

# 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.

The paper is undoubtedly needed. Summarizing graphs/charts are clear.

However, some aspects should be significantly improved.

== Abstract ==

If you state here, "The extracts reduced feed and water consumption in rats but caused no physical signs of toxicity or death, nor did they have any significant effects on weight, biochemical, or hematological parameters when compared to controls," it is worth showing the doses and extract concentrations to rapidly judge if the data are or are not attractive to the reader.

== Background ==

It is worth presenting the current knowledge on the chemical composition and its implications on the expected actions/toxicity of analyzed plants.

== Methods ==

The following parts should be additionally explained:

2.1. Bioethical Commission acceptance scan should be attached as Supplementary Data.

2.3. If the plants were identified, plant names should be reported, at least here, with botanical extensions/ author citations (e.g., *Rhamnus prinoides* L'Hér.). Providing the synonyms should increase the traceability of this paper.

2.4. The amounts of plant material / produced extracts are unknown. These should be added.

2.4. How often were the extracts prepared / re-warmed from the frozen, pending portion? The stability of the infusion should be separately proved. See later remarks in the Discussion.

2.5. The cage area and number of rats per cage (1/cage or more?) should be defined.

2.6.-2.7. The strategy to control the effectiveness of dose delivery per rat is unclear.

2.6.-2.7. Results of postmortem histopathological analysis should be measured and provided.

There is no detailed information on the blood aspiration and hematological parameters testing strategy in Methods. Why were the hematological parameters not evaluated in the acute toxicity assay?

To summarize, according to described conditions, the experiment is hard to be repeated in an unrelated laboratory.

== Results ==

Table 1. The value for LYM/RP/225 seems unnoticed.

Table 2. The values for Na<sup>+</sup>/K<sup>+</sup> may be affected by extract constituents. Does it not seem significant for prolonged use of *Clematis hirsuta* leaves and *Rhamnus prinoides* roots?

== Discussion ==

Quoting literature LD50 values without providing DERs/DSRs of the extracts is pointless. It is much better to prepare the interpretation for the readers + reference.

The Discussion is at least partial. If in the treated group, there is a lower feed and water consumption compared to the control, while the weight is gaining + there is a tendency to disturb the K<sup>+</sup>/Na<sup>+</sup> balance - there are some explanations. One is the water retention in the body due to sodium retention. Thus, the following questions must be answered:

- How were the daily effective dosages of water and feed measured?
- Why the frequency/volumes of urination were not checked during the study?
- Why the frequency/amounts of defecation were not checked during the study?

The average value for LYM/RP/225, 10-fold higher than for control, seems unnoticed and uninterpreted.

Considering the possible changes in extract composition, at least the following questions must be answered:

Was it effectively proved that there were no changes in the composition of the extract when freezing and re-warming extracts made with boiling water before the animal assay?

What changes in transparency/color of extracts were observed while re-warming from frozen, pending portions?

What changes in the transparency/color of extracts were observed during the watering period when extracts were stored at room temperature?

Finally, what was the initial and final composition of extracts using LC methods?

*Rhamnus prinoides* is known to be used as provided but is also known to contain laxative anthraquinones like other members of the *Rhamnaceae* family (doi: 10.3390/ph13040055). The question is if the amounts in such root extract are significant enough to produce this and other Na<sup>+</sup>/K<sup>+</sup> imbalance effects. This level can be checked before the animal assay. On the other hand, the results obtained for its leaves' extracts, containing probably also tannins were opposite (doi:

10.1101/2022.09.19.508518).

== Conclusions ==

In light of the above findings and remarks, I would not state the same as the Authors. Yes, there were no sudden deaths, but that is not all. If not every result is easy to be interpreted, it should be stated.

== Funding statement ==

OK, if no external funds were received. However, all experiments have value; thus, suitable info should also be added if conducted using normal-way spending of statutory funds of the entities employing the Authors.

== References ==

O. Guideline, F.O.R. Testing, and O.F. Chemicals - quoting these names, some twice, does not assure the reader of the Authors' scientific prowess.