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.

Reviewer comments

- Mistake correction:

Part/ Section	Correct from.	To.
Title	Toxicological evaluation of aqueous extracts of Clematis hirsuta and Rhamnus prinoides	Toxicological evaluation of aqueous extracts of *Clematis* *hirsuta* and *Rhamnus prinoides*
Abstract, Keywords, (and all over the methodology section)	*Wistar*	Wistar
Abstract (and all over the manuscript)	*p≤0.05*	*p<0.05*
Background (and all over the manuscript)	of*C . hirsuta*	of *C. hirsuta*
Background (and all over the manuscript)	respectively	, respectively
Results	between*C . hirsuta*	between *C. hirsuta*

- **In addition to:**

1. Methods:

1.1. It is preferred to rename this section (from "Methods" to "Materials and Methods").

1.2. The reference and method of shade drying should be explained in details, i.e. the temperature, the surface, etc.

1.3. In Methods subsection 2.6. Acute toxicity, 21 rats were used and divided equally into 3 groups. Thus, each group should contain 7 rats (not 5 rats as the authors mentioned).

1.4. In Methods subsection 2.6. Acute toxicity, authors should mention that animals were well observed during the first 24 hours, as well as the 14 days of the study for any signs of acute toxicity.

1.5. In Methods subsection 2.7. Subacute toxicity, authors should explain how the animals were treated by the end of the four-week period, fasting, anesthesia, blood sampling, whether the biochemical indices were determined in serum or plasma, and how them be collected and stored till analyzed, etc.

1.6. The method of calculating BWG%, feed and water consumption should be explained. Similarly, the methods followed for hematological and biochemical analysis should be mentioned accompanied with the references.

2. Results:

2.1. In "Results" section, page 7/15, subsection 3.2. Subacute toxicity (lines 2, 3, 4), authors mentioned "Furthermore, the weight gain in extract-treated rats was not significantly different *(p>0.05)* from the weight gain in untreated (control) rats in the second week of treatment". This comment does not agree with the results understood from figure 4 (A, B). That's because only the low and the medium doses of each extract (25 and 75 mg/kg CH and RP) did not induce significant changes compared to control group in the 2nd week of treatment, while the high dose (225 mg/kg) caused significant increase.

2.2. In "Results" section, page 7/15, subsection 3.2. Subacute toxicity (lines 6, 7), authors mentioned "*C. hirsuta* aqueous leaf extract-treated rats gained significantly more weight than untreated (control) rats in the fourth week of treatment *(p=0.0003, p<0.0001,* and *p=0.0004* for 25mg/kg, 75mg/kg, and 225mg/kg". This comment does not agree the results understood from figure 4 (A). That's because the p value (0.0003) does not agree with the star numbers (**) put on the column of the group treated with 25 mg CH/kg in the 4th week of treatment.

2.3. In "Results" section, page 8/15, under Fig. 4, authors are recommended to insert a caption including not only the title of the figure, but also what is the meaning of (*), (**), (***) or (****), i.e. to what it refers.

2.4. In "Results" section, page 8/15, subsection 3.2. Subacute toxicity (lines 2, 3, 4 below fig. 4), authors mentioned "Rats given 75mg/kg and 225 mg/kg of *R. prinoides* aqueous root extract gained significantly more weight than untreated (control) rats in the 3rd week of treatment *(p<0.0001,* and *p<0.0001* for 75mg/kg and 225 mg/kg respectively*)*. According to this comment, the P values are the same, while the heights and star numbers on the columns representing the two groups, in Fig. 4B, are different. There is a contrast!!

2.5. In tables (1, 2), the standard error values were markedly high for some parameters. For example, and not exclusively, MON values for RP group (225 mg/kg), EO, BAS, and PLT. The (mean± *SEM*) value of LYM for RP group (225 mg/kg) is very high compared to other groups including the control and has the same letter. How?. The letters used to express the significance of differences for some parameters in both tables are not logic (BAS, PLT, ALP, chloride, creatinine, Na, total protein, urea). Statistics should be revised.

2.6. The authors used the small letter 'a' in most parameters to refer to the lowest value and in others to refer to the highest value (as MON). The authors should use the same method in referring to the values in all tables, and this should be explained in the captions.

2.7. The abbreviations in each caption should be arranged according to their order in the tables.

3. Discussion:

The discussion already explored the significance of the work results. However, in Line 10 the authors said'In the

subacute toxicity protocol, there were no significant changes in weight gain, hematological, or biochemical parameters between *Clematis hirsuta*-treated rats and controls". This sentence disagrees with the results represented in Fig.4 (A) and tables 1, 2. For example, in Fig. 4 (A), all *Clematis hirsuta*-treated groups (25, 75 and 225 mg CH/kg), recorded significant increase in weight gain % compared to control group in the 4th week of treatment, and some of these doses induced significant effects also at the 2nd and the 3rd weeks. The same discrepancy was noticed in page 12/15 as authors said "Furthermore, there were no significant differences in weight gain, hematological, or biochemical parameters between *R. prinoides* aqueous root extract-treated rats and controls". As noticed in Fig 4 (B), all *R. prinoides* aqueous root extract-treated groups (25, 75 and 225 mg RP/kg), recorded significant increase in weight gain % compared to control group in the 4th week of treatment, and some of these doses induced significant effects also at the first three weeks. Similarly, in table 2, all *Clematis hirsuta*-treated groups (25, 75 and 225 mg CH/kg), recorded significant decrease in potassium level compared to control group. Revision is needed, and these significant subacute effects should be accounted.

4. Conclusion:

To be sure, no plant extract is safe in general. The safety of the extracts under study should be specified by the maximum dose used.

5. References: Revision is recommended since the following:

5.1. The main information of some references is not complete.

5.2. Some references are websites and are not validated.