Author's response to reviews

Title: Evaluation of Hypoglycemic Potential of Tridax procumbens (Linn.)

Authors:

   Hemant Pareek (hemantipr@yahoo.com)
   Sameer Sharma (sameer2592474@yahoo.co.in)
   Balvant S Khajja (balvantsingh@yahoo.co.in)
   Kusum Jain (kusum236974@yahoo.co.in)
   GC Jain (jain-gc@uniraj.ernet.in)

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Author's response to reviews: see over
Reviewer's report

Title: Evaluation of Hypoglycemic Potential of Tridax procumbens (Linn.)

Version: 1 Date: 8 January 2009

Reviewer: Karl WK Tsim

Reviewer’s report:
In this manuscript, the anti-diabetes activity of Tridax procumbens was investigated. Diabetic rat model was employed in the study. The oral administration of the extract from the whole plant exhibited hypoglycemic activities including reducing fasting blood glucose level, increasing oral glucose tolerance and correcting body weight. No acute toxicity was found in the study. The data shown in this manuscript were convincing, however, there are still several aspects need to be improved before publication.

Major comments:

1. Since the traditionally, the leaf powder is used for treating diabetes, but in this study, 50% methanol extract of the whole plant was employed. The authors need to explain the rational of this choice of the materials.

   The rationale behind using whole plant was based on the literature where in per reference no. 13 ‘the effect of various extracts (whole plant extract, aqueous extract, butanol extract and ether fraction) of this plant has been studied in dead space wound models. Compared to various extracts, the whole plant extract has the greatest pro-healing activity with increase in tensile strength and lysyl oxidase activity. Aqueous extract was also effective in increasing lysyl oxidase but to a lesser degree (Udupa et al, 1991). We did so to determine whether T. procumbens (which is a small herb) completely possesses the anti-diabetic properties or not, so that in further studies we may take different plant parts and subsequently can go for active component. No information is available about the current use of this plant extract in folk medicine in diabetic cases, but In Literature there are reports that T. procumbens have flavanoids contents therefore we used methanolic extract so that the extract may contain maximum amount of compounds including flavanoids

2. In the methods as on page 4, it would be better to provide the age of the medicinal herb used.

   The plant is a native of tropical America and naturalized in tropical Africa, Australia and Asia including India, but it has been introduced to tropical, subtropical, and mild temperate regions worldwide. The plant is used by tribes/villagers to arrest bleeding from cuts and bruises in animals since time immemorial.

3. In the result part, at the very beginning, it would be better to describe the physical condition of the animal model used.

   The physical conditions of the animal model is described in Methods page no. 5 under heading of ‘Experimental animals’
4. All the data in this manuscript were shown in table format; however, it would be better to include graphs for the convenience of readers. The data shown in tabular format so related field scientific people may get the accurate information, however we understand that graphical representation is better for understanding the effects.

5. In the background part in page 3, it was mentioned that the limitation of the conventional treatment of diabetes using sulfonylureas and biguanids, such as side effect and fail to significantly alter the complications. Therefore, it would be necessary to include the potential activity of this medicinal herb against the complications of diabetes. The authors cannot comment upon the necessity of inclusion of this plant, unless it is thoroughly examined for Anti-diabetic effects.

**Level of interest:** An article whose findings are important to those with closely related research interests

**Quality of written English:** Acceptable

**Statistical review:** Yes, but I do not feel adequately qualified to assess the statistics.

**Declaration of competing interests:** 'I declare that I have no competing interests'

**Title**
The author should modify the title. It should be: “Evaluation of hypoglycemic and anti-hyperglycemic potential of Tridax procumbens”

The title has been modified as suggested and now it shall read as: “Evaluation of hypoglycemic and anti-hyperglycemic potential of Tridax procumbens”

**Material and methods:**

The specimen voucher number of the plant must be given.
The specimen voucher number of the plant has now been provided in the manuscript i.e. RUBL 20534

The ONE TOUCH glucometer is not accurate mainly for research purpose.
The only prudent way for analysis of blood glucose in small samples was to use glucometer as in live rat it is very difficult to get adequate quantity of sample. Repetitive estimation using method based on Auto-analyzer & other methods often used in research would require relatively high quantity of blood sample. In ONE TOUCH glucometer this
can be performed with a very small sample and in few papers we have observed researchers using ONE TOUCH glucometer, therefore we used this instrument.

**What was your motivation to choose two doses (250 and 500 mg/kg)? An explanation needs to be given why these concentrations were used. How do these concentrations relate to traditional use?**

The rationale behind using these two concentrations was to determine whether doubling the quantity of drug affects the pattern of glucose reduction or the overdosing can have hypoglycemic effects. The 250mg/kg dose was selected on the basis of information available in the literature i.e. at this dose the plant extract is reported to have wound healing activity.

**Which extract is currently used in folk medicine? Is it the methanolic extract? Or aqueous extract?**

No information is available about the current use of this plant extract in folk medicine in diabetic cases, but in literature there are reports that T. procumbens have flavanoids contents therefore we used methanolic extract so that the extract may contain maximum amount of compounds including flavanoids.

**In toxicity the author should determine the LD50 (Lethal dose 50). For justify doses used, the author must provide references. The World Health Organization request to use doses up to 5 g / kg. The number of animals used, the sex, in acute toxicity and the parameters studied are not significant to conclude that the extract is not toxic.**

The authors have not determined the LD50, however in reference no. 12 (Diwan et. al.) the LD50 was calculated and they have also found that at 2g/kg b.wt. no sign of toxicity was visible.

**Which method (or apparatus) did the authors use to evaluate the behavioural parameters (grooming, mood, hyperactivity, sedation, loss of righting reflex, respiratory rate and convolution).**

The animals were observed continuously in normal/standard laboratory conditions i.e. in cages in animal house itself for the initial 4h and intermittently for the next 6h and then again for 24h and 48h after the drug administration. Only visible symptoms were observed, no special apparatus was used.

**Results and Discussion:**

**In table 1, the reference compound should be included**

The correction is made as suggested

**Table 2: The normal control values should be included**

The correction is made as suggested

**In table 3, the statistical analysis should be done between the initial (0d) and the final (30d) values.**

The correction is made as suggested
Table 4: The authors have to explain how the percentage of glucose reduction is not dose-dependent? Is it as compared to initial values or to values 7 days before?
The correction is made as suggested

In table 5, the statistical analysis should be done with the diabetic control value at 120 min
The correction is made as suggested

In discussion the author said that: “the increase in body weight in diabetic rats can be attributed to the improvement in insulin secretion”. But probably to the insulin like effect of the plant extract.
The correction is made as suggested