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.

Manuscript entitled: *Toxicological evaluation of aqueous extracts of Clematis hirsuta and Rhamnus prinoides*

Comments and suggestions

Abstract

- *First sentence*

The authors should change the following sentence "***Clematis hirsuta* leaves and *Rhamnus prinoides* roots have a long history of medicinal use in Nyeri County, Kenya**" for "***Clematis hirsuta* Guill. & Perr. (Ranunculaceae) leaves and *Rhamnus prinoides* L'Hér. (Rhamnaceae) roots have a long history of medicinal use in Nyeri County, Kenya**" (In the first mention of the scientific names of the plants in the text, the authors must incorporate the person who described each species and their corresponding plant family)

- *Third sentence*

Change the sentence "**The acute and subacute toxicity of *Clematis hirsuta* aqueous leaf and *Rhamnus prinoides* aqueous root extracts in Wistar rats was investigated in this study**" for "**The acute and subacute toxicity of *C. hirsuta* aqueous leaf and *R. prinoides* aqueous root extracts in Wistar rats were investigated in this study**" (In the following mentions of each specie in the text, it is advised that the authors place the name of the specie following the next example: *C. hirsuta* and *R. prinoides*; they must abbreviate the genera name, without incorporating who described each species nor their corresponding plant family).

- *Last sentence*

Change the sentence "**These findings indicate that oral administration of *Clematis hirsuta* aqueous leaf and *Rhamnus prinoides* aqueous root extracts to Wistar rats is generally nontoxic**" for "**These findings indicate that oral administration of *C. hirsuta* aqueous leaf and *R. prinoides* aqueous root extracts to Wistar rats are generally nontoxic**."

Furthermore, I suggest incorporating more information in the section of results in the abstract because it is the most important part of the work, and it is the one that will capture the attention of the lectors. There are contents that are not relevant for the reading of the abstract, e.g., when the authors explained how the data is summarized.

Background

- *First paragraph*

When the authors mention the specie *Thevetia peruviana*, *Chenopodium ambrosioides* and *Argemone mexicana*, I suggest that in addition to the scientific names, they include who described each species and its corresponding family. Such information can be obtained from http://www.worldfloraonline.org/ or another web site.

- *Second paragraph*

I suggest changing the sentence "***Clematis hirsuta*** **is a small climbing shrub that grows to a height of 4 m in Kenya, Uganda, Tanzania, and Saudi Arabia**" for "***Clematis hirsuta*** **Guill. & Perr. is a small climbing shrub that grows to a height of 4 m in Kenya, Uganda, Tanzania, and Saudi Arabia.**"

- *Third paragraph*

I suggest changing the following sentence "***Rhamnus prinoides*** **on the other hand is a dense evergreen shrub that can grow to 7.5 m in height. It is found in India, Ethiopia, Eritrea, South Africa, Cameroon, Congo, Angola, and Kenya and belongs to the Rhamnaceae family**" for "**On the other hand, *Rhamnus prinoides* L'Hér. (Rhamnaceae) is a dense evergreen shrub that can grow up to 7.5 m in height. It is found in India, Ethiopia, Eritrea, South Africa, Cameroon, Congo, Angola, and Kenya.**"

- *Fourth paragraph*

I suggest changing the first sentence of the fourth paragraph "**Leaves and roots of *Clematis hirsuta* and *Rhamnus prinoides* respectively are used to treat type 2 diabetes in Nyeri County, Kenya**" for "***C. hirsuta*** **leaves and *R. prinoides* roots are used to treat type 2 diabetes in Nyeri County, Kenya**".

I suggest changing the third sentence "**The current study sought to determine the safety of *Clematis hirsuta* leaves and *Rhamnus prinoides* roots in Wistar rats by measuring changes in weight, biochemical, and hematological parameters**" for "**The current study sought to determine the safety of *C. hirsuta* leaves and *R. prinoides* roots in Wistar rats by measuring changes in weight, biochemical, and hematological parameters**."

Methods

- *2.2. Materials*

Changing the sentence "**Leaves of *Clematis hirsuta* and roots of *Rhamnus prinoides* were sourced from Nyeri County**" by "**Leaves of *C. hirsuta* and roots of *R. prinoides* were sourced from Nyeri County**".

- *2.3. Medicinal plant collection and identification*

For future reference, I recommend that the authors include in this section the number of the vouchers by which the

specimens were deposited at the Herbarium of the University of Nairobi

- *2.5. Experimental animals*

In this section I have a doubt. Why did they use only female rats? Why did not they determine the potential toxicity of both extracts both in male and female rats?

- *2.6. Acute toxicity*

I do not understand how many lots of animals and which doses were used for determining the acute toxicity. Did they only use the dose of 2000 mg/kg?

I would like that the authors could be more explanatory in this point.

Results

- *3.1. Acute toxicity*
- *3.1.1. Mean feed consumption*

I consider that the following sentence '**Figures 1 A and B compare the mean feed consumption in rats given 2000 mg/kg of *Clematis hirsuta* aqueous leaf extract or *Rhamnus prinoides* aqueous root extract to the mean feed consumption in rats given only distilled water**" is repetitive in the expression of the results and I suggest deleting it.

I suggest changing "**The mean feed consumption of rats in the *C. hirsuta* treatment group was significantly lower than that of rats in the control group (p=0.0222). Figure 1A. The mean feed consumption of rats in the *R. prinoides* treatment group was significantly lower than that of rats in the control group (p=0.0014). Figure 1B**". for "**The mean feed consumption of rats treated with *C. hirsuta* (*p*=0.0222) and *R. prinoides* (*p*=0.0014) was significantly lower than the control group (see Figures 1A and 1B)**"

- *3.1.2. Mean water consumption*

I consider that the following sentence "**Figures 2 A and B compare the mean water consumption in rats given 2000 mg/kg of *Clematis hirsuta* aqueous leaf or *Rhamnus prinoides* aqueous root extracts to the mean water consumption in rats given distilled water only**" is repetitive in the expression of the results, and I suggest deleting it.

I suggest changing the sentence "**The mean water consumption of rats in the *C. hirsuta* treatment group did not differ significantly from that of rats in the control group (p=0.1187). Figure 2A. The mean water consumption in the *R. prinoides* treatment group was significantly lower than that in the control group (p=0.0010). Figure 2B**". for "**Against the control group, the mean water consumption of rats treated with *C. hirsuta* did not differ significantly (*p*=0.1187), however, in the *R. prinoides* treatment group such consumption was significantly lower (*p*=0.0010) (see Figures 2A and 2B)**"

- *3.1.3. Mean body weight*

As the previous comments, I consider that the following sentence "**Figures 3 A and B compare the mean body weight gain in rats given 2000 mg/kg of *Clematis hirsuta* aqueous leaf or *Rhamnus prinoides* aqueous root extracts to the mean body weight gain in rats given only distilled water**" is repetitive.

- *3.1.4. Physical examination of rats for signs of toxicity*

Replace "***Clematis hirsuta***" with "***C. hirsuta***" as appropriate.

Replace "***Rhamnus prinoides***" with "***R. prinoides***" as appropriate.

- *3.2. Subacute toxicity*

In the following sentence "**During the experimental period for the subacute toxicity study (28 days), there were no signs of toxicity or mortality in treated rats. The results showed that the extracts' LD50 was > 225 mg/kg**". Is such affirmation referent to both extracts? specify.

In respect to the following information supplied by the authors "**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. Figure 4A. Rats given 75 mg/kg and 225 mg/kg C. hirsuta aqueous leaf extract gained significantly more weight than untreated (control) rats in the third week of treatment (p=0.0011 and p=0.0011 for 75 mg/kg and 225 mg/kg respectively). Figure 4A. 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**". In this point, I consider that the graphics are representatives of the results obtained in reference to the variations in the body weight gain by which this text is repetitive with the graphed in the figures 4A and 4B. I recommend to the authors choosing one of the manners of results expression to avoid duplication.

Same comment for the text included after Figure 4

In the references of Figures 4A and 4B, I suggest incorporating the significance of the symbols *, **, ***, **** and the abbreviature "ns" as a note. For example: * p<0.05, ** p<0.01, ...

- *3.3. Hematological values*

Replace in the references of the Table 1 "***Clematis hirsuta***" by "***C. hirsuta***" and "***Rhamnus prinoides***" by "***R. prinoides***."

I recommend to the authors that the references to the hematological analyzes (WBC, HCT, MON, NEU, LYM, EO, MCV, BAS, PLT, RBC, HGB, MCH and MCHC) at the end of the Table 1 be placed according in the order in which appear in such table.

- *3.4. Biochemical values*

Replace in the references of the Table 2 "***Clematis hirsuta***" by "***C. hirsuta***" and "***Rhamnus prinoides***" by "***R.***

prinoides."

I recommend to the authors that the references to the biochemical analyzes (ALT, AST and ALP) at the end of the Table 2 be placed according in the order in which appear in such table.

Discussion

Replace "***Clematis hirsuta***" with "***C. hirsuta***" as appropriate.

Replace "***Rhamnus prinoides***" with "***R. prinoides***" as appropriate.

In general terms, I had seen that the discussion of the work is poor. I hoped for a more exhaustive and deep discussion, a stronger contrast of the results with other investigation works (it is a personal appreciation).

Furthermore, when the authors make the following comment in the last paragraph of the discussions *"Future work should examine the extracts' pathological effects on major organs in rats ...."* I want to know ("in a personal way") why they did not examinate and analyze the organs of same animals that they used in such toxicological studies.