Reviewer's report

Title: The Juice of Fresh Leaves of Catharanthus roseus Linn. Reduces Blood Glucose in Normal and Alloxan Diabetic Rabbits

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Reviewer: Mt T Pepato

Level of interest: A paper whose findings are important to those with closely related research interests

Advice on publication: Accept after discretionary revisions

1) Comments: This is a paper which investigates the effect of oral treatment of normal and alloxan-diabetic rabbits with the juice of fresh Catharanthus roseus leaves on their blood-glucose levels over a period of 24 h. Some further clarification, principally on the fasting of the animals, would be a valuable addition to the paper, to strengthen the data for its important findings. The importance of the paper lies in the more prolonged (in normal animals) and potent (in diabetic animals) reduction in glycemia achieved with the juice, in comparison with a dose of glibenclamide. These results apparently support traditional usage and justify further study of this plant. As far as I am aware, the content of this paper is not duplicated elsewhere and this work is not too small an advance on previous work from the same group, nor does it need to be made part of a more substantial follow-up.

Taking into consideration the 7 items in the Guidelines for referees of your Journal, I would like to mention the following:

a) Discretionary revisions:
- Perhaps in future work, owing to hormonal changes that occur in female, it would be advisable to use males alone in the experiments, and not a mixed sex group as in this study.
- The authors mention that C. roseus has various names in different places - it may be of interest to give the name used by English-speaking growers: 'Madagascar periwinkle' ('The Plant Book' by D J Mabberley (2nd edition, Cambridge Univ Press, 1997)

b) Compulsory revisions:
Background to Abstract
1st sentence: the authors mention that C. roseus is used in folk medicine all over the world. This sentence should include for what this plant is used (diabetes...? other conditions..?)

Background
1- Referring to the 1st sentence: "Diabetes mellitus .... 10% .... over the World"; this statement should have a supporting reference.
2- Second paragraph: "hot water decoction" is tautologous, as water decoction is always done with hot water; thus, this phrase should be simply "water decoction".

Material and Methods
Plant material
1- Add the serial number of the voucher specimen that was kept in the herbarium.
2- The words "of our Department" at the end of the 2nd sentence suggest that the Department of Pharmaceutical Sciences has its own herbarium, even though the authenticity was checked in Dept of Botany. If the herbarium is in the latter, this should read "of the Botany Department" or "of our University" (if only one exists).

Animal Experiments
1- For what reason were the rabbits fasted for so long a time, ie. 18h + 24h (total of 42h)? Isn't this rather a long period of fasting, which in itself might lead to a state of hypoglycemia? Also, why 24h without water?
The motive for this procedure must be explained in the text.
2- Although most articles use the term 'oral administration' by itself, it would be more correct, to allow readers to reproduce the method in other laboratories, to specify it more precisely - was a gastric tube (gavagem) used, or what, and was the volume 3 mL?
3- In the 2nd paragraph, it should be stated that groups I to IV consist of normal animals, as well as the normal control, group V.
4- It should be stated whether glibenclamide was administered by the same route as the leaf juice (and in the same volume).
5- The dose of glibenclamide given to the normal animals was the same as that given to the diabetic ones, ie. 40 mg/kg body weight. Considering that animals fasted for periods up to 42 hours, did this dose not provoke severe hypoglycemia, and in consequence some deaths among the rabbits? The authors should comment on these points.
6- 1st sentence of the 3rd paragraph: please add the mean value for blood glucose found in groups VI to X, before alloxan was administered to them.
7- Last paragraph: please add the route(s) by which C. roseus was given to animals in groups VI, VII and VIII, and glibenclamide was given to group IX.

Collection of blood and analytical procedure
1- The total volume of blood collected in samples should be added to the text, together with confirmation that it was within the allowed volume, for the sampling not to affect the readings.

Results
1- 1st paragraph: There is a mistake in the value given for the 20 hour peak in the normal group (value in the text does not coincide with that in the table). Please correct this error.
2- 2nd paragraph: "The percent reduction in blood glucose was higher in the diabetic condition compared to the normal state". Was this reduction significantly higher? If so, this fact should be added, and if the statistical test has not been done, it needs to be carried out in order to maintain the above sentence in its present form, otherwise it should read ".....there was a tendency for reduction in blood glucose to be higher in the diabetic........" or "....reduction in blood glucose tended to be higher ............" etc.
Such results as these (Table 2), as well as the fact that the juice had a more prolonged effect (at 1.0 ml/kg) than the glibenclamide dose in the period 18-24 h after treatment (Table 1), could be highlighted and commented on in the Discussion section.
3- The initial mean concentration of blood glucose in each group (before administration of C. roseus, glibenclamide or water) needs to be provided, either in the text or the tables.

Discussion
1- A reference should be provided for the statement in para. 3, line 4....."and these compounds are active in mild alloxan-induced diabetes, whereas they are inactive in intense alloxan diabetes (nearly
all b-cells have been destroyed)".
2-Regarding the validity of the following claim: "Since our results showed that glibenclamide reduced blood glucose levels in hyperglycemic animals, the state of diabetes was not severe", as well as the comments immediately following this, it is crucial to show that the mean initial glucose levels (before glibenclamide was given) in the glibenclamide-treated groups were close to those in juice-treated groups.

References
The norms of the Journal should be followed with respect to the author-names, with the surname first and then the initials. Also, the first digit in page numbers should not be repeated (ex: 243-275 should be 243-75). Also, several book references did not follow the norms - please examine the norms carefully.

**Competing interests:**

None declared.