Reviewer's report

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

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Reviewer: Akihiro Hagiwara

Reviewer's report:

Comments to Author:

Author: Ramaswamy, R.S.. et al.

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

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

1. P8L11-12&L26-29 (section 2.7. Repeated dose 28-day toxicity study).
   This study was performed according to the OECD guideline, TG 407. However, functional observations should be conducted in the fourth exposure week was not described in the present study.

2. P10L19-20 (section 3.2. Acute oral toxicity study) and Fig.2.
   The term of “…body weight gain …” should be corrected to “…body weight change…”, since body weight curves were presented in Fig.2.

3. P10L28-29 (section 3.3. Repeated oral toxicity study:) and Table 2
   The term of “…body weight gain …” should be corrected to “…body weight change…”, since only average body weights ± SEM, but not body weight gain, were presented in Table.2.

   Body weight data at commencement (Week 0) should be noted in Table 2. Body weight retardations were apparent in 600 mg/kg/day from week 1 to 4, and in 900 mg/kg/day from week 1 and 2. No statistical significance? Is there any reason?

   Please delete all data concerning MF (n=10) in Table 2, because of no meaning of combination. In footnote, a sentence of “Significance with…..and **P<0.01 vs control group” should be deleted, since no significant change was appeared in Table 2.

4. P10L29-P11L1 (section 3.3. Repeated oral toxicity study:) and Tables 3 & 4
   Statistical analysis could not be performed for food and water consumption data, because sample size of each group was one (animals were housed in groups of 3-5/cage)(refer P7L12-15). How did you calculate average and SEM? Please note in footnote accurately, such as n=1 cage of 5 animals per group.

5. P11L1-4 (section 3.3. Repeated oral toxicity study:) and Table 5
The description cited a reference “…were found to be well within the clinical range of rats [18] in …” should be moved to Discussion section. In this section, results of statistical significant parameters should be noted clearly.

6. P11L4-11 (section 3.3. Repeated oral toxicity study:) and Tables 6 and 7
   Same as comment #5.

7. P11L14-16 (section 3.3. Repeated oral toxicity study:) and Table 9
   Table 9 should be deleted, since no histopathological alteration was found in any animal/sex/group.

8. P11L16-19 (section 3.3. Repeated oral toxicity study:)
   A sentence of “Representative histopathological…(Fig not shown).” should be deleted, because of no meaning. However, histopathological findings of control and treated animals killed at recovery phase should be noted.

9. Tables 2~8
   Data should be tabulated for males, and then for females, separately, because comparison between groups must be done for each gender. Gender is the important factor to evaluate the data. Please delete all data concerning MF (n=10), because of no meaning. In footnote, a sentence of “Significance with…..and **P<0.01 vs control group” should be deleted, since no significant change was appeared in the table.

**Level of interest:** An article of limited interest

**Quality of written English:** Acceptable

**Statistical review:** No, the manuscript does not need to be seen by a statistician.