my opinion the language of this paper is perfectly adequate for an English-language scientific publication.

I am English, a Science graduate from Sheffield University and have MSc 's from Birkbeck College and Sheffield Hallam University. Recently, I have taught English at Cultura Inglesa, one of the more respected language schools in Brazil.

I hope this information may be of use to the Editors and Referees.

Yours sincerely,

(Timothy Roberts).
We are extremely grateful for the care taken in reviewing our manuscript and your suggestions for changes, which have helped us make substantial improvements to the text. We have tried to incorporate these suggestions and to reply fully to your questions. The manuscript has been completely revised, taking into account the recommendations and demands of the referees.

Major Compulsory Revisions

1. The authors’ conclusion “absence of toxicity” is very presumptive. They just showed changes in a narrow spectrum of biomarkers representing hepatic, renal and muscular cytotoxicity. Serum levels of LD and CK does not stand for liver toxicity, since they can be less specific than AST and ALT as marker of hepatic injury. It could be true in the case of ACE in kidney.

Answer: We agree that we used a narrow spectrum of biomarkers (LD and bilirubin) to show hepatic cytotoxicity and that AST and ALT are more specific markers of liver injury than LD. However, as we argue in the Discussion (previous manuscript, pages 6-7, Discussion, para. 2, 5th and 6th sentences; new manuscript, Discussion, page 9, para. 1, 5th and 6th sentences; page 11, para 4, 2th sentence), earlier data from our laboratory (Ref. 19) indicated that diabetes in itself can bring about changes in the serum activities of AST and ALT. Thus, it would have made the interpretation of the results more complex if we had also used these enzymes as markers of hepatic toxicity, as the possible changes in their levels due to the herbal treatment would be added to the changes produced by diabetes. On the other hand, diabetes in itself did not alter LD activity, nor the serum level of bilirubin (Ref. 19). This was the reason we used, in the present study, a narrow spectrum of biomarkers well-known for this experimental model. However, in the revised manuscript, we have now explained our criteria more clearly.

NB: We cannot find in our manuscript the idea that serum levels of CK stand for liver toxicity, implied in your phrase “Serum levels of LD and CK does not stand for liver cytotoxicity”.

2. The experimental data shown in this paper do not provide the values of non-diabetic control fed with BF, which can give an important parameter for comparison with the values of diabetic groups.

Answer: In the revised manuscript we present the values of non-diabetic control fed with BF. In the original manuscript we did not include the results of studies of toxic effects of Bauhinia forficata on the normal groups because:


b) From the results of an earlier study of ours (Mori, MD, Baviera AM, Ramalho LTO, Vendramini RC, Brunetti IL, Pepato MT: Temporal response pattern of biochemical analytes in experimental diabetes. Biotechnol Appl Biochem 2003, 38: 183-191) we knew that the enzyme activities being tested in the present study do not change with time in diabetic animals. Hence, we felt that
for the normal group it was not important to report activities of enzymes that do not change in the diabetic group.

Minor Essential Revisions

1. In title (Bauhinia forficata: absence of toxicity during long term treatment of experimental diabetes) "Absence of toxicity" is too conclusive. Their results could not support such an affirmation.

Answer: The title was changed.

2. In the section of Background, the final aim of this study is not clearly revealed to the reader, whereas it can be assumed in the Abstract and the last paragraph of the Discussion.

Answer: We have tried to make the final aim of this study clearer to the reader.

3. Many sentences of this paper are too long and sophisticated to be followed. The should refine English spelling used.

Answer: We have endeavoured to make the corrections requested above, as well as all those listed below:

- P2, last L, serum glucose and urinary glucose: words repeated
- See page 3, para 2, line 4.
- P5L5, all serum enzymes: unnecessary word
- See page 6, line 2.
- P5L7, CK alone: unnecessary word
- See page 6, line 3.
- P8L2, no higher: wrong word
- See page 9, para 1, line 13.
- Reference 4, Pepato Mt (?)
- See page 13.
We are extremely grateful for the care taken in reviewing our manuscript and your suggestions for changes, which have helped us make substantial improvements to the text. We have tried to incorporate these suggestions and to reply fully to your questions. The manuscript has been completely revised, taking into account the recommendations and demands of the referees.

Major comments:
1. I would suggest a change in the manuscript title to: "Absence of toxicity after one-month treatment with Bauhinia forficata decoction in streptozotocin-induced diabetic rats". I do not think that one month is a long-term treatment.  
Answer: This has been done, except that we substituted Evaluation for Absence to combine your suggestion with that of another referee.

2. Abstract (Results and Discussion): according to the data in figure 3, AMS activity was increased and not decreased in both groups (DC and DT) at day 33, as compared to day 0.  
Answer: We agree and a correction has been made. The text has also been changed to include the normal rat groups. Nevertheless, group DC on day 33 was not significantly different from DC on day 0. Both the data and the result of the statistical test have been checked.

3. In the Methods section (Decoction preparation), last 2 lines: it is not clear what is meant by "The final yield was 87% by volume....". How the yield was determined?  
Answer: The yield was determined volume for volume (i.e. final vol. of filtrate as a fraction of the initial litre of water). This information was added in Methods (decoction preparation, line 9).

4a. Animals and their treatment: it should be interesting to list in the References any publication about Brazilian Ethics Committees.  
Answer: This has been done. See page 4, Section Animals and their treatment, para. 1, line 4 and page 13, ref.7.
4b. Besides, I would like to know whether streptozotocin was really injected into the jugular vein, since usually a penis or tail veins are used for that purpose.  
Answer: At the start of the experiment, the young rats weigh around 129.5±1.2 g. The penile and caudal veins of rats weighing less than 180 g have a very narrow bore and tend to burst when the needle is inserted, so we used the jugular.

5a. Decoction administration, 1st paragraph: what is the purpose for water administration to both groups (DC and DT) on the first six days after STZ injection?  
Answer: We needed the 6-day period to measure all the experimental variables in all 50 animals and make the necessary calculations, to choose the rats that would go into the matched diabetic groups (pairs of rats with similar severity of diabetes).  
5b. The way water or the B. forficata decoction were administered is not clear. How the volume drunken was determined?  
Answer: We added the word "metabolic" before "cage" to clarify this (page 5, Section Decoction administration, para. 1, line 16). The metabolic cage is especially suited to monitoring liquid and food intake and volume of urine excreted. One component of these cages is a graduated drinking bottle. Each rat had its own
cage; those housing a diabetic rat were equipped with two bottles, filled daily with 100 mL of water or decoction, as appropriate for the group of rats, while normal rats had one bottle of 100 mL water or decoction daily, in accordance with their group. After 24 hours, the level of the meniscus in each bottle was read to obtain the volume drunk.

6. Decoction administration, 2nd paragraph: It is confuse. How was the blood collected for the measurements of serum enzymes after 19 days of treatment (25 days after STZ injection)? Why at both times (at days 19 and 33), blood was not collected the same way, i.e. through the orbital plexus, as most people do?

   Answer: We agree it is confused. New information has been added to Decoction administration (page 5), para. 1, lines 3-4, where we refer for the first time to the collection of blood. We chose to take blood from the tail throughout the experiment because this method is recommended for longitudinal studies and is considered stress-free. Here are some references on the topic of blood collection from the tail:


   The sampling method used on the day of sacrifice (day 33) was different from the other times, since bilirubin was also assayed on that day, by a technique requiring a larger volume of blood than could be taken from the tail.

7. Results, 2 last paragraphs: besides a significant increase in AMS activity in the DT group, as compared to DC group, I have the feeling that a significant decrease was also shown in the DT group at day 33 in ACE values. Please check the statistics.

   Answer: The rise in AMS activity in DT was not significant relative to DC, but was so relative to day 0, i.e. before the start of treatment (see Fig. 4). We have checked the statistics.

   Regarding ACE, we checked the values used in the statistical test, the result of the statistical test, and did not find the difference suggested in the Comment.

8. Table 1, end of footnote: it would be better to say that, "all values are mean ± SD obtained at the 4th day after STZ and before starting Bauhinia forficata treatment". Besides, I do not understand why figure 1 represents data from LD as well as bilirubin. Perhaps, it would be better if these data were shown in 2 different figures.

   Answer: The end of footnote on Table 1 was altered as suggested.

   Figure 1 originally contained both LD and bilirubin data because both are related to liver function and we thought in this way to economise space in the journal. However, we have followed your suggestion and split the data between Figs. 1 and 2.

9a. Discussion, 1st paragraph: It has to be rewritten in order to make it clear.
Answer: We have tried to clarify this point (see pages 8-9, Discussion, para. 1). It is important that the study start with the same severity of diabetes in groups DC and DT. This was verified by the similarity between the physiological and metabolic data obtained from these two groups, before treatment started (day 0).

9b. Besides, all the discussion section has to be revised as far as orthographic and grammatical errors are concerned.
Answer: This has been done.

10. Discussion, 2 last pages: the discussion on AMS activities in both DC and DT groups has to be improved, since the data show a significant increase in this enzyme activity with time in both groups, as well as a greater increase in AMS activity at day 33 in the DT group, as compared to the DC group at the same period of time.
Answer: The data show a significant increase in activity of AMS in groups NC and NT on days 19 and 33, relative to day 0, as well as in DT on day 33, relative to day 0. They don't show a significant increase in activity of AMS in group DC on day 19, nor on day 33, relative to day 0. The data don't show a significant increase in AMS activity on day 33 in the DT group, compared to the DC group over the same interval. There is only a tendency for a greater increase in AMS in the DT group than in the DC group on day 33. We have tried to discuss this adequately in the text.

11. Discussion, last page: I feel that the ACE data deserve to be checked regarding statistics, since it seems that values are significantly decreased in the DT group at day 33.
Answer: We have checked the values used in the statistical test, the results of the statistical tests and found no significant differences between the following pairs of ACE activities, involving day 33: DC (day 33) X DT (day 33); DT (day 33) X DT (day 19); DT (day 33) X DT (day 0). For this reason, we have not changed the text of the discussion on ACE.

12. Finally, questions such as those related to AMS and ACE measurements have to be stressed in the Discussion section.
Answer: This has been covered in answers to Comments 10 and 11.

13. All the text including References have to be revised, concerning English language errors.
Answer: This has been done.
We are extremely grateful for the care taken in reviewing our manuscript and your suggestions for changes, which have helped us make substantial improvements to the text. We have tried to incorporate these suggestions and to reply fully to your questions. The manuscript has been completely revised, taking into account the recommendations and demands of the referees.

Major Compulsory Revisions

1. What is the status (higher or lower) of the activities of serum LD, CK, AMS and ACE in the untreated diabetics before the start of the treatment, when compared with those of normal healthy controls?
Answer: The activities were similar. This is now presented in the manuscript.

2. Whether the leaf decoction of Bauhinia forficata is hypoglycemic in normal healthy controls? If it is not so, is the toxicity of B. forficata studied in normal healthy animals?
Answer: No, the leaf decoction of *Bauhinia forficata* is not hypoglycemic in normal healthy controls, as can be verified in: Pepato MT, Keller AM, Baviera AM, Kettelhut IC, Vendramini RC, Brunetti IL: Anti-diabetic activity of *Bauhinia forficata* decoction in streptozotocin-diabetic rats. *J Ethnopharmacol* 2002, 81: 191-197.
The toxicity of *B. forficata* in normal rats was studied in normal healthy animals and the results are presented in the revised manuscript.

3. The changes in the body weights of both groups of rats during and at the end of the treatment are not given. The body weight changes correlate with the control of diabetes and other effects of the plant product.
Answer: Now the body weights of all groups, normal (treated and untreated) and diabetic (treated and untreated), of rats during and at the end of the treatment have been included. Comments relating to these weights can be found in Methods, Sections: Animals and their treatment and Decoction administration; in Results, last para; in Discussion, page 11, para. 2 and in Figure 6.

4. The discussion part of the paper does not contain any references on the toxic effects of the phytochemical constituents of the plants. e.g.Garg etal.(Vet Hum Toxicol.1992) reported nephro- and hepatotoxicity with tannins in cattle.
Answer: This was added in Discussion, page 11, para 3.