Author’s response to reviews

Title: Acute and Sub-Acute Oral Toxicity of Dracaena Cinnabari Resin Methanol Extract in Rats

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Author’s response to reviews:

We would like to thank the reviewer for the careful and thorough reading of this manuscript and for the thoughtful comments and constructive suggestions, which help to improve the quality of this manuscript.

Comment / Vikas Kumar –
Reviewer 1:
1. The authors have nicely investigated the acute and sub-acute toxicity of Dracaena cinnabari resin methanol extract in rodents.

Response:
We appreciate the reviewer comment. Thank you.

Comment / Sreenivasan Sasidharan -Reviewer 2:
1. Provide the appropriate references for the dosages used in the "Acute oral toxicity". Or explain how this dosages were selected?

Response:
We appreciate the reviewer comment. The selection of the doses was according to the OEDC 423 guideline instruction (paragraph no. 19 in OECD 423 guideline).
P Shende, YA Kulkarni, R Gaud, K Deshmukh, R Cavalli, F Trotta and F Caldera [1] studied the acute toxicity and used the doses of 300 mg/kg and 2000 mg/kg, while R Ramaswamy, N Prathyusha, R Saranya, H Sumathy, K Mohanavalli, R Priya, J Venkhatesh, C Babu, K Manickavasakam and S Thanikachalam [2] used the doses of 2000 mg/kg; both studies used OECD guidelines 423 as a reference.

Thus, this study has been done to give an overview on the doses that can be used in the future study.

2. Provide the appropriate references for the dosages use in the "Sub-Acute Oral Toxicity". Or explain how this dosages were selected?

Response:

We appreciate the reviewer comment. OECD 407 guideline (as stated in paragraph no. 3) the determination of oral toxicity using repeated doses (28 day) may be carried out after initial information on toxicity that has been obtained by acute toxicity testing. In addition, according to the OECD 407 guideline (as stated in paragraph no. 10), this test provides information on the selection of concentrations for longer term studies. It appears that the doses of sub-acute oral toxicity test usually lower than that used in acute oral toxicity test. P Shende, YA Kulkarni, R Gaud, K Deshmukh, R Cavalli, F Trotta and F Caldera [1] used a dose of 300 mg/kg/day for subacute oral toxicity test (28 days). Another study by R Ramaswamy, N Prathyusha, R Saranya, H Sumathy, K Mohanavalli, R Priya, J Venkhatesh, C Babu, K Manickavasakam and S Thanikachalam [2], selected doses of 300, 600 and 900 mg/kg/day for subacute oral toxicity test (28 days). Both studies used acute oral toxicity with a highest dose of 2000 mg/kg, and their tested materials were tolerated up to 2000 mg/kg.

There are no fixed doses in the literature for the subacute oral toxicity test. Therefore, due to the following reasons: 1) based on our result of the acute oral toxicity test (DC could be well tolerated up to the dose 2000 mg/kg body weight), 2) the dose of 2000 mg/kg considered the highest dose in the acute toxicity test that used in this study, 3) because the duration of the test is 28 days (no single dose like acute oral toxicity) and the animals should be given that dose daily and 4) for animal welfare purpose, doses of DC resin methanol extract lower than 2000 mg/kg such as 500 mg/kg (low), 1000 mg/kg (medium) and 1500 mg/kg (high) body weight were chosen for subacute oral toxicity test. Both studies used OECD guidelines 407 as a reference.

Reference added (Methods - Sub-Acute Oral Toxicity, line 162, page 8).
3. Provide the P values in the text of the results as (p<0.05) or p>0.05).

Response:

We agree with the reviewer comment. Thus, the information provided changed according to your advice.

P values as p > 0.05 or p < 0.05 was added. (Result, line 231, 232 page 11, line 234, 235, 238, 239, 241, 242 page 12, line 290, 291 page 17, line 306 page 18, line 309 page 19).

4. Please label Histopathological Observation in Figure 1 and Figure 2

Response:

Done. (Result - Histopathological Observation, line 353-356 page 22 and 357-360 page 22).

Comment / Ming Xue - Reviewer 3:

1. The introduction section is long, some contents are not related with toxicity, and this part needs to be condensed or rewritten. Response:

We appreciate the reviewer comment. Some parts in the introduction have been condensed according to your advice. (Background, line 81-84 page 5).

2. What are the major active components in the Dracaena Cinnabari resin methanol extract? At least, the authors should indicate the spectra data of main components using the HPLC methods.

Response:

We appreciate the reviewer comment. There are phytochemical studies done and reported that DC resin has led to the isolation of several active compounds belonging to the flavanoids, homoisoflavanoids, chalcones, sterols and terpenoids. Some homoisoflavanoids and chalcones, isolated from the resin exhibited a strong antioxidant activity [3]. (Background, line 77-80 page 5). Another study by D Gupta, B Bleakley and RK Gupta [4] also reviewed the chemical constituents of DC resin. (Background, line 81-82 page 5).

As DC resin has been a famous traditional medicine since ancient times in many cultures, the present study has been done to detect scientifically if this plant may cause any apparent acute or subacute toxicity and concur with the use of this plant by native people as an herb. Furthermore,
this study will play a role in drug discovery and the development of Dracaena Cinnabari as a therapeutic medicine.

3. The dose of Groups 4 received the DC resin methanol extract is at 1500 mg/kg body weight, instead of two scale at 2000 mg/kg. Why?

Response:

Repeated dose 28-day oral toxicity study test provides information on the selection of concentrations for longer term studies.

The dose of 2000 mg/kg considered the highest dose in the acute toxicity test that used in this study and because the duration of the subacute oral toxicity test is 28 days (no single dose like acute oral toxicity) and the animals should be given that dose daily, and for animal welfare purpose we used dose of 1500 mg/kg body weight as the highest dose level during this test. In addition, based on some articles that used acute and subacute oral toxicity, the authors selected the doses for subacute oral toxicity test lower than that used in the acute oral toxicity. P Shende, YA Kulkarni, R Gaud, K Deshmukh, R Cavalli, F Trotta and F Caldera [1] used a dose of 300 mg/kg/day for subacute oral toxicity test (28 days). Another study by R Ramaswamy, N Prathyusha, R Saranya, H Sumathy, K Mohanavalli, R Priya, J Venkatesh, C Babu, K Manickavasakam and S Thanikachalam [2], selected doses of 300, 600 and 900 mg/kg/day for subacute oral toxicity test (28 days). Both studies used acute oral toxicity with a dose of 300 and 2000 mg/kg as per OECD 423 which is same as the present study. It appears that the doses of subacute oral toxicity test are usually lower than that used in acute oral toxicity test. This in turn, will help us to determine the dose level in our future study. We have a plan to evaluate the chemopreventive properties of DC resin methanol extract which will take at least ten weeks oral administration of this material.

Reference added (Methods - Sub-Acute Oral Toxicity, line 162, page 8).

4. How are about the LD50 values of the acute oral toxicity in mice for DC resin methanol extract? The authors should compare the LD50 data with the rat sub-acute oral toxicity data.

Response:

We appreciate the reviewer comment. However, in the present study, we focus on the acute and subacute oral toxicity tests in the rat as per OECD guideline 423 and 407, respectively, to help us in selecting the safe doses for longer term studies in the future. In addition, the LD50 was not one of our objectives.
In fact, we follow the OECD 423 guideline with the test procedure of a starting dose of 300 mg/kg body weight (ANNEX 2c), so, the next dose selected was 2000 mg/kg as the highest dose. There is no study has been conducted the toxicity of DC resin methanol extract and this is the first study evaluated the acute and sub-acute oral toxicity of this plant.

5. The results showed that water intake was significantly higher in the DC resin methanol extract treated groups compared to the control. The reasons should be investigated or discussed.

Response:

We appreciate the reviewer comment. We discussed increase water intake in DC resin methanol extract treated groups compared to the control in the discussion part “DC resin extract can produce vasodilatation (hypotension) due to relaxation of smooth muscles of blood vessels [5] which in turn stimulate thirst and increase water intake [6].” (Discussion, line 434, 435, page 26).

We could rewrite this part as follow, if accepted: DC can produce hypotensive effect due to the vasodilation or to increased glomerular filtration rate which results in increased urinary excretion [5]. It is reported that under reduced blood pressure, young rats drank significantly more water compared to the rats in control conditions [6]. Hypotension is a potent stimulus of thirst in rats [7-9].

6. Results section: the authors should check the data.

Response:

Done

7. Discussion in general: this section should be parallel to the results.

Response:

Done
References


