Median Lethal Dose (Ld_{50}) Test of Analgesic Herbal Jatropha Curcas L. Latex on Animal Models

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Abstract

Dental pain is a common illness suffered by society. One of the plants that are commonly used for dental pain relief is the latex of Jatropha curcas L. Scientific data regarding the analgesic effect of Jatropha curcas L. latex has been widely studied, but there is no scientific data about the safety of its use. This study aimed to evaluate of the safety usage of Jatropha curcas L., by performing an acute toxicity test to obtain a median lethal dose (LD50).

Mus musculus Swiss-Webster strain was divided into 6 groups (control group was given PGA 10%, and the first to the fifth group were given latex orally at doses of 30, 40, 50, 60, 70 g/kg body weight respectively). The mortality of the animal was measured every day for 14 days. The data were depicted with log-probit graphic method for determined value LD50.

LD50 was 50 g/kg body weight. Conclusion: The latex of Jatropha curcas L. was classified as non-toxic material.

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Introduction

Dental pain is a common illness suffered by society. Riskesdas (2018) reported that the largest proportion of dental problems in Indonesia is toothache (tooth decay/cavities/pain) with a percentage of 45.3%.[1] According to Lipton et al (1993), 22% of Americans in the United States experienced orofacial pain and the most common pain was toothache, which was estimated to have occurred in 12,2% of the population.[2]

To overcome dental pain, people in a rural areas often use herbal medicine instead of visiting the dentist. One of the herbal medicine that commonly used for dental pain relief is the latex of *Jatropha curcas* L.(Fig 1). To abolish the pain, the latex was dripped to the cavity. In addition, to cure the dental pain, this latex is commonly used as a strong laxative, or as a mouth rinse to treat the bleeding gum.[3,4]

The tendency of increased usage of herbal medicine should be supported as an alternative way to avoid the side effects caused by chemical drugs. Scientific data regarding the analgesic effect of *Jatropha curcas* L. latex has been widely studied; it was reported that the latex had the ability to decrease the level of PGE_2 in inflamed tooth pulp of *Macaca nemestrina*. It was also reported that either the latex or the extract had an analgesic effect through the writhing test.[5] As a standardized herbal medicine, however, the plants should be safe. This study, therefore, was aimed to evaluate the safety of this herbal, especially the acute toxicity (LD_{50}), one of the parameters in determining the safety of the material.

Materials and methods

The latex were obtained from one and half years old *Jatropha curcas* L. plants; the trunks of the plant was incised, 30 – 40 cm long, and the latex were collected in the vial of 20 mL and immediately covered with aluminum foil and stored at a temperature of -20°C before experimentation. (Fig 2).

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Figure 1. *Jatropha curcas* L. plants.

Figure 2. The Collecting Latex of Jatropha curcas L.

Figure 3. *Mus musculus Swiss-Webster* strain.

The experimental doses used were 30; 40; 50; 60; 70 g/kg body weight and prepared by making the suspension of the latex with pulvis gummi arabicum (PGA) 20% (Bratachem) and aqua destillata.[6]

Sixty male and female *Mus musculus*, *Swiss-Webster* strains, 2 – 3 months old, and 20 – 30 grams, were used in this study. (Fig 3). This study was approved by the Unpad Pharmacy Laboratory Ethics Approval. The animals were first acclimatized for seven days and were divided into six groups (ten animals each), which consisted of five experimental groups and one control group. Before experimentation, the animals fasted for 18 hours.[6]

Fifteen milliliters of the latex of each dose were administered orally. To determine the LD_{50}, the amount of mortality was noted every day for 14 days. Data (the amount of mortality) was plotted at a graph-paper of log-probit for determining LD_{50} value. LD_{50} value is determined at the point of intersection between the dose (at the axis) and the 50% of mortality (ordinate).[7] The experimental group with associated dose was depicted in Table 1.

Group	Dose
Control	PGA 20%
I	30 g/kg BW
II	40 g/kg BW
III	50 g/kg BW
IV	60 g/kg BW
V	70 g/kg BW

Table 1. Experimental Group and the Dose of Latex of *Jatropha curcas* L.

Results

The cumulative mortality of male and female *Mus musculus* are depicted in Table 2 whereas its pattern of mortality can be seen in Figure 4 (male) and Figure 5 (female).

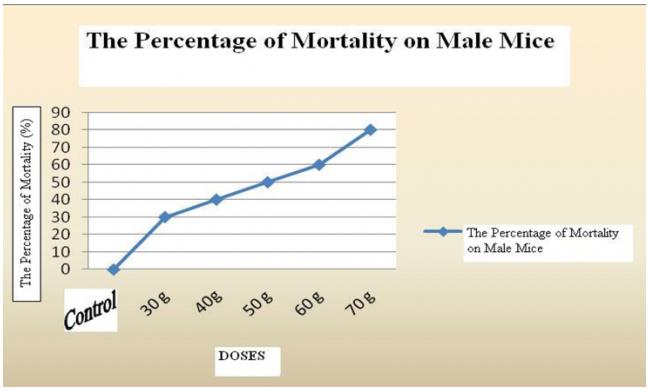

Figure 4. The Graphs Pattern Percentage Line of Mortality on Male Mice.

Group	Treatment	Animal (10)	Result									
			1 day	2 days	3 days	4 days	5 days	6 days	7 days	9 days	13 days	14 days
Control	Control	Male	0	0	0	0	0	0	0	0	0	0
Dose 1	30 g	Male	10	10	10	20	20	30	30	30	30	30
Dose 2	40g	Male	20	20	40	40	40	40	40	40	40	40
Dose 3	50 g	Male	20	30	30	30	40	50	50	50	50	50
Dose 4	60 g	Male	60	60	60	60	60	60	60	60	60	60
Dose 5	70 g	Male	80	80	80	80	80	80	80	80	80	80
Control	Control	Female	0	0	0	0	0	0	0	0	0	0
Dose 1	30 g	Female	20	20	20	20	20	20	20	20	20	20
Dose 2	40g	Female	20	40	40	40	40	40	40	40	40	40
Dose 3	50 g	Female	30	30	50	50	50	50	50	50	50	50
Dose 4	60 g	Female	60	60	60	60	60	60	60	60	60	60
Dose 5	70 g	Female	80	80	80	80	80	90	90	90	90	90

Table 2. The Percentage of Cumulative Mortality of *Mus musculus.*

Note: In the group of males and females, 50% of animal death occurred at the same dose group 50 g/kg BW, the male groups occurred at 6 days and the females at 3 days.

It was revealed that there was no mortality in the control group, either on male or female animals. The LD_{50}, either on male or female animals is 50 g/kg BW. The highest mortality was at the dose of 70 g/kg BW where the mortality of the male is lower than the mortality of the female.

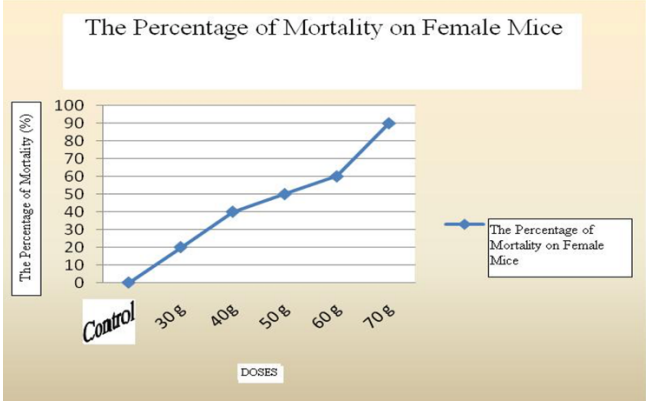

Figure 5. The Graphs Pattern Percentage Line of Mortality on Female Mice.

Discussion

Acute toxicity test is a test that must be performed to determine the safety of the single-use of a drug or the substance which is aimed to use as a medicine in human use.[8,9] It includes the determination of median lethal dose (LD_{50}), the assessment of body weight, and the spectrum of acute toxicity. The latter is aimed to evaluate the effect of the material to be tested on the biologic system of the human body such as abnormal behavior, tremor, convulsion, motoric un-coordination, salivation, lacrimation, or Straub. Weather or not the tested materials have an adverse effect on those systems, usually will be disclosed by this kind of test.

In toxicology, the median lethal dose, LD_{50} (abbreviation for 'Lethal Dose', 50%), of a substance is the dose required to kill half of the members of a tested population. The LD_{50} is frequently used as a general indicator of a substance's acute toxicity.[10,11,12] LD_{50} was first introduced as an index by Trevan in 1927.[13,14]

In this study, LD_{50} either for male or female mice, was 50 g/kg body weight. This study, however, is not by the previous study, which was stated that the LD_{50} for the latex of *Jatropha curcas* L. was 5 g/kg body weight.[15] The reason for this difference is probably due to the method of the experiment; the previous study administered the sample intragastrically, whereas in this study, the samples were administered orally. Lu (2006) stated that to be safe, the LD_{50} of the material should be greater than 15 g/kg BW and oral administration is a common and easier method.[16]

In this study, we used one type of rodent provided it included male and female of 10 animals in each experimental group, this is according to Lu (1995) that acute toxicity test could be performed at least one species.[17] Ideally, the acute toxicity test should be performed in two different animal species i.e. one rodent and one non-rodent.[18] This study, however, experimented on male and female rodents and it was revealed that there was no different result between them.

Conclusions

Considering the above factors it can be concluded that the materials, having the LD_{50} at 50 g/kg body weight (BW), as long as concerning the lethal dose, are considered safe. But, the final judgment should be made after the other test, such as the body weight test and the test to evaluating the effect on the body system has been performed. It is suggested to perform the test of change of the body weight and the effect of the material to the biologic body system.

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Declaration of Interest

The authors report no conflict of interest.

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