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ACUTE ORAL TOXICITY STUDY OF CLINACANTHUS NUTANS IN MICE

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ABSTRACT

Clinacanthus nutans Lindau (Family: Acanthaceae) has attracted public interest recently due to its high medicinal values for the treatment of cancer, inflammation and various skin problems. This study was aimed to determine the oral LD_{50} value of the methanol leaves extract of *C. nutans* and identify the targeted organs in mice. This acute oral toxicity study was conducted in accordance to OECD 423 guidelines by using male Swiss albino mice weighing 25-35 g. First group was served as control group which received distilled water (vehicle) while second and third group were orally treated with single daily dose of 0.9 g/kg and 1.8 g/kg of methanol leaves extract of *C. nutans*, respectively. All the animals were closely observed for 14 days. Body weight for each mouse was recorded at day-0, day-3, day-7 and day-14. Relative organ weights for liver, kidney, spleen, lung and heart were also determined. All the results were presented as mean ± standard deviation and analyzed using Dunnett's Test after ANOVA test. From the results obtained, no mortality was observed in both treatment groups either post 24 hours or 14 days of oral administration of *C. nutans*. Body weight for each mouse and relative organ weight showed insignificant difference when compared to the control group. In conclusion, acute exposure of 1.8 g/kg of *C. nutans* was safe in male mice without causing any adverse effects or mortality. The oral LD_{50} of methanol leaves extract of *C. nutans* was suggested to be greater than 1.8 g/kg bw in male mice.

INTRODUCTION: *Clinacanthus nutans* Lindau belongs to the family of Acanthaceae, is a small shrub that native to tropical Asia countries [1]. It has local name known as Sabah snake plant or Belalai Gajah [2]. It is commonly consumed in the form of herbal tea for the treatment of diabetes mellitus, fever, diarrhoea and dysuria [3]. In the recent year, the pharmacological properties of *C. nutans* such as anti-viral, anti-oxidant, anti-inflammatory have been previously reported [4-7]. Several compounds such as *C*-glycosyl flavones, sulfur-containing glycosides, cerebrosides and a monoacyl-monogalactosylglycerol have been identified from the leaves of *C. nutans* by using different solvent systems [8-10]. Literature search showed that no toxicity study has been conducted on the methanol leaves extract of *C. nutans* either in animals or humans. So far, only one toxicity study has been conducted on ethanolic leaves extract of *C. nutans* at 1.3 g/kg bw using mice [11]. Thus, this study provides preliminary data to other researchers to elicit the safe and appropriate safe doses for pre-clinical study.

The objectives of this study were to examine the possible acute oral toxic effect of 1.8 g/kg bw of methanol leaves extract of *C. nutans* in male mice and to determine the LD_{50} value of *C. nutans* in experimental animals. The present study was carried out based on the method recommended by OECD 423 guideline [12].

METHODS AND MATERIALS:

Plant Materials: The fresh leaves of *C. nutans* were collected from Seremban, Negeri Sembilan and dried in Postgraduate Research Laboratory, UCSI University. The leaves were blended in fine powder and were extracted by methanol using maceration method [13]. All the extracts were concentrated and kept in the dessicator until used.

Selection of Experimental Animals: A total of 20 mice weighing 25 to 35 g body weight were used in this acute oral toxicity study according to OECD 423 guideline [12]. The present acute oral toxicity study was approved by the Faculty Ethical committee. Each group consisted of five animals (n=5) and allowed to access to food and tap water *ad libitium*. All the mice were randomly assigned into the respective group. Treatment groups were orally treated with a single dose of 0.9 g/kg and 1.8 g/kg of methanol leaves extract of *C. nutans*, respectively while the control group was treated with the respective vechile, distilled water.

All the animals were closely observed *via* cage-side observation for first four hours post treatment and twice daily the day after until day-14 [14]. Body weight change was measured on day-0, day-3, day-7 and day-14. All the mice were sacrificed and several ogans, i.e. liver, kidney, heart, lung and spleen were removed on day-15. The relative organs weight was calculated. All the results were analysed using Dunnett's test by comparing with the respective control group. $p<0.05$ was considered as significant difference compared to control group [15].

RESULTS: All the male mice that received 0.9 and 1.8 g/kg of methanol leaves extract of *C. nutans* did not show any toxic signs and abnormal behavioural changes post 24 hours of *C. nutans* treatment and during 14 days observation duration. In additional to that, *C. nutans* treated mice in this study showed no significant effect on body weight and relative organ weight after 14 days observation period (**Table 1 and 2**).

TABLE 1: EFFECT OF METHANOL LEAVES EXTRACT OF *CLINACANTHUS NUTANS* ON BODY WEIGHT CHANGES AND MORTALITY IN MALE MICE

Grouping (g/kg)	Body weight Changes (g)				% Mortality
	Day-0	Day-3	Day-7	Day-14	
Control	25.8±2.3	26.9±2.3	27.8±1.5	31.3±3.4	0
***C. nutans* (0.9)**	25.1±1.8	26.6±1.7	28.0±2.5	30.0±1.1	0
***C. nutans* (1.8)**	27.7±1.9	29.6±2.9	31.2±2.4	31.9±1.1	0

Value = mean ± standard deviation; n=5. Analyzed using Dunnett's test

TABLE 2: EFFECT OF 1.8 G/KG OF METHANOL LEAVES EXTRACT OF *CLINACANTHUS NUTANS* ON RELATIVE ORGAN WEIGHTS IN MALE MICE

Grouping (g/kg)	Relative organ weight (g/100g body weight)				
	Liver	Kidney	Heart	Spleen	Lung
Control	4.84±0.20	1.44±0.02	0.44±0.04	0.63±0.10	0.72±0.09
***C. nutans* (0.9)**	5.31±0.37	1.61±0.19	0.51±0.05	0.71±0.07	0.68±0.09
***C. nutans* (1.8)**	5.50±0.73	1.39±0.06	0.43±0.04	0.56±0.08	0.73±0.01

Value = mean ± standard deviation; n=5. Analyzed using Dunnett's test

DISCUSSION: Acute toxicity study could be able to provide important information to identify the targetted organs of test substances after acute exposure [16]. From the results obtained, the exact LD_{50} value could not be determined due to no mortality was observed. However, it is believed that the LD_{50} of methanol leaves extract of *C. nutans* is greater than 1.8 g/kg in mice. Based on the classification of chemical toxicity as described in the OECD 423 guideline, the toxic profile of *C. nutans* is classified as closely to category 5 which is low acute toxicity hazard [12]. This has been the first study conducted on the acute oral toxicity of *C. nutans* in mice and these findings are very crucial for choosing the therapeutic dose in clinical trials.

Derelanko (2000) has postulated the inter-species dose convertion based on the equivalency of surface area [17]. The conversion factor of mouse (20g) to human (60 kg) is 1/12. Thus, the 1.8 g/kg of *C. nutans* in mice is equivalent to 0.15 g/kg (or 150 mg/kg) in humans. However, it is too early to conclude the safety of *C. nutans* since it has been studied only in experimental animals but at least it provides some correlation between information obtained from animal study to human usage. Many interspecies factors such as different expression of enzymes, metabolic rates and physiological changes between mice and humans need to be carried out to confirm our suggestions. Further evaluation of the sub-chronic oral toxic effect in rats needs to be carried out for better understanding about the mechanisms of *C. nutans*.

CONCLUSION: For conclusion, single oral administration of methanol leaves extract of *C. nutans* did not cause any mortality and adverse effects in male mice. It could be concluded that a single dose of 1.8 g/kg of *C. nutans* was safe to both male and female mice and the LD_{50} value of *C. nutans* was greater than 1.8 g/kg bw in mice.

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