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.

Preliminary observations: The article discusses the toxicological evaluation of the aqueous extracts of*Clematis hirsuta* and *Rhamnus prinoides*, which are traditionally used in Africa. While clematis is used for inflammation and pain management, the latter is used for various conditions such as respiratory and gastrointestinal tract infections.

Overall comments:

Impactful comments: (Mandatory to make revisions before consideration for publication):

1. The title needs revision: the title should include the species, organ (of the plant) used, and animal species used, together with the studies conducted. I have provided a title example below, which the authors can consider. But if they can develop a better one, that should also be fine.
    1. Acute and sub-acute toxicity evaluation of aqueous extracts of*Clematis hirsuta* (leaves) and *Rhamnus prinoides* (roots) in Sprague-Dawley rats.
2. It is important to insist that the information provided in the article needs to be more specific. There needs to be more clarity on the following (These details are required in the article during revision).
    1. From the Materials section, I can assume that the plant's leaves and roots (for the latter) were used to prepare the aqueous extract(s). however, in the other sections, it is specified as materials. Were there any other plant organs which were used for evaluation? Kindly replace the word "materials" with a specific organ name, as there could be confusion.
    2. If the aqueous extract was made of a mixture of both plant leaves/roots together (if both species leaves/parts were mixed ratio of mixing is required).
    3. Or the aqueous extract was prepared individually for both the leaves and separately administered to the animal in different groups.
    4. Or, after individual extraction, they were mixed at a certain ratio and administered to the animal groups.
    5. Or they were individually administered to the animal in groups (ratio not required in this case).
    6. Because of the above points specified, I cannot conclude if the LD50 and the NOEL/NOAEL of the subacute toxicity study are for the mixture of extracts or the individual ones. I do not see a NOAEL value specified. Please establish a NOAEL/NOEL value based on the parameters evaluated.

7. Table 1 lists out values of 6 groups, from which I can understand that the groups are dosed individually with extracts and not mixtures in ratios. Please add a table showing the doses and the subsequent group tested accordingly. I have provided an example below. (List all the groups with the reespective dose and the plant species against the respective dose groups.

Dose groups		
Group 1	XXX dose	Plant species name
Group II	XXX dose	

1. From the above point, are the LD50 values also for individual extracts? Kindly add a statement that the LD50 values are individual if that is the case.
2. Kindly include the guidelines (Guideline numbers) used for the up-and-down method and subacute toxicity studies. There are OECD guidelines for both of these endpoints.
3. In conclusion, please add that "Further evaluations are required to evaluate the effect of the extract on major organs" (or a similar but more detailed statement is required).
4. The key conclusion is to include the LD50 values and the NOEL values of the extracts individually. Please specify the values in the conclusion section. (On priority).
5. Kindly look into the language of the manuscript on priority. Some significant revisions are required in using prepositions and the manuscript's language construct. Please use Grammarly or some software to correct these.
6. There are many mismatches in the space provided between words. In certain places, it is given as "from*Clematis flammula*" (without space), whereas it has to be "from *Clematis flammula*" (with space). Kindly correct these mismatches (Supported by Grammarly).

Information comments: (Not so mandatory but good to have) (please note that these responses will not affect the consideration for acceptance):

1. I notice that the authors have used the up-and-down method for calculating the LD50 of the extract. Kindly mention the guideline number OECD 423 as an up-and-down procedure similar to the guideline. I have listed a few concerns below (I assume this must have happened because of restricted funding and the consideration of not wasting animals).

    1. As per OECD Guideline no 423 (up-and-down method) for acute toxicity to calculate the LD50, if you start the study at 2000 mg/kg as the limit dose when no deaths are seen, why was the testing for 5000 mg/kg NOT done?
    2. The value >2000 mg/kg is acceptable because 5000 mg/kg is higher than 2000 mg/kg. However, testing at 5000 mg/kg could have been of better help in deciding the high dose in the subacute toxicity study, where the high-dose could have been 500 mg/kg (unlike 225 mg/kg tested in the subacute toxicity study) if there were no deaths at LD50 5000 mg/kg.
    3. Given that this is traditional medicine and there are high chances of overdosing without a physician's attention, it is important to evaluate it to the highest threshold possible to ensure no toxicity is seen. Kindly adapt these strategies

in the upcoming evaluations.

Overall Impression: The work is a albeit primitive, is a novel effort to ensure the safety of the end consumers who use this traditional medicine daily/whenever required. However, significant research and effort should go into designing the study (specifically regarding the toxicity aspects of the extract). While I understand that the study reactions seen could have been because of restricted funding. Yet, the authors have done a good job of evaluating the extract's safety by assessing the biochemical parameters. In the future, the work will expand onto evaluating the critical effects on the major organs.

Final conclusion: The article requires significant revision (as suggested in the above points) and can be accepted for submission after the recommended revisions. I would like to look at the manuscript after revision.