Author's response to reviews

Title: Acute toxicity and the 28-day repeated dose study of a Siddha medicine Nuna Kadugu in rats

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Version: 6 Date: 31 July 2012

Author's response to reviews: see over
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To
The Editor-in-Chief
BMC Complementary and Alternative Medicine.

Dear Sir/Madam,

Subject: Manuscript entitled “Acute and repeated dose 28-day oral toxicity studies of a Siddha medicine “Nuna Kadugu” in Sprague Dawely rats” - Submission of revised manuscript – Regarding

Herewith we are submitting the revised form of the manuscript entitled “Acute and repeated dose 28-day oral toxicity studies of a Siddha medicine “Nuna Kadugu” in Sprague Dawely rats” with a new title “Acute toxicity and the 28-day repeated dose study of a Siddha medicine “Nuna Kadugu” to be considered for publication in BMC Complementary and Alternative Medicine. We had made appropriate changes as suggested by the reviewers. The response to reviewers comments is appended with the covering letter.

With the submission of this revised manuscript I, as corresponding author oblige to state that the above mentioned manuscript has not been published elsewhere or accepted for publication elsewhere or under editorial review for publication elsewhere and also declare no conflicts of interest amid the authors. Animal experimental protocol was approved by Institutional Animal Ethical Committee (IAEC), Sri Ramachandra University, Chennai, India and the guidelines of “Guide for the Care and Use of Laboratory Animals” (Institute of Laboratory Animal Resources, National Academic Press 1996; NIH publication number #85-23, revised 1996) were strictly followed in animal handling and care.
Looking forward for your favorable consideration

Thanking you,
Yours Sincerely,
Ramaswamy
(Corresponding author)
Response to Reviewer Comments

Reviewer's report

1. Reviewer: Akihiro Hagiwara

Comments to Author:

The introduction and discussion sections are well described, but the materials and methods section and results section should be precisely noted.

1. P1, Title Typographical error “Dawely” should be corrected.

Author response: As suggested by one of the reviewer, now title has been changed to “Acute toxicity and the 28-day repeated dose study of a Siddha medicine “Nuna Kadugu” in rats”.

2. P2L14. (Abstract-Methods section) “at 0.3, 1, 4, 24 h…” should be “0.5, 1, 4, 24 h…”

Author response: As per the reviewer comment, the corrections were made in the manuscript.

3. P2L19-21. (Abstract-Results section) - This sentence “There were no gross...” need to improvement.

Author response: As per the reviewer comment, the sentence was modified in the manuscript.

4. P6L12-15 (section 2.5. Experimental animals - Husbandry) Room temperature and relative humidity should be noted for values recorded during the course of study, but not target values set for the study.

Author response: We performed and maintained the Room temperature and relative humidity for repeated dose (28 day) study as per the OECD 407 guidelines. In the guidelines, it was given that the temperature in the experimental animal room should be 22°C (± 3°C) and relative humidity should be 30% - 70%.
5. P8L27-29 (section 2.8. Histopathology)

Please note clearly that histopathological examination were not done for any organ in animals of low and mid dose groups, since no treatment-related changes were found in the highest dose.

Author response: As per the OECD 407 guideline, full histopathology should be carried out on the preserved organs and tissues of all animals in the control and high dose groups. These examinations should be extended to animals of all other dose groups, if treatment-related changes are observed in high dose group. In our study, we performed hisopathological examinations in high dose group and there were no treatment related changes. Hence we didn't performed histopathology examination of low and mid dose groups.

As per the reviewer comment, the sentence was clearly explained in the manuscript.

6. P6L12-15 (section 2.5. Experimental animals - husbandry:, and P7L17, 18) There was a discrepancy that animals were housed in groups (3-5/cage)(refer P6L12-15), but animals were housed individually (refer P7L17, 18).

Author response: Actually, we were housed the animals individually. The sentence was now modified in the manuscript.

4. P16, Table 1a Need explanation of “S.No”, “NK”, and “-” ~ “+++”.

Author response: As per the reviewer comment, we included foot note in the Table 1a.

5. P18~20, Tables 2 – 4. Unit (g, g/rat or g/rat/day etc) of each parameter should be noted

Author response: As per the reviewer comment, we included the units with explanation in Table 2-4.
2. Reviewer: Nicola Stagg

Reviewer's report:

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.

Author response: In Siddha practice, 3g/day of NK was a human efficacy dose it’s not a rat dose if this human dose is converted to rat dose it will be < 300mg/kg. There is no exposure of NK greater than 1000 mg/kg for rat even for an efficacy study. Hence we followed the category V in 423 guideline for the 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.

Author response: A 28-day repeated oral toxicity study was performed according to the OECD guideline, TG 407 (Revised - 18 December 2007) with minor modifications. In Siddha practice, 3g/day of NK was recommended for the treatment of vitiligo in adult humans. The rat dose of 270mg/kg was arrived from the human (3g/day) dose based on body surface area conversion. From that dose we did two higher dose levels. Hence NK was administered at three dose levels i.e., at 300, 600 and 900 mg/kg/day.
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.

Author response: In our study, we used NK satellite group to check both reversibility and delayed onset of toxicity. We observed the animals throughout the study for any signs of toxicity (till 42 days) and mentioned in the manuscript. But there were no signs of toxicity noted in high dose NK (28 days) as well as high dose NK satellite group (42 days), it can be inferred that NK will not produce delayed onset of toxicity.

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.

Author response: Thank you for your valuable comments, Traditionally Nuna kadugu are used for the treatment of skin diseases there were no report for its mode of action. In continuation of toxicity studies we are also planned to execute the efficacy studies in the animal models to identify its mode of action.

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

Author response: As per reviewer suggestions, the values for male, female and combined are now included separately in the manuscript of all the Table.

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: Rubiciaceae) IS commonly KNOWN as "Nuna" in Tamily. Please see edits on attached pdf

Author response: As per the reviewer suggestion, now we reworded the sentences in the manuscript and made all the corrections in the 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.

Author response: As per the reviewer suggestion, we changed the note in the manuscript.