

Review of: "Toxicological evaluation of aqueous extracts of Clematis hirsuta and Rhamnus prinoides"

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Potential competing interests: No potential competing interests to declare.

After evaluating the manuscript, I don't think that the protocol used in this manuscript was well prepared. I suggest adding the missing information then submit the manuscript.

- The first question that came to my mind is whether the authors performed the LD50 study or not. The authors
 mentioned at page 7 that the LD50 of the extracts were ≥ 2000 mg/kg. The authors should mention the procedure of
 determining LD50 in details and the exact LD50 for each extract.
- 2. Based on the results of LD50, the doses that were used for conducting the experiments (25, 75 and 225 mg/kg) are not properly selected. It would be good if the authors used 75, 150 and 225 mg/kg.
- 3. The discussion is short. In order to make convincing discussion, the extracts of the two plants should be analysed using LCMS. The components of the plant extracts differ based on several factors including the country, so it is important to let the readers know the components of the extract being grown in this country. In addition, not all the analyzed parameters were discussed in the discussion part.
- 4. Why did the authors use different parameters to be measured in the acute and sub-acute studies?. For instance, analysing haematological and biochemical parameters in sub-acute but not acute study.
- 5. The experimental procedure should be written in detail. It is not written whether the animals got the extract in drinking bottles continuously in all the days or through the oral gavage and what is the timing of providing the extracts to the animals.

Suggestions to improve the manuscript:

- 1. The authors should write the name of the plants italic in the title.
- 2. The authors should write the name of plants in the same way and as abbreviated *Ç. hirsute, R. prinoides*) in all the manuscript except in the first time of the appearance of these words in the abstract and the introduction.
- 3. It is better to delete the details of statistical analysis from the abstract. Specifically, the sentences (The data from the acute toxicity study was summarized as mean±standard deviation and analyzed using the unpaired Student's t-test. The data from the subacute toxicity study was summarized as mean ±standard deviation and analyzed using Two Way ANOVA and Tukey's post hoc test. The significance level was set at p≤0.05).
- 4. The authors should use one Y scale in Figures 3A and 3B.
- 5. The authors should write the words in the same way in all the manuscript. For instance, either subacute or sub-acute.
- 6. No need to keep repeating untreated (control) rats in some pages of the manuscript. The authors can write it at the



beginning then use the word control group in all the manuscript.

- 7. The authors should use the phrase treated group instead of treatment group. Also, it is better to use the phrase treated rats or treated group instead of using treatment group rats.
- 8. Replace the sentence [provided fifty-six 8-10-week-old female *Wistar* rats] with [provided 8-10-week-old female *Wistar* rats (N=56)].
- 9. Use the same way of writing the doses in all the manuscript. For instance, consider making spaces between the numbers and the unit when writing the doses (e.g. 25 mg/kg and not 25mg/kg).
- 10. Replace the sentence (Toxicology data on *C. hirsuta* presented in the current study was a mixed bag) with (Toxicology data on *C. hirsuta* presented in the current study was mixed).
- 11. Replace the sentence (hotly debated topic) with (debated topic).
- 12. Replace the sentence (and to attract pollinators) with (and for attracting pollinators).
- 13. When explaining the results of table 1 in the text (at page 9), put the abbreviations of the analyzed parameters in the text to be similar to the way of explaining the parameters for table 2 (at page 10).
- 14. Replace the sentence [Secondary metabolites, which plants use for defense] with [Secondary metabolites that the plants use for defense].

Good Luck

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