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Evaluation of the sub-acute oral toxic effect of methanol extract of *Clinacanthus nutans* leaves in rats

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ABSTRACT

Objective: To examine the possible sub-acute toxic effect after 14 d oral administration with 0.3 g/kg, 0.6 g/kg and 0.9 g/kg of methanol leaves extract of *Clinacanthus nutans (C. nutans)* on liver and kidney functions in young male Sprague Dawley (SD) rats. **Methods:** This study was carried out according to OECD 407 guideline. A total of 24 young healthy male SD rats aged between 8-12 weeks old were used throughout the experiments. Each group consisted of six rats (n=6). All animals were observed twice daily until day-14. Body weight, food consumption and water intake were measured on day-3, 7 and day-14. Blood samples were collected and used to analyse the serum levels of AST, ALT, ALP, bilirubins, urea and creatinine in rats. Relative organ weights for liver, kidney, heart, spleen and lung were calculated. **Results:** From the results obtained, all the serum biochemical parameters, food and water intake, relative organs weight showed no significant changes when compared to the control group. No lethality and abnormal behavioural changes were seen in both control and treatment groups during experiment. **Conclusions:** For conclusion, repeatedly dosing of *C. nutans* extract at 0.3g/kg, 0.6g/kg and 0.9 g/kg up to 14 d was proven safe in male SD rats without causing any adverse effects and organ damages in male SD rats.

1. Introduction

Clinacanthus nutans (C. nutans) Lindau (Family: Acantaceae) or locally known as Belalai Gajah in Malaysia, is a small shrub native to tropical Asia[1]. It has been used in Thailand as a traditional medicine for the treatment of skin rashes, insect and snake bite[2]. This plant has gained high popularity among Malaysians of its high medicinal value in treating cancer[3]. *C. nutans* has been reported to possess anti-oxidant, anti-inflammation and anti-viral activity[4-7]. Putwatana *et al* had reported that *C. nutans* was more effective than benzydamine in preventing and relieving radiation-induced oral mucositis in head and neck cancer patients[8].

Literature search showed that inadequate toxicity information available for *C. nutans*. Up to our best knowledge, only one study has reported on the toxicity of ethanolic extract of *C. nutans* leaves tested in mice and rats. In that study, the ethanolic extract of *Clinacanthus nutans* leaves at 1.3 g/kg bw showed no acute toxic effect in mice and repeatedly feeding with 1 g/kg bw in rats for 90 d showed no any abnormalities of the internal organs in rats[9]. However, the toxicity of methanol leaves extract of *C. nutans* has not been reported previously. This attracts our interest to evaluate the possible toxic effect of methanol extract of *C. nutans* leaves in rats to enhance the understanding regarding safety profiling of *C. nutans*. Hence, the objective of the present study is to determine the possible sub-acute (14 d) toxic effect of *C. nutans* on liver and kidney functions in Sprague Dawley (SD) young male rats. In the present repeated-dose oral toxicity, all the rats were orally treated with methanol extract of *C. nutans* leaves (0.3, 0.6 and 0.9 g/kg) for a period of 14 d.

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2. Methods and materials

2.1. Chemicals

Diethyl ether and methanol were supplied by local chemical supplier. All the chemicals used were at industry grade.

2.2. Plant material

Fresh leaves of *C. nutans* were purchased from Herbal park, Seremban. All the dried leaves were blended into fine pieces and macerated with methanol at room temperature for 3 d. Extracts were concentrated using rotary evaporator under reduced pressure at 40 °C, followed by freeze drying at −50 °C. Extracts were kept in desicator until use.

2.3. Sub-acute (14 days) oral toxicity study

All the procedures involved animal testing in this study were reviewed and approved by Faculty Research Ethics Committee. This toxicity study was carried out according to OECD 407 guidelines (2008)[10]. A total of 24 male SD rats aged between 8 to 10 weeks old were used. All the rats were acclimatised in an air-conditioned animal transit room maintained at (23 ± 2) °C with 12 h light/dark cycle prior 5 d of experiment. First group served as control group and group 2 to 4 were treated with single dose daily with 0.3 g/kg, 0.6 g/kg and 0.9 g/kg methanol extract of *C. nutans* leaves, respectively for 14 d. Cage-side observations were conducted twice daily[11]. Body weight of each rat, water intake and food consumption were recorded on day-0, day-3, day-7 and day-14. Blood samples were collected *via* cardiac puncture from each rat on day-15 after overnigh fasting. Blood serum was separated and used for biochemical analyses. Biochemical analyses was conducted using Cobas c 311 analyser which was a fully automated, random access analyser for clinical chemistry and homogeneous immunology (HIA)[12]. All the rats were sacrificed to obtain relative organs weight for liver, kidney, lung, spleen and heart.

2.4. Statistical analysis

Results were presented as mean ± standard deviation. Results for the toxicity study were analysed using analysis of variance (ANOVA) followed by Dunnett's test. $P<0.05$ was considered as significant difference when compared to the respective control group.

3. Results

The rats that received 14 d treatment of *C. nutans* leaves extract did not present noticeable signs of toxicity at any of the doses. No significat differences on serum biochemical parameters, relative organ weights, body weight gain, food intake and water consumption were observed between *C. nutans* treatment groups and control group (Table 1-3).

4. Discussion

Barle et al have mentioned the purpose to perform toxicological experiments in animals is to determine the effect of an action on a biological system which can be used later to extrapolate the doses and effects on humans[13]. The present study was different from the toxicological study on *Clinacanthus nutans* carried out by Chavalittumrong *et al*

Table 1
Sub-acute (14 d treatment) effect of *C. nutans* leaves extract on body weight changed, food consumption and water intake in male sd rats.

Grouping (g/kg)		Day-0	Day-3	Day-7	Day-14
Body weight changed (g)	Control	118.4±16.85	144.0±17.39	175.6±22.33	211.9±22.83
	0.3 (*C. nutans*)	119.1±12.53	144.0±12.01	175.5±11.19	210.1±12.36
	0.6 (*C. nutans*)	123.5±22.86	151.9±24.67	184.1±26.50	227.4±33.92
	0.9 (*C. nutans*)	123.9±18.51	148.5±20.47	178.4±28.54	217.4±31.43
Water intake (mL/rat/d)	Control	9.8±2.12	18.3±1.18	25.0±1.18	29.6±2.95
	0.3 (*C. nutans*)	7.8±2.12	20.4±2.95	25.0±2.36	25.4±0.59
	0.6 (*C. nutans*)	11.5±2.12	17.9±4.13	27.5±2.36	32.5±7.07
	0.9 (*C. nutans*)	12.1±1.77	17.9±1.77	27.1±4.13	34.6±4.13
Food consumption (g/rat/d)	Control	10.8±1.05	13.3±0.21	18.6±0.49	19.2±1.06
	0.3 (*C. nutans*)	11.4±1.46	13.6±0.38	19.5±0.73	18.6±0.29
	0.6 (*C. nutans*)	11.1±1.97	14.8±1.69	19.9±0.54	20.8±1.97
	0.9 (*C. nutans*)	10.0±0.65	13.4±1.75	18.7±1.88	20.7±0.26

n=6, Analysed using Dunnett's Test.

Table 2

Sub-acute (14 d treatment) effect of methanol extract of *C. nutans* extract on serum bochemical parameters in male sd rats.

Parameters (Unit)		Grouping (g/kg)			
		Control	0.3 (*C. nutans*)	0.6 (*C. nutans*)	0.9 (*C. nutans*)
Kidney function tests	Urea (mM)	5.3±1.06	5.2±0.92	5.1±1.05	6.0±0.27
	Creatinine (μM)	47.7±5.32	44.2±3.66	41.8±1.94	61.0±26.68
Liver function tests	Alkaline phosphatase (IU/L)	310.2±78.4	274.5±78.93	233.7±52.11	251.7±85.16
	Alanine aminotransferase (IU/L)	53.3±8.14	56.7±9.31	68.5±13.22	66.7±13.00
	Aspartate aminotransferase (IU/L)	123.8±27.67	178.8±98.67	163.2±86.63	112.2±33.09
	Total bilurubin (μM)	0.8±0.36	0.6±0.45	0.9±0.28	1.0±0.37

n=6; Data = mean ± standard deviation. Results were analysed using Dunnett's test.

Table 3

Sub-acute (14 d treatment) effect of methanol extract of *C. nutans* extract on relative organ weights in male sd rats.

Relative organ weights (g/100 g)	Grouping (g/kg)			
	Control	0.3 (*C. nutans*)	0.6 (*C. nutans*)	0.9 (*C. nutans*)
Liver	5.30±1.06	5.20±0.92	5.10±1.05	6.00±0.27
Kidney	47.70±5.32	44.20±3.66	41.80±1.94	61.00±26.68
Heart	310.20±78.40	274.50±78.93	233.70±52.11	251.70±85.16
Lung	53.30±8.14	56.70±9.31	68.50±13.22	66.70±13.00
Spleen	123.80±27.67	178.80±98.67	163.20±86.63	112.20±33.09

n=6; Data = mean ± standard deviation. Results were analysed using Dunnett's test.

in terms of the solvent used for extraction. The toxicological study carried out by Chavalittumrong *et al* used ethanol as solvent while methanol was used as the extracting solvent in this study[9]. A study showed that methanol was the best solvent to extract the highest amounts of total phenolic content from different parts of plants compared to ethanol, water, acetone, ethyl acetate and chloroform[14]. This indicating the correlation between dielectric constant of solvent and the total phenolic content.

Up to our best knowledge, this study was the first toxicology study reported on the methanol extract of *C. nutans* leaves in rats. Since no oral toxicity study had been previously reported on methanolic extract of *C. nutans* leaves, the starting dose of was selected based on the recommendation given by OECD 423 guideline (2001)[15]. According to OECD 423 guideline, the starting dose could be selected from either one of the four dose levels, *i.e.* 5, 50, 300 or 2 000 mg/kg body weight. Therefore, 300 mg/kg was chosen as the starting dose and the other two doses, *i.e.* 600 mg/kg and 900 mg/kg were chosen by doubling and tripling the selected starting dose. OECD 407 guideline (2008) was chosen for this study due to animal wellfare consideration[10]. This is in agreement to the ethical principle practiced by the Americal Veterinary Medical Association to reduce the numbers of animals used for research and development[16].

Majority of the exogenous compounds administrated via gastroinstetinal tract will be delivered to the liver through portal vein for metabolism and kidney for elimination[17]. Liver and kidney are the two main organs for investigation in oral toxicity study. For liver function test, four serum hepatic biochemical parameters, namely alanine aminotransferase (ALT), alkaline phosphatase (ALP), aspartate aminotransferase (AST) and total bilirubin were analysed in this study. AST and ALT are common markers used to diagnose the hepatocyte integrity[18]. On the other hand, increases in the serum ALP and bilirubin levels would indicate the presence of cholestasis[19]. For kidney function test, two serum renal biochemical parameters, namely urea and creatinine were analysed in this study[20]. From the results obtained, all doses of methanolic extract of *C. nutans* leaves ranging from 300 to 900 mg/kg bw showed no significant influence on all serum biochemical parameters when comparing all treated groups to the control group, indicating that *C. nutans* leaves showed no substancial toxic effect on male rat liver and kidney.

There are few statistical methodologies or formulas currently available to extrapolate the animal data to humans. World Health Organization (WHO) guideline has correlated the inter-species difference according to the duration of treatment. Experimental animals that treated with test substance for 14 d consecutively is equivalent to human consumption for lesser than a week[21]. Acceptable daily intake (ADI) is used by Food and Agricultural Materials Inspection Center (FAMIC) to determine the level that is harmless to humans based on the non-observable adverse effect level (NOAEL) value obtained from animal study[22]. Based on the results obtained from all parameters measured, the NOAEL of methanol extract of *C. nutans* leaves was 900

mg/kg in rats. Thus, the ADI is determined to be 9 mg/kg [900 mg/kg ÷ 100] in humans. However, this information is served as preliminary data generated from sub-acute toxicology study in male rats. More extensive studies such as chronic study and toxicokinetics evaluation need to be carried out to further confirm the safety of *C. nutans* leaves extract.

For conclusion, 14 d oral administration of methanol extract of *C. nutans* leaves to male rats were proven safe without causing any adverse effects and damages to liver and kidney in male SD rats.

Conflict of interest statement

We declare that we have no conflict of interest.

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