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## Acute and Sub Chronic Oral Toxicity Assessment of the Ethanolic Extract from the Rind of *Nephelium lappaceum* in Rats

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### ABSTRACT

*Nephelium lappaceum* is a tropical fruit native to Malaysia. The rind of *N. lappaceum*, is having extremely high antioxidant and free radical scavenging activities. The ethanol extract from the rind of *Nephelium lappaceum* was evaluated for acute and sub-chronic toxicity study in Sprague Dawley rats. In the acute study, a single oral administration of *N. lappaceum* rind extract (50, 200, 1000 and 2000 mg kg<sup>-1</sup>) was administered to rats for 14 days. In the sub chronic toxicity study, the extract was administered to rats (500, 2000 mg kg<sup>-1</sup>) for 28 days. There was no mortality, or adverse effects observed in rats. There was no significant difference observed in relative organ weights and the biochemical analysis (serum urea, creatinine, ALP, AST and total protein). Histological observation of liver and kidney also did not reveal any significant changes. In conclusion, present study showed that the lethal dose of ethanol extract of *Nephelium lappaceum* rind is more than 2000 mg kg<sup>-1</sup> and there is a huge margin of safety for the therapeutic use. No-observed-adverse-effect-level (NOEL) of the extract is considered to be up to 2000 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days in rats.

**Key words:** Rat toxicity, body weight, organ weights, liver enzymes

### INTRODUCTION

*Nephelium lappaceum*, also known as rambutan is native to Southeast Asia and belongs to the family (Sapindaceae) (Wall *et al.*, 2006). It is a bright red, oval shaped fruit, with seed, hairy peel with long, soft spines. This fruit is available between June to August in tropical countries. Almost all parts of the plant namely, roots, bark and leaves have various medicinal uses and in the production of dyes. The dried rind of this fruit has been used as traditional medicine as well as in the production of soap. The previous studies have shown antibacterial activity (Thitilertdecha *et al.*, 2008), anti-herpes simplex virus type 1 activities (Nawawi *et al.*, 1999) and antihyperglycemic activity (Palanisamy *et al.*, 2011) of *N. lappaceum* rind. Unlike the pulp of *N. lappaceum*, which possesses low antioxidant activity (Leong and Shui, 2002), the peel of *N. lappaceum*, is rich in phenolic content. Rambutan peel is also utilised in an activated carbon form to use as an adsorbent for the removal of Malachite Green (MG) dye which is an environmental toxin (Ahmad and Alrozi, 2011). The isolated compounds from *N. lappaceum*

identified are ellagic acid, corilagin and geraniin. Geraniin is reported as the major bioactive compound which has high antioxidant activity (Palanisamy *et al.*, 2008). Though the peel of *N. lappaceum* has been reported to possess therapeutic value, there are no reports about its toxicological evaluations. It is necessary to determine the toxicological effects in order to ascertain the safety of this medicinal plant. Hence, we have taken up this study with an objective to evaluate the acute and sub chronic toxicity of ethanolic extract of the *N. lappaceum* rind in Sprague-Dawley rats.

## **MATERIALS AND METHODS**

**Plant material:** *N. lappaceum* rind was obtained from the markets in Kuala Lumpur, Malaysia and was authenticated by the herbarium of the Forest Research Institute of Malaysia (FRIM, Malaysia). One kilogram of the plant material was washed with water and dried at 40°C in the oven and powdered using Fritsch dry miller (Palanisamy *et al.*, 2008). Ethanol extraction was carried out by using ethanol at 1:10 (w/v) at room temperature in the orbital shaker. The suspension from the ethanol extraction was filtered and concentrated using rotary evaporator. The extract was kept at -4°C until used. The extract was dissolved in saline (NaCl; 0.9%) upon administration.

**Experimental animals:** Male Sprague Dawley rats, aged 6 weeks and weighing between 150 and 200 g were used in this study. All rats were obtained from the animal house, School of Medicine and Health Sciences, Monash University Malaysia. They were housed one rat per cage and maintained at room temperature (25±2°C) with 12:12 h dark/light cycle and the rats had free access to tap water and food. The experiment procedures used in the study followed the Animal Care and Ethics Guidelines of International Medical University, Malaysia.

**Acute toxicity study:** Acute toxicity study was carried out based on the guidelines of the Organization for Economic and Co-Operation Development (OECD, 2001a, b). Rats were divided into five groups of six animals each and fasted overnight prior to the experiment. The doses were chosen from literature based on the effective doses applied especially on the hepatoprotective and renoprotective activity.

**Group 1:** Administered vehicle (saline 0.9% w/v p.o.)

**Group 2:** Administered *N. lappaceum* extract (50 mg kg<sup>-1</sup> p.o.)

**Group 3:** Administered *N. lappaceum* extract (200 mg kg<sup>-1</sup> p.o.)

**Group 4:** Administered *N. lappaceum* extract (1000 mg kg<sup>-1</sup> p.o.)

**Group 5:** Administered *N. lappaceum* extract (2000 mg kg<sup>-1</sup> p.o.)

After administration of the extract, animals were observed during 1, 2, 4 and 6 h and then daily for 14 days for the general behavior change and mortality.

**Sub chronic toxicity study:** Guideline 407 (2001a) was adopted for 28 days repeated oral toxicity study. Animals were randomly divided into 5 groups of 6 rats each.

**Group 1:** Administered vehicle (saline 0.9% (w/v) p.o.)

**Group 2:** Administered *N. lappaceum* extract (500 mg kg<sup>-1</sup> p.o.)

**Group 3:** Administered *N. lappaceum* extract (2000 mg kg<sup>-1</sup> p.o.)

After administration of the ethanolic extract, all the rats closely observed for any toxic symptoms or abnormalities. Besides, the body weight, food consumption and water intake were recorded daily. At the end of the treatment all the animals were anesthetized using diethyl ether and blood samples were collected via cardiac puncture.

**Measurement of biochemical parameters in rats:** The blood obtained via cardiac puncture was allowed to clot before centrifuging at 3000 rpm for 10 min (Atsamo *et al.*, 2011). Serum obtained was used for analysis of Alanine Aminotransferase (ALT), Alkaline Phosphatase (ALP), urea, total protein and creatinine.

**Absolute and relative organ weight:** Upon sacrificing the rats, vital organs such as the heart, liver, lung, kidney and the pancreas were removed, washed with saline and weighed before fixing in 10% buffered formaldehyde solution. The relative organ weights was calculated and compared with the control group (Gomes *et al.*, 2012).

**Histopathological observation:** Samples of the liver and kidney were processed in the tissue processor and embedded in paraffin wax. Four micrometer sections were cut using microtome and stained with haematoxylin and eosin (H and E). The stained tissues were observed under light microscope for any histopathological changes (Veerappan *et al.*, 2007).

**Statistical analysis:** Statistical analysis was done using one way ANOVA followed by the post hoc Dunnett's test (SPSS version 16), where the data were compared with the control. All data points are expressed as the Mean $\pm$ SD. Value of  $p < 0.05$  was considered statistically significant.

## RESULTS

Figure 1 shows body weight of both control and rats treated with *N. lappaceum* increased constantly. The weight gain in the control group increased compared with the treatment groups. There is no statistically significant differences in rats treated during 14 days and rats in the recovery period.

Figure 2 shows food consumption between the control group and the treatment group in 14 days acute toxicity study was not significantly affected. But, rats treated with 2000 mg kg<sup>-1</sup> *N. lappaceum* rind extract for 28 days showed significant difference in food intake at week 4 compared to control group. Moreover, water consumption also showed no significant difference in all the rats compared to the control group.

There were no significant differences in relative organ weights of the liver, lung, kidney, heart and the pancreas of treated group with the control (Table 1). The liver weight of rats treated with highest dose (2000 mg kg<sup>-1</sup> *N. lappaceum*) showed lower value compared to the control group but it did not reach the level of statistical significance.

The data in Table 2 shows that *N. lappaceum* treatment caused no significant differences in the control and treatment group of rats for urea, creatinine, ALP, ALT and total protein levels.

The morphology of the liver and kidney was studied in all the animals for the acute and sub chronic toxicity. There was good maintenance of the kidney structure in all groups. The glomerular

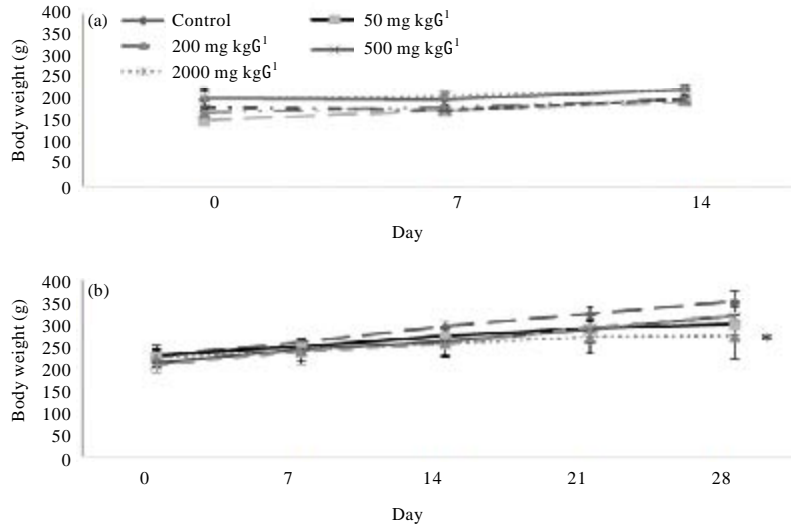


Fig. 1(a-b): Body weight of control and *Nephelium lappaceum* rind extract administered rats during (a) 14 day acute and (b) 28 day sub-chronic toxicity study, Values are Mean±SD of four rats in each group

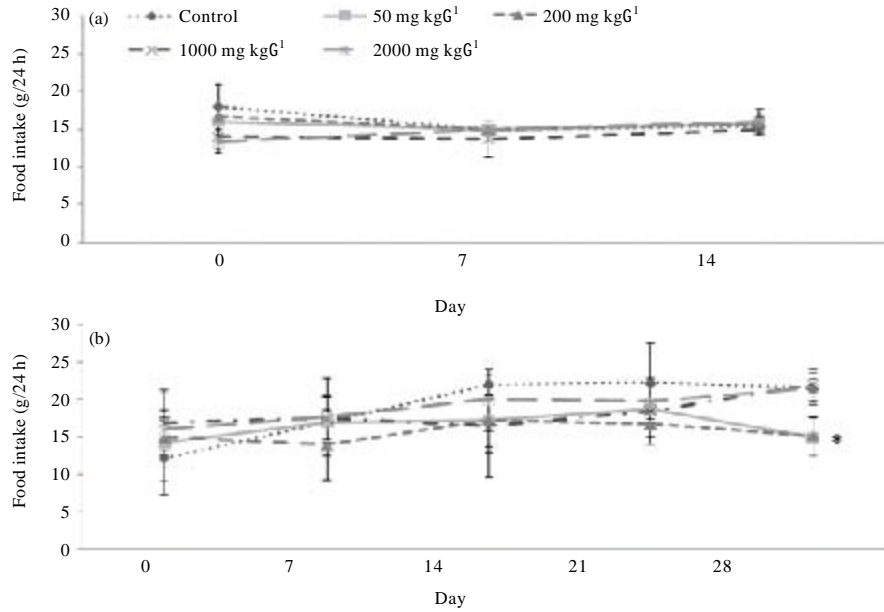


Fig. 2(a-b): Effect of *N. lappaceum* rind on food intake of rats during (a) 14 day acute and (b) 28 day sub-chronic toxicity study, Data expressed in Mean±SD

and tubular structures in all groups were very well preserved. Interstitium and blood vessels were unremarkable. No obvious pathological features were observed in these groups. The hepatic architecture was adequate with clusters of normal hepatocytes and normal orientation of the central vein and the portal triad. No evidence of inflammation, degeneration or necrosis was seen in all these groups (Fig. 3, 4).

Table 1: Effect of *N. lappaceum* rind extract on relative organ weight of rats

Toxicity	<i>N. lappaceum</i> (mg kg <sup>-1</sup> )	Relative organ weight (g)				
		Liver	Lung	Kidney	Heart	Pancreas
Acute: 14-day toxicity	Control	3.5±0.4	0.7±0.1	0.5±0.1	0.4±0.0	0.4±0.0
	50	4.1±0.1	0.9±0.2	0.5±0.0	0.4±0.0	0.5±0.1
	200	4.0±0.2	0.9±0.1	0.5±0.1	0.3±0.1	0.4±0.1
	1000	3.9±0.2	0.8±0.2	0.5±0.0	0.4±0.0	0.4±0.1
	2000	3.3±0.2	0.9±0.1	0.4±0.0	0.3±0.0	0.5±0.1
Sub-chronic: 28 day toxicity	Control	3.8±0.3	0.6±0.1	0.4±0.1	0.3±0.0	0.4±0.1
	500	3.5±0.4	0.6±0.2	0.4±0.1	0.3±0.0	0.4±0.0
	2000	3.2±0.6	0.7±0.1	0.4±0.0	0.3±0.0	0.3±0.1
	500	3.5±0.3	0.6±0.1	0.4±0.0	0.4±0.0	0.3±0.0
	2000	3.4±0.3	0.6±0.0	0.4±0.0	0.3±0.0	0.3±0.0

Values are Mean±SD of 6 rats in each group

Table 2: Effect of *N. lappaceum* rind extract on various biochemical analyses of rats

Toxicity study	<i>N. lappaceum</i> (mg kg <sup>-1</sup> )	Urea (mg dL <sup>-1</sup> )	Creatinine (mg dL <sup>-1</sup> )	ALP (IU L <sup>-1</sup> )	ALT (IU L <sup>-1</sup> )	Total protein (g dL <sup>-1</sup> )
Acute: 14 day toxicity	Control	13.9±2.9	0.7±0.1	225.7±560.6	58.4±31.1	5.4±1.0
	50	12.5±1.6	0.7±0.0	277.3±180.9	63.0±50.8	5.9±0.2
	200	14.7±2.8	0.8±0.1	276.7±340.9	50.7±90.8	5.7±0.3
	1000	19.1±4.4	0.8±0.1	298.7±210.4	49.0±30.6	6.3±0.2
	2000	19.6±0.8	0.8±0.1	216.0±740.5	41.4±00.5	6.3±0.3
Sub-chronic: 28 day toxicity	Control	17.3±2.0	0.6±0.0	274.5±120.4	47.5±14.0	7.0±0.2
	500	16.5±1.2	0.7±0.0	258.3±490.2	45.9±14.7	7.2±0.1
	2000	16.5±1.2	0.6±0.1	324.8±175.8	52.1±13.3	6.8±0.4
	500	16.6±1.2	0.6±0.0	301.5±141.7	48.5±90.4	6.8±0.2
	2000	15.4±1.1	0.6±0.0	224.0±130.3	49.6±50.3	7.0±0.2

Values are Mean±SD of 6 rats in each group

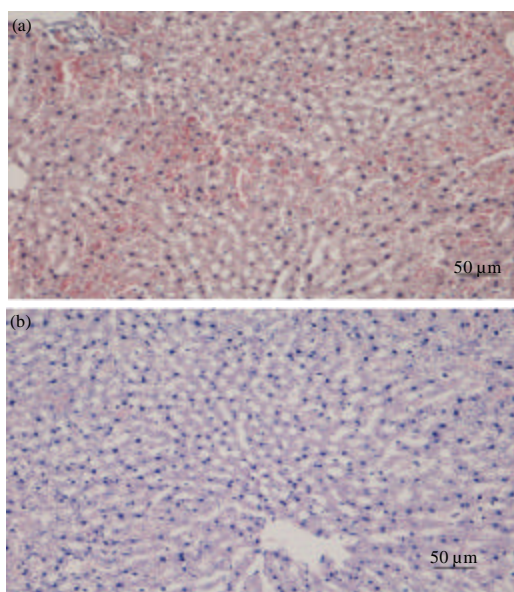


Fig. 3(a-b): Photomicrograph (H and E, 200x) showing normal architecture of liver (a) Acute and (b) Sub-chronic group

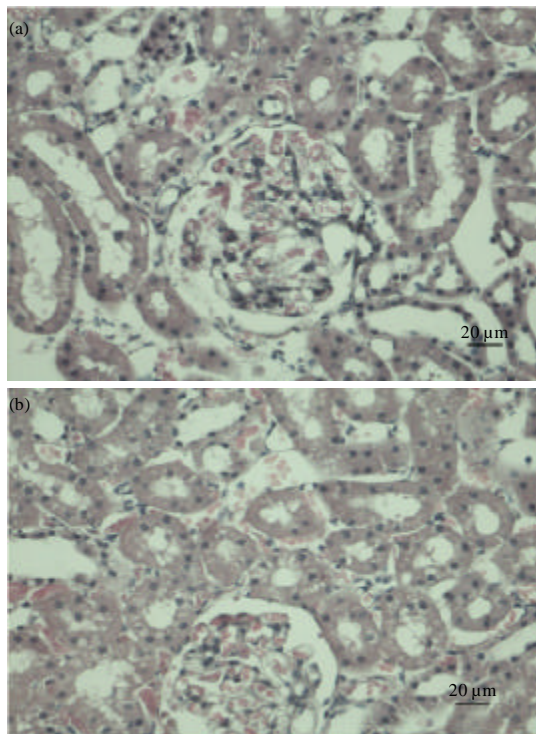


Fig. 4(a-b): Photomicrograph (H and E, 400x) showing normal architecture of kidney (a) Acute and (b) Sub-chronic group

## DISCUSSION

*Nephelium lappaceum* rind is widely used as medicinal plant in Southeast Asia. However, toxicity study and adverse effects of this plant with the scientific prove is not available. The present study evaluated the acute toxicity and sub chronic toxicity of ethanolic extract of *N. lappaceum* rind. Oral administration of the extract of a single dose for 14 days showed no toxic effect on the body weight, relative organ weights and on the biochemical parameter as well as the food and water intake. There was no mortality during the study period. Therefore toxicity level of *N. lappaceum* extract classified as more than 2000 mg kg<sup>-1</sup> based on the OECD classification.

All the rats continued to gain weight during 14 days observation period and no significant changes in the food and water intake was observed when compared to the control group. General toxicity can be accessed via organ weight measurements, in which changes in the body weight and organ weight is a sensitive indicator of toxicity (Thanabhorn *et al.*, 2006; Norazmir and Ayub, 2010). According to Atsamo *et al.* (2011), frank toxic effect could not be determined when there is no significant difference in the relative organ weight, which is proportional to the body weight. Moreover, toxicity indication can be seen in organ weights rather than absolute weight of rats (Harizal *et al.*, 2010; Patel *et al.*, 2008). In this study, no significant changes were observed in relative organ weights of all the treated groups compared to the control group. Therefore, it can be suggested that the body weight decrease in rats treated with *N. lappaceum* rind at dose 2000 mg kg<sup>-1</sup> is insignificant.

Serum AST and ALP enzymes present in the liver is used as a marker to detect chronic liver disease (Hor *et al.*, 2012). ALP is mainly present in bile, liver, kidney, bone and placenta and there

will be a significant elevation of this enzyme in liver injury (Betti *et al.*, 2012). Serum ALP is a useful indicator to diagnose intra hepatic and extra hepatic bile obstruction in the liver. Although, ALP level is elevated in the sub chronic treatment with 2000 mg kg<sup>-1</sup> *N. lappaceum* rind extract compared to the control group, it did not show any statistically significant difference. As reported by Konan *et al.* (2007), ALP seems to have no clinical relevance as it is affected by the age and developed in the adulthood. Fat accumulation in hepatocytes usually takes place in liver injury and leads to increase in the relative liver weight as well as liver enzymes such as ALP and AST (Chin *et al.*, 2008). Based on our study, the relative weight of liver showed no significant difference when compared with the control group. This observation could indicate that the ethanolic extract of *N. lappaceum* has protective effect on liver function study. Similarly, no changes were observed in ALP, urea, creatinine and total protein level which is a good indicator of liver and kidney functions. The biochemical analysis was further supported by the histopathology findings which revealed no lesion or pathological changes in the liver and kidney of treated rats. Therefore, the ethanolic extract of *N. lappaceum* rind did not cause any significant damage to the liver and kidneys and was indeed well tolerated by the rats.

Thus, the present work evaluated the acute and sub chronic toxicity of the ethanolic extract of *N. lappaceum*. The results of this study demonstrated that the ethanolic extract of *N. lappaceum* may be considered relatively safe of any toxicity. Peel waste of *N. lappaceum* has very significant potential due to its powerful antioxidant properties. Because of the non-toxic effects of this extract on the organs systems, there is a clear potential for the utilization of *N. lappaceum* rind as a food additive or for therapeutic use.

## CONCLUSION

In conclusion, the present investigation demonstrated that the ethanolic extract of *N. lappaceum* rind at level up to 2000 mg kg<sup>-1</sup> day<sup>-1</sup> did not cause any adverse effects and considered as nontoxic and safe. No-observed-adverse-effect-level (NOEL) of the extract is considered to be up to 2000 mg kg<sup>-1</sup> day<sup>-1</sup> for 28 days in rats. This was further supported by biochemical parameters, body weight, relative organ weights and histological findings. Thus, the lethal oral dose of *N. lappaceum* is classified under category five, which is not at or below 2000 mg kg<sup>-1</sup>. This study provides valuable data on the toxicity profile of ethanolic extract of *N. lappaceum* rind that would be useful in further pharmacological studies.

## ACKNOWLEDGMENT

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