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Acute Toxicity (Oral) Information of *Litsea elliptica* Blume Essential Oil

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***Abstract*—*Litsea elliptica* Blume (Lauraceae), known as Medang pepijat in Malaysia. It has been proven to have promising insecticidal activities. *L. elliptica* essential oil was evaluated for its acute toxicity (14 days) by the oral route in Sprague-Dawley rats. In this study, acute oral toxicity of the essential oil using female Sprague-Dawley rats (10/group) has been investigated. To determine median lethal dose (LD50) and non-observed-adversed-effect level (NOAEL), the essential oil was administered orally in six different doses (single dosing), 500, 1000, 2000, 2500, 3000 and 4000 mg/kg per body weight (bw). Control group was administered orally of distilled water. Mortality rate and toxic symptoms been observed in all groups for 14 days. Body and selected organs weight have also been measured. From the results, oral acute LD50 was estimated to be 3488.86 mg/kg/bw and classified using WHO recommended classification in the U group. Dose-dependent toxic symptoms (hypoactivity, piloerection and lacrimation) were showed in this study. There were also significantly changes of weekly body and organs (kidney and heart) weight. The acute NOAEL was revealed at 500 mg/kg/bw. In conclusion, *Litsea elliptica* essential oil is safe at dose of 500 mg/kg/bw for acute oral toxicity study.**

Keywords-component; Median lethal dose (LD50), Litsea elliptica Blume (Lauraceae), Non-observed-adversed-effect level (NOAEL), Acute oral toxicity, Natural insecticide.

I. INTRODUCTION

The great potential of natural products industry can be applied to a wide range of items such as herbal or traditional medicine, natural pesticides, biopharmaceutical and others. The uses of plants for pesticidal purpose are getting increasingly popular as they are believed as being beneficial and free of side effects. The usages of natural products as pesticides are most promising of environmentally desirable and economically profitable. While, highly toxics inorganic pesticides provide a broad range of toxic effect, essentially indestructible and contaminated the world.

However, most of the information available to the consumer about several natural products does not have any scientific data support. Natural products have their use as medicament based simply on a traditional folk use that has been perpetuated along several generations.

Litsea elliptica (Family: Lauraceae) is a well known tropical tree used in herbal or traditional medicine in Thailand and Indonesia [`1]. In Indonesia, *L. elliptica* crushed leaves were applied around the forehead for treatment of headache [2]. As for in Thailand, *L. elliptica* or its common name, Thammung, has been proved to have chemopreventatives activities, thus explain the reduced incidence of gastric cancer in that country [3]. Furthermore, a significant antimutagenicity activity was also proven [4].

While in Malaysia, *L. elliptica* or its common name, Medang pepijat was first reported by [5] to have potential insecticidal activity using its essential oils. This report then supported by [6] research founding of its repellence properties against *Aedes aegypti* bites. The recent studies on the insecticidal properties also shown that methanol extracts of *L. elliptica* leaves has a significant effects as larvicidal [7] and adulticidal [8].

To date, only a few studies have been performed about the toxicological potential of *L. elliptica* essential oil to mammalian. Therefore, preliminary toxicological investigation of this essential oil has been performed in female Sprague-Dawley rats. The aim of this study was to examine the acute toxicity effects of *L. elliptica* essential oils before the commercialization as natural product pesticide can be done.

II. MATERIALS AND METHODS

The leaves were originally collected from Pasoh Forest, Negeri Sembilan. Voucher specimen numbered as FRI41999 was deposited at the Herbarium of the Forest Research Institute Malaysia, Kepong. Then, the leaves were dried at room temperature and grounded to produce smaller particles. Clevenger apparatus was used to extract the essential oil by steam distillation for at least 8 hours.

Sprague-Dawley female rats were used in this investigation. Seven groups of ten rats each were obtained from Laboratory Animal Resource Unit, Faculty of Medicine, Universiti Kebangsaan Malaysia. Body weights have been determined to range from 180 to 220 gram. Then, the rats were divided into six groups of ten female rats randomly. They were housed doubly and allowed to adapt to the cage conditions for a week before the experiment. They were fed

ad libitium with a standard laboratory diet (Mouse pellet 702 P, Gold Coin Sdn. Bhd.) and free access to drinking tap water.

The study was conducted according to USEPA Health effects test guidelines on acute oral toxicity [9] with some modification. Six doses concentration of the essential oil were selected; 500, 1000, 2000, 2500 3000 and 4000 mg/kg/bw and one group of control receiving distilled water. Rats were orally single dosing by gavage using a feeding needle. Rats were fasted overnight prior to dosing.

Observation of toxic symptoms were made and recorded daily until day-14. Body weight, food and water intake were recorded prior to dosing and weekly. Mortalities were also recorded and the dead animals were necropsied. At day-14, all surviving animals were sacrificed, necropsied and lung, kidney, spleen, heart, and liver were weighted. Pathological observations of these organs were performed on gross and recorded.

The LD_{50} was calculated using probit analysis, SPSS 11.5. One-way ANOVA was employed to analyze the data statistically. Comparisons were carried out using Scheffe pos hoc test. All values were expressed as mean ± SEM. Differences were considered statistically significant when $p<0.05$.

III. Results And Discussion

Mortality was observed in groups of 2500, 3000 and 4000 mg/kg/bw with one, five and six death respectively. Using probit analysis, LD_{50} was estimated to be 3488.86 mg/kg/bw (Figure 1). Therefore, essential oils of *L. elliptica* leaves is suggested to be classified in the U group as unlikely to present acute hazard in normal use by WHO recommended classification guidelines of pesticides [10].

According to the study, some pharmacological activities of essential oils from *Litsea elliptica* leaves were revealed. Throughout the study, rats from group 1000, 2000, 2500, 3000 and 4000 mg/kg/bw showed hypoactivity. Rats in group of 3000 and 4000 mg/kg/bw also showed lacrimation and piloerection. Only rats in group of 500 mg/kg/bw did not show any toxic signs.

As it comes to end of intoxication process, the reaction of the proximate toxicant with biochemical targets was translated into physiological responses. According to single oral dosing of the essential oil, some toxicity symptoms has been observed as result of a dose-related decrease on autonomic (ANS) and central nerves system (CNS). The symptoms are hipoactivity, lacrimation, and piloerection.

Lacrimation is one of the toxic symptoms showed as results to actions on ANS by stimulation by asetilcoline [11]. It is also revealed in exposure to organophosphate insecticides [12]. The blockage of asetilcolinesterase that can be stimulated by this insecticide can also lead to death [13]. This might be the reason of death to rats from group of 3000 and 4000 mg/kg/bw. As for piloerection and hipoactivity, CNS depressions were suggested. It showed decrease in locomotors activity that was controlled by CNS [14].

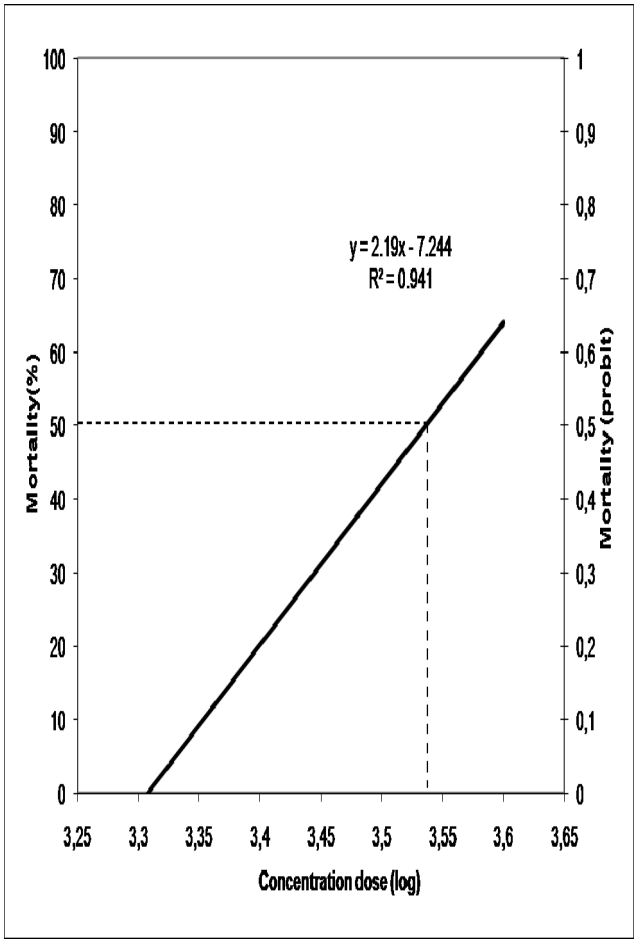

Figure 1. Probit analysis of *L. elliptica* essential oil showed the LD_{50} was estimated to be 3488.86 mg/kg/bw.

The essential oil showed some similarity in toxicity symptoms to organophosphate insecticides such as malathion, diazinon and chlorpyrifos which also have some neurotoxic symptoms [15]. Beside observation on toxicity symptoms throughout the study, weekly body and selected organs weight also been studied. On day-7, there was significant body weight decrease in group 3000 mg/kg/bw compared to control ($p<0.05$). While on day-14, there were significantly decrease in body weight in group 2000 until 3000 mg/kg/bw compared to control ($p<0.05$) and 3000 mg/kg/bw compared to 500 and 1000 mg/kg/bw ($p<0.05$) (Figure 2).

As for selected organ weight, the study showed some significance changes only in renal and heart weights (Table 3). These significant changes can also be related to changes in food (Figure 4) and water consumption (Figure 5) weekly and the toxic symptoms.

According to [16], changes in body and organ weights can show the toxic effects of the xenobiotic. From the study, *L. elliptica* essential oil administered acute orally at minimum dose of 2000 mg/kg/bw can be suggested to significantly decrease body weight. It might be associated with the toxic symptoms that occurred during the study

(hipoactivity, lacrimation and piloerection) that can be suggested to lead the rats to become anorectic. It also been proven with the decrease weekly water and food consumption observed during the study.

The increase in both absolute and relative kidney and heart weights of the rats treated with *L. elliptica* essential oil at minimum dose of 2500 mg/kg/bw observed in the present study could be associated with kidney and heart edema or hypertrophy, since the edema can induce an increase on organ weight [17].

In conclusion, the acute oral administration of *L. elliptica* essential oil did not induce significant alterations at concentration dose of 500 mg/kg/bw and this dose has been suggested to be the NOAEL (Non-observed-adverse-effect level).

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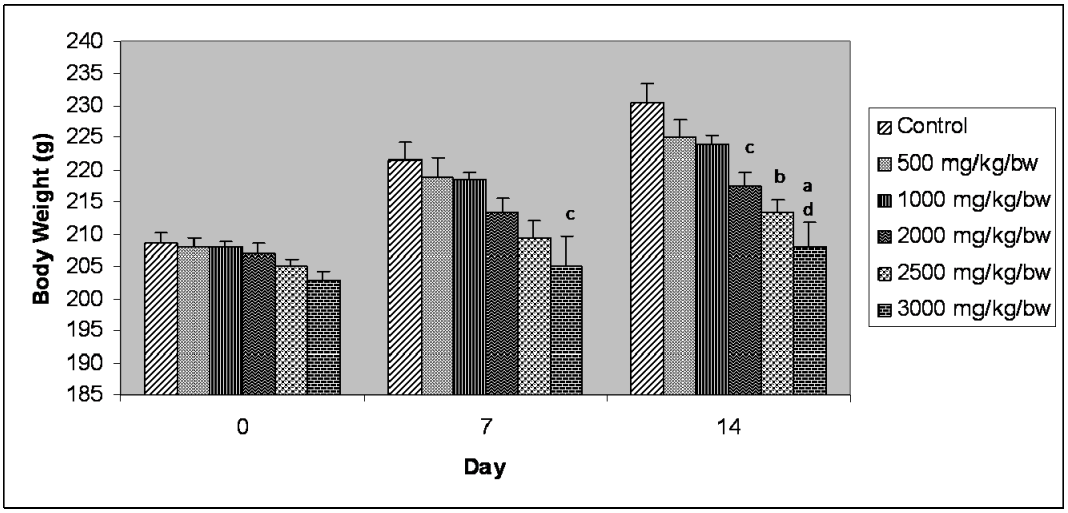

a Significant at p<0.001as compared to control group

b Significant at p<0.01as compared to control group

c Signifikan pada p<0.05 as compared to control group

d Signifikan pada p<0.05 as compared to 500 dan 1000 mg/kg/bw group

Figure 2. Comparison of mean body weight between all groups (except 4000 mg/kg/bw group) after 14 days of study

Parameter	Group (min±SD)					
	Control	500 mg/kg/bw	1000 mg/kg/bw	2000 mg/kg/bw	2500 mg/kg/bw	3000 mg/kg/bw
Absolute weight (g)						
1 Liver	7.81 ± 1.04	7.84 ± 1.29	8.67 ± 0.90	8.41 ± 1.29	8.35 ± 0.77	8.52 ± 0.78
2 Kidney	1.34 ± 0.06	1.34 ± 0.07	1.03 ± 0.05[a]	1.01 ± 0.08[a]	1.28 ± 0.10[b]	1.51 ± 0.11[b,c,e]
3 Spleen	0.44 ± 0.10	0.48 ± 0.06	0.45 ± 0.08	0.48 ± 0.16	0.50 ± 0.11	0.49 ± 0.18
4 Heart	0.32 ± 0.03	0.33 ± 0.03	0.35 ± 0.03	0.36 ± 0.03	0.39 ± 0.05[d,g]	0.39 ± 0.07[f]
Relative weight (%)						
1 Liver	3.40 ± 0.48	3.49 ± 0.58	3.87 ± 0.38	3.86 ± 0.56	3.92 ± 0.40	4.09 ± 0.26
2 Kidney	0.58 ± 0.04	0.60 ± 0.04	0.46 ± 0.02[a]	0.46 ± 0.03[a]	0.60 ± 0.05[b]	0.73 ± 0.06[a,b,e]
3 Spleen	0.19 ± 0.04	0.27 ± 0.03	0.20 ± 0.03	0.22 ± 0.07	0.23 ± 0.05	0.24 ± 0.10
4 Heart	0.14 ± 0.01	0.15 ± 0.02	0.15 ± 0.01	0.16 ± 0.02	0.18 ± 0.02[d]	0.19 ± 0.03[f]

a Significant at p<0.001 as compared to control and 500 mg/kg/bw groups

b Significant at p<0.001 as compared to 1000 dan 2000 mg/kg/bw groups

c Significant at p<0.001 as compared to 2500 mg/kg/bw group

d Significant at p<0.01 as compared to control group

e Significant at p<0.05 as compared to control and 500 mg/kg/bw groups

f Significant at p<0.05 as compared to control group

g Significant at p<0.05 as compared to 500 mg/kg/bw group

Figure 3. Comparison of mean organ weight between all groups (except 4000 mg/kg/bw group) after 14 days of study

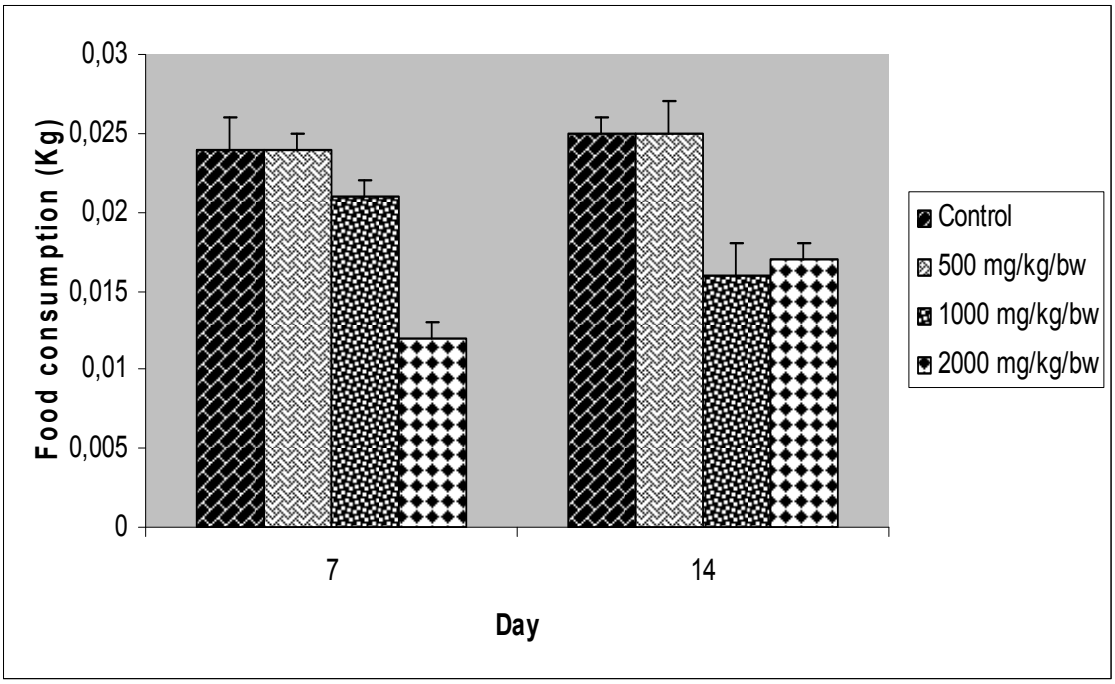

Figure 4. Comparison of mean food consumption (Kg) between all groups (except 2500, 3000 and 4000 mg/kg/bw group) after 14 days of study

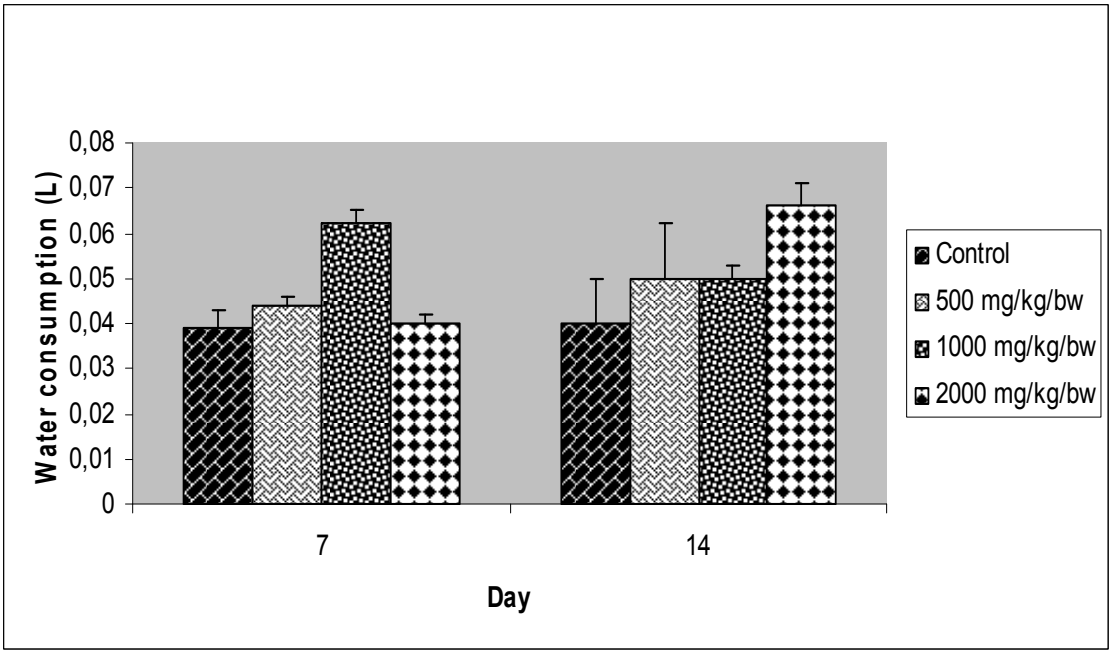

Figure 5. Comparison of mean water consumption (L) between all groups (except 2500, 3000 and 4000 mg/kg/bw group) after 14 days of study