Reviewer's report

Title: Acute and repeated dose 28 day oral toxicity studies of a Siddha medicine Nuna Kadugu in Sprague Dawely rats

Version: 5 Date: 19 June 2012

Reviewer: Nicola Stagg

Reviewer's report:

When I did my first review of the following manuscript in February, I provided comments/questions in the comment section to the editors online and attached an edited version of the manuscript. In my second review of the manuscript, I noted that they had not addressed any of the comments/questions I had listed. They only edited the manuscript based on the attached edited version that I provided. I requested that they specifically address my comments.

---------------------------------------

Referee 1’s first review for the manuscript in February:

The manuscript submitted by Ramaswamy et al., entitled "Acute and Repeated dose 28-day oral toxicity studies of a Siddha medicine "Nuna Kadugu" in Sprague Dawley rats" presents the results of acute and 28-day repeated oral rodent toxicity studies to evaluate human safety of a herbal medicine, Nuna Kadugu used for the treatment of vitiligo.

The acute oral and 28-day toxicity studies were OECD guideline compliant and were a good choice for assessing potential human safety to NK. The data was presented well. I have a few points to consider in the revision.

1. The limit dose of 2000 mg/kg bw was appropriate for the acute oral, but I'd suggest including in the discussion how this dose compares to human exposure. The fact that you saw no toxicity at this acute high dose is a great finding, but what does it mean to human exposure if they are prescribed 3 g/day. You could report it as margin of exposure. I'm not sure there is any value in presenting the classification, but if you choose to keep it in I would suggest emphasizing that cat V represents the lowest category of toxicity.

2. It was unclear how the dose levels were selected for the 28-day repeated dose study. It is stated that the rat dose of 270 mg/kg was arrived from the human dose based on body surface area conversion - Freireich et al. 1996. I think more detail needs to be provided to justify this. Typically for OECD studies, a 100 fold safety factor would be applied to convert human exposure to rodent exposure - 10 fold for rodent to human variability and 10 fold for variability across humans (10 X 10 = 100). I would be interested to see how the dose levels compare using the two approaches.

3. Including a satellite group was a good idea, but the main purpose of doing this
should be to evaluate reversibility and that wasn’t mentioned. You didn’t see any toxicity after 28-days anyway, but satellite groups that don’t get vehicle or treatment are included for evaluating reversibility mainly.

4. What is the mode of action for the efficacy of NK for vitilago? That’s not mentioned at all and that should lead you to focus on what a potential mode of action for toxicity might be. Does it target the immune system? If that’s the case, then you might emphasize the evaluate of the thymus and spleen for any effects as well as the hematology results.

5. It wasn’t clear that the results would be presented as male and female combined but all the tables just so single endpoints/dose/parameter

6. I think there needs to be some rewording throughout the manuscript. The sentences need to be succinct and the grammar needs to be improved.
   i.e. abstract paragraph 1 - first sentence is not a complete sentence. Morinda Pubescens (Family: Rubiacaeae) IS commonly KNOWN as "Nuna" in Tamily. Please see edits on attached pdf

7. Were no pvalues less than 0.05 observed. It should be stated on all figures and tables that statistics of pvalue less than 0.05 were evaluated.