SUBACUTE TOXICITY STUDY OF *CLINACANTHUS NUTANS* ETHANOLIC EXTRACT *IN VIVO*

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ABSTRACT *Clinacanthus nutans* (Belalai Gajah) leaves extract has been used extensively as a traditional remedy in the Asian region. Despite of that, there was no standard measurement has been reported so far. Thus the application of the extract might be associated with risk of toxicity. The present study was designed to investigate the possible toxicity risk of *C. nutans* extract on the murine model *in vivo*. Sub-acute toxicity study was carried out following repeated oral administration of *C. nutans* ethanolic extract at daily doses of 50, 300, 2000 and 5000 mg/kg body weight for 28 days. From this study, the results showed that there were no significant differences in body weight, relative organs weight and physiological changes in control and treated groups. These results were further confirmed by observing histological changes on kidney and liver tissue where the results revealed that *C. nutans* extract exerts no toxic effect at concentration of 50 mg/kg body weight, whereas at the concentrations of 300, 2000 and 5000 mg/kg, a mild degree of toxicity was shown on both organs. These results indicated that *C. nutans* ethanolic extract at the concentration ranging from 300 to 5000 mg/kg body weight induced mild hepatic and renal toxicity in mice.

Keywords: *Clinacanthus nutans*; ethanolic extract; sub-acute toxicity.

1. INTRODUCTION

Recent trends in the application of Complementary and Alternative Medicine (CAM) therapies have led to a growing prevalence in US adults from approximately 36% in 2002 to 38.2% in 2007 [1]. One such practice is through the consumption of herbal based product, namely *Clinacanthus nutans.*

Scientifically named as *Clinacanthus nutans* (Burm. f) Lindau, it is commonly acknowledged as 'Belalai Gajah' in Malaysia. *C. nutans* fresh leaves have been used extensively as traditional remedies to treat various illnesses, namely skin rashes, insect and snake bite, herpes simplex virus (HSV), varicella-zoster virus (VZV) lesions and dysentery [2,3]. Its health promoting properties has successfully drawn public interest, leading to its commercialization into products such as capsule, herbal tea, and concentrated extract that is abundantly available in the market [4].

Despite of that, most of these products surprising do not come with standard measurement. This might expose consumers to a possible risk of toxicity with its prolonged use. Previous studies reported that the methanolic extract of *C. nutans* at the highest dose of 2500 mg/kg did not cause any toxic effect on the liver and kidney of mice, thus suggesting its no-observed-adverse-effect level (NOAEL) to be greater than the tested dose [5]. Therefore, the objective of this study is to investigate the possible subacute oral toxicity effect of *C. nutans* ethanolic extract in mice at dose up to 5000 mg/kg body weight for 28 days.

2. MATERIALS AND METHODS

2.1. Plant Material

The crude extract of *C. nutans* leaves was obtained from Dr Lim Vuanghao, Universiti Sains Malaysia, Penang. The extract was stored in the refrigerator at -20˚C for further use.

2.2. Experimental animals

Healthy female BALB/c mice at age of 8-10 weeks old were purchased from Universiti Kebangsaan Malaysia (UKM) and housed under standard environmental conditions. The experimental protocols of the animals were approved by the UKM Animal Ethics Committee [Ethical number: 72/2016].

2.3. Experimental design: Repeated dose 28-days oral toxicity study in mice

The study was conducted in accordance to OECD Guideline 420 by dividing the animals into groups of mice administered daily with 30% ethanol and 10% Tween 20 that served as positive control and delivery vehicle group respectively. This is followed with treated groups received *C. nutans* ethanolic extract at doses of 50, 300, 2000 and 5000 mg/kg body weight throughout the testing period. During the study, all animals were closely monitored for abnormal behavior and changes in body weight and feeding pattern.

2.4. Organs Weight and Histopathological analysis

After sacrification, vital organs like liver and kidney were harvested, blotted and weighed on analytical balance for absolute organ weight. They were then fixed in 10% neutral buffered formalin, processed, sectioned, stained with hematoxylin–eosin staining and examined under a light microscope for histological examinations. The relative organ weight (ROW) of each organ was calculated as follows [6].

$$\text{ROW} = \frac{\text{Absolute organ weight (g)}}{\text{Fasted body weight on sacrifice day}} \times 100$$

2.5. Statistical analysis

Statistical analysis was performed using Graphpad Prism version 7. Differences in parameters were evaluated using a One-way Analysis of Variance (ANOVA) followed with Dunnet's post hoc test.

3. RESULTS AND DISCUSSION

3.1. Body Weights and Feeding Pattern

Along with the increasing trend of *C. nutans* application as primary health care among consumers, there is an urge of interest to ascertain on its toxicity profile. Previous study on ethanol extraction has only been carried out in lower dose up to 250 mg/kg in 90 days of sub chronic testing period [7]. Considerable uncertainty still exists on the relationship between the dosage and course of treatment. Therefore, this study evaluates on much higher dosage with a shorter testing period of 28 days. Current study showed no mortality or physical signs of toxic effect were observed in treatment dose up to 5000 mg/kg and control groups. Similarly, there was no significant difference were observed on physical parameters like the mean of food and water intake (Table 1) and body weight gain (Figure 1) between the treated and control groups. Any alteration in body weight is believed to be associated with adverse effects of drugs and chemicals [8]. From the results obtained, it was appeared that all these parameters were not affected by the administration of *C. nutans* extract, thus evidencing a normal metabolism in the test animals.

Table 1. Weekly food and water intake of mice orally administered with *C. nutans* for 28-days. Results were expressed as mean ± SD, n=5.

Parameters	Groups (mg/ml)	Week 1	Week 2	Week 3	Week 4
Water intake (ml/day)	Positive control	3.60 ±1.75	3.29 ± 1.01	3.45 ± 1.09	4.14 ± 0.49
	Delivery vehicle	3.91 ±2.16	2.91 ± 1.03	2.54 ± 0.22	2.80 ± 0.28
	50	2.66 ± 1.30	2.31 ± 0.84	1.91 ± 0.11	2.69 ± 0.47
	300	5.11 ± 3.56	2.43 ± 0.57	2.66 ± 0.50	2.69 ± 0.27
	2000	3.34 ± 2.13	3.74 ± 1.33	4.09 ± 0.55	4.20 ± 0.83
	5000	3.43 ± 1.89	3.91 ± 0.86	4.06 ± 0.28	3.63 ± 0.78
Food intake (g/day)	Positive control	2.23 ± 1.25	3.34 ± 1.51	3.77 ± 1.25	3.06 ± 1.36
	Delivery vehicle	2.51 ± 1.49	2.77 ± 0.47	2.54 ± 0.32	2.97 ± 0.54
	50	2.17 ± 1.11	2.60 ± 0.16	2.74 ± 0.39	2.57 ± 0.76
	300	3.00 ± 1.37	3.06 ± 0.57	2.89 ± 0.23	2.13 ± 0.70
	2000	2.35 ± 1.61	2.54 ± 0.71	2.74 ± 1.55	2.31 ± 1.24
	5000	2.03 ± 1.16	3.40 ± 1.41	4.43 ± 1.20	4.23 ± 1.15

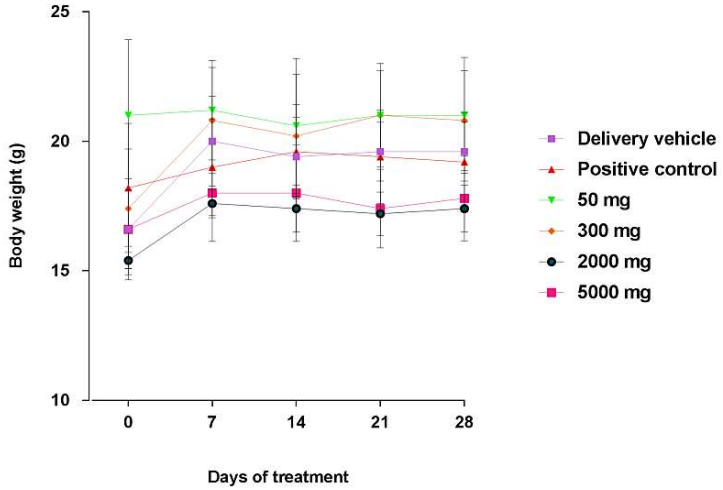

Figure 1. Weekly body weight measurements (g) of mice orally administered with *C. nutans* for 28-days. Results were expressed as the mean ± SD, n=5.

3.2. Organ Weights

Liver and kidney played critical roles in detoxification and excretion processes in biological system [9]. In this study, the results displayed non-significant changes for both organs in all treated groups as compared to the control (Table 2). In general, any change in the liver and kidney weight might be related to the organs injury, including swelling, atrophy or hypertrophy [10]. However, the results still need to be further validated by histological assessment to confirm on the findings.

Table 2. Relative organs weight of mice orally administered with *C. nutans* for 28-days. Results were expressed as mean ± SD, n=5.

Dosage administration (mg/kg)	Relative organ weight	
	Liver	Kidney
Positive control	5.915 ± 0.937	1.369 ± 0.255
Delivery vehicle	5.490 ± 0.756	1.252 ± 0.227
50 mg	5.802 ± 0.653	1.424 ± 0.173
300 mg	5.912 ± 0.730	1.672 ± 0.315

2000 mg	5.981 ±0.263	1.536 ±0.167
5000 mg	6.012 ± 0.762	1.400 ±0.110

3.3. Histopathological Examination

Histopathological examinations of the livers and kidneys had shown no signs of toxicity in the treatment group at a concentration of 50 mg/kg and control groups, but there were occasionally slight degeneration and necrosis in the cellular structure of both organs in treatment groups ranging from 300 to 5000 mg/kg of *C. nutans* extract (Images not shown). There were mild renal tubular lesions with the presence of congested blood vessel in the kidney, whereas droplets of fatty infiltration were displayed in hepatocellular of the liver. These findings are in line with previous studies that reported oral administration of *C. nutans* caused histopathological lesions to both organs [7].

4. CONCLUSION

It could be concluded that supplementation of *C. nutans* ethanol extract at doses of 300 to 5000 mg/kg body weight induced a mild degree of hepatic and renal toxicity in mice. Therefore, it may have potential health risk in a long run use. However additional studies in biochemical assays and hematological test are necessary to further support the findings.

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