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ACUTE TOXICITY TEST OF ETHANOL EXTRACT OF SUNGKAI LEAF (*Peronema canescens* Jack) IN WHITE MICE (*Mus musculus*)

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Abstract:

Sungkai (Peronema canescens) is a type of plant that can be used in traditional medicine in Jambi, Indonesia. The purpose of the study was to determine the value of LD₅₀ from the results of the acute toxicity test of LD₅₀ ethanol extract of Sungkai leaves (Peronema canescens) in male and female white mice (Mus musculus). This research method is experimental research in the laboratory used 50 mice comprising 25 males and 25 females which are divided into 5 groups, normal control (Na-CMC 1%), ethanol extract (625 mg/kg body weight), ethanol extract (1,250 mg/kg body weight), ethanol extract (2,500 mg/kg body weight) and ethanol extract (5,000 mg/kg body weight). The test preparation is administered orally through the gastric sonde and is administered only once in 24 hours. The Indonesian Pharmacopoeia Edition III method was used to calculate the value of LD₅₀ in this study. The results of the acute toxicity test of LD₅₀ Sungkai leaf ethanol extract are included in the safe category in the male animal group. In the group of female animals, an LD₅₀ value of 6.50 mg/kg body weight was got. They include it in the category of very toxic and dangerous for the group of female animals. On the data on the ratio of organ weights in each research organ. Only in the male group, there was no noticeable difference (P>0.05) in each organ, but in the female group of animals, there were noticeable differences (p<0.05) in the heart, liver, lungs, kidneys, and stomach organs.

Keywords: *Sungkai Leaf (Peronema canescens); Jambi; Acute Toxicity Test LD₅₀; Ethanol Extract; White Mice (Mus musculus).*

1. Introduction

Traditional medicine should be used to maintain health empirically, even today it is still used. The development of science on the type of disease will have a positive effect on the development of science related to drugs derived from plants (1). Of the 20,000 plant species that exist, about 80% are used by the population as medicine worldwide (2). Plants have content and properties for health. The plant parts used can be leaves, stems, roots, tubers, or maybe also all parts of the plant or commonly known as herbs (3,4). Based on previous research, the plant produces active components that are used for health care or medicine and produces different properties according to their uses (4–6).



Some of the advantages of using plants as traditional medicine are that these ingredients are obtained without a doctor's prescription, and the raw materials are easily obtained from the surrounding nature and can be prepared by the user himself. One example of a plant that has medicinal properties is the sungkai leaf plant. The Sungkai leaf plant is a tropical plant that we can find in everyday life (7). Sungkai leaves are commonly used as immunomodulatory (8) So they can be used to improve the body's defense system. Based on phytochemical test research that has been carried out by several researchers, sungkai leaf extract contains active substances in the form of peronemins, catechol, quinic acid, isovanillic acid, and guaiacol (9).

During the COVID-19 pandemic that attacked the world, it was felt that there was a need to trace scientific studies about medicinal plants that were also used by the public to improve the body's defense system, therefore it was important to test the acute toxicity of LD₅₀ on sungkai leaves, considering the frequent use of sungkai leaves as traditional medicine.

The acute toxicity test is part of a preclinical test, to measure the toxicity effect of a compound that will occur within 24 hours after a single dose (10). The LD₅₀ toxicity test is tested to determine the lethal dose value of a substance, with this the researcher is interested in researching the acute toxicity test of LD₅₀ sungkai leaves in white mice.

2. RESEARCH METHODS

Tools and Materials

Rotary Evaporator (IKA RV10[®]), glassware (pyrex[®]), Sungkai leaves (*Peronema canescens*) obtained in Jambi City, male and female white mice (*Mus musculus*), 96% ethanol, aqua dest, hot water, NaCMC, food and drink mice.

Ethical Clearance

Ethical Clearance is a written statement given by the Research Ethics Commission for research involving living things and which states that a research proposal is feasible to be carried out according to 7 WHO standards. Ethical Clearance was carried out at the Ethics Committee of the Health Service Poltekkes of the Ministry of Health, Jambi. Ethically feasible information that has been carried out consecutively for acute toxicity tests LD₅₀ sungkai leaf extract (*Peronema canescens*) on white mice (*Mus musculus*) No. LB. 02.06/2/29/2022.

Extraction

The extraction process of sungkai leaves that have been cleaned, dried, and pureed, the next step is extraction by maceration method using 96% ethanol (1: 10 w / v) in a dark bottle closed, then allowed to stand for 24 hours. Furthermore, filtering and squeezing are carried out, then the pulp is added again with 70% ethanol solvent until the sample is completely submerged, this soaking and filtering process is carried out for 3 days with 3 solvent changes or carried out until the solvent color is clear. After all the filtrate produced is then mixed, then the next step is concentration using a rotary evaporator at a temperature of 50⁰C until finally the results of the viscous extract are obtained. The basic working principle of the rotary evaporator is to trigger the evaporation of the extraction solvent and convert the solvent back into a liquid form. The condensation process occurs with the help of a censor until the solvent is accommodated in a separate state with a sample called an extract(11–16).

Acute Toxicity Test LD₅₀

This research is a laboratory experimental study where sungkai leaf extraction activity will be tested using maceration methods, phytochemical screening test, and acute toxicity test LD₅₀ sungkai leaf ethanol extract on white mice (*Mus musculus*) male 25 experimental animals and females as many as 25 experimental animals, each of which is subdivided into five treatment groups. A total of 10 experimental groups were obtained, where each group consisted of five experimental animals with a body weight of 20-25 grams (17) each (male and

female mice were separated). The group was treated with the administration of sungkai leaf ethanol extract with doses of multiples as follows:

- Control group: not given treatment (fraction) and only given 1% Na-CMC.
- Group F1: given 625 mg/kg body weight of sungkai leaf ethanol extract.
- Group F2: given 1,250 mg/kg body weight of sungkai leaf ethanol extract.
- Group F3: given 2,500 mg/kg body weight of sungkai leaf ethanol extract.
- Group F4: given 5,000 mg/kg body weight of sungkai leaf ethanol extract.

Before acute toxicity testing of LD₅₀ test animals were acclimatized for 7 days and fed and drank ad libitum. The acclimatization process aims to create test animal conditions in an environmental atmosphere (18), The acclimatization process is carried out at the Pharmacology Laboratory of the Health Polytechnic of the Jambi Province Pharmacy Study Program. The test animals were satisfied the day before the 18-hour LD₅₀ acute toxicity test but were still given ad libitum drinking. The goal is to ensure that no food intake can affect the series of testing processes at acute toxicity values LD₅₀ (19). The administration of sungkai leaf ethanol extract to animals is carried out orally through sonde and is only given once with a pre-designed dose. Observations in this study were carried out for 24 hours. Test animals are sacrificed to determine the presence of changes or damage to the organs of mice. The mice are dislocated on the occipital section, then the mice are dissected to take the heart, lungs, stomach, liver, and kidneys. Next, the organ is weighed to calculate the weight ratio of the mice organ.

3. RESULT AND DISCUSSION

Result

Toxicity Test Results

Data obtained from the LD₅₀ toxicity test of sungkai leaf ethanol extract (*Peronema canescens*) on male and female white mice (*Mus musculus*) of the Swiss strain Webster are shown in the following table form.

Table 1. Male mice toxicity test results

Dose (mg/kg body weight)	Number of Dead		Pi
	Experimental Animals	Experimental Animals	
625 mg/kg body weight	0	5	0
1250 mg/kg body weight	0	5	0
2500 mg/kg body weight	0	5	0
5000 mg/kg body weight	2	5	0.4

Tabel 2. Hasil uji toksisitas mencit betina

Dose (mg/kg body weight)	Number of Dead		Pi
	Experimental Animals	Experimental Animals	
625 mg/kg body weight	2	5	0.4
1250 mg/kg body weight	4	5	0.8
2500 mg/kg body weight	5	5	1
5000 mg/kg body weight	5	5	1

LD₅₀ value of sungkai leaf ethanol extract

It was obtained that the number of deaths in test animals from all 4 doses of multiples of sungkai leaf extract produced a sequence of mortality rates, namely 0,0,0,2 in the male group, while in female mice produced a mortality rate, which was 2,4,5,5. Of these deaths, only male mice can be counted as LD₅₀. Based on the calculation table of LD₅₀ Indonesian Pharmacopoeia Edition III (20), with the formula:

Calculation formula LD₅₀:

$$m = a - b (\sum pi - 0,5)$$

Information:

m = Log LD₅₀.

a = Log the lowest dose logarithm that causes 100% mortality per group.

b = Sequential dose log differences.

$\sum pi$ = Number of animals that died at dose divided by animal test dose.

The results of the acute toxicity test of LD₅₀ ethanol extract of sungkai leaves on white mice using the Indonesian Pharmacopoeia Method Edition III obtained an unbiased LD₅₀ value calculated in the male animal group. This is because, although it has been tried at the highest doses, there is still no data on 100% mortality in that group. This opens up opportunities for the continuation of this study using higher doses. In the group of female animals, an LD₅₀ value of 6.50 mg/kg body weight was obtained. It falls into the category of extremely toxic and tends to be dangerous for the female group of animals.

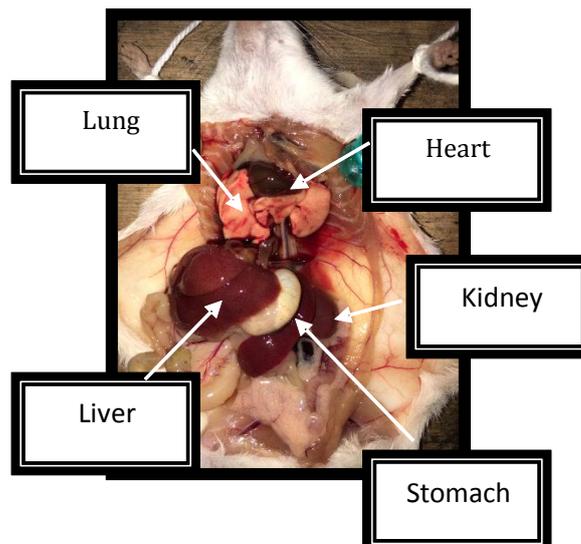


Figure 1. The shape and location of the mice organ

Organ Weight Ratio Results

Table 3. Results of the weight ratio analysis of the mice organ of the Duncan and Bonferroni test.

Organ	Dose (mg/kg body weight)	Sig. Female	Sig. Male
Heart	625	>0,05	>0,05
	1250	<0,05	>0,05
	2500	<0,05	>0,05
	5000	<0,05	>0,05
Liver	625	>0,05	>0,05
	1250	>0,05	>0,05
	2500	<0,05	>0,05
	5000	>0,05	>0,05
Lungs	625	>0,05	>0,05
	1250	<0,05	>0,05
	2500	<0,05	>0,05
	5000	<0,05	>0,05
Kidney	625	>0,05	>0,05
	1250	>0,05	>0,05
	2500	<0,05	>0,05
	5000	>0,05	>0,05
Stomach	625	>0,05	>0,05
	1250	>0,05	>0,05
	2500	<0,05	>0,05
	5000	>0,05	>0,05

DISCUSSION

An acute toxicity test conducted using the Indonesian Pharmacopoeia method Edition III (20) is a test to measure toxicity by determining the value of LD₅₀. The value of LD₅₀ can be known by calculating the number of animals that died within 24 hours (21) after multiples of doses in test animals, namely doses of 625, 1,250, 2,500, and 5,000 mg/kg body weight (22).

Of the death rates, the highest number of deaths is in female mice, which is due to males and females, there is a difference in sensitivity to toxicity (23) and because of the different hormones between males and females. These differences will always be directly influenced by the endocrine system (24), which functions in producing hormones that communicate between cells (25). Therefore it can be stated that sex differences affect the LD₅₀ value.

Death in test animals is possible on an ethanol extract of sungkai leaves containing flavonoid compounds (26). Where this flavonoid compound is included in the phenolic group, which is known if flavonoids are at excessive levels in the cell, it will be able to cause a functional group –OH (hydroxyl) in flavonoids will bind to integral proteins on the cell membrane (27). This causes the cessation of active transport which results in the process of ion entry will be uncontrollable in the cell, so this can cause cell death (necrosis) (28). The number of dead cells will result in a state of disruption of the body's metabolism so that it can trigger disruption of the normal functioning of organs in the body. The results of the analysis of organ weight ratio data using SPSS, Duncan, and Bonferroni further tests were used. Duncan's further test to see the difference in the value of different numbers in different subsets statistically, while for Bonferroni it is used as a verification stage if the data shown by Duncan cannot be fully used.

In the weight ratio of the heart organs in the female group, there was a noticeable difference ($p < 0.05$) in the dose group of 1250mg/kg body weight, 2500 mg/kg body weight, and 5000 mg/kg body weight. Meanwhile, in the



dose group of 625 mg/kg body weight, there was no such data. This states that organ weights in the dose group of 1250mg/kg body weight, 2500 mg/kg body weight, and 5000 mg/kg body weight are predicted to have a noticeable effect. This shows that in this dose group it can potentially damage the heart organs if consumed at that dose. These results can potentially trigger damage to the heart muscle and decreased activity of the experimental animal heart organs (29,30). In the male group, the influence on organ weight did not have a statistically noticeable impact ($p>0.05$). These two groups of animals can certainly be attributed to the number of animals that died at the time of the LD₅₀ test.

In the weight ratio of liver organs in the female group, it was shown that only the dose group of 2500mg/kg body weight showed a noticeable impact ($p<0.05$) on differences in animal organ weights. Meanwhile, in the animal group, 5000mg/kg body weight showed no statistically noticeable difference. This can certainly be a reason for the need for further testing at the stage of histopathology of organs, especially the liver organs (31,32). Because of course, it will be very early if toxicity data decisions are made only from the data on the weight ratio of animal organs. In the group of male animals, safe values are indicated, but this should be carried out further tests to the stage of organ histopathology.

In the ratio of lung organ weights in the female group, the results showed the same value as the heart organ data, where at doses of 1250 mg/kg body weight, 2500 mg/kg body weight, and 5000 mg/kg body weight showed a noticeable difference ($p<0.05$) in the ratio of lung organs (33). These results can also be attributed to the number of deaths in the group when the LD₅₀ test was carried out. In the group of male animals, in the entire group, the dose of administration did not show a noticeable difference ($p>0.05$) in the lung organs.

In the ratio of the weight of the kidney organs in the group of female animals, results were obtained similar to the data of the liver organs. Where only at a dose of 2500 mg/kg body weight showed a noticeable difference ($p<0.05$) in the weight ratio of the test animal organs. For this reason, it is necessary to advance the safety test of this drug to the realm of organ histopathology to investigate and ensure damage to organ cells (32,34,35). In the group of male animals, there was no noticeable difference ($p>0.05$) in all variations in the dose of administration of this drug.

In the gastric weight ratio of mice in the female group, only at a dose of 2500 mg/kg body weight showed a noticeable difference ($p<0.05$) while in other doses, there was no noticeable difference (36,37). The data obtained on the stomach organs is similar to that obtained in the liver and kidney organ data. In the male animal group, data were obtained that showed no noticeable difference ($p>0.05$) where which showed safety in the male animal group.

4. CONCLUSION

From the series of research processes that have been carried out, it was found that the LD₅₀ value of sungkai leaf ethanol extract using the Indonesian Pharmacopoeia Method Edition III in the female animal group of 6.5 mg/kg body weight is very, very toxic, while for male mice, a higher dose of acid is needed to find the acute toxicity value of LD₅₀, this indicates that the use of sungkai extract in the male group is safe to give. On the data on the ratio of organ weights in each research organ. Only the male group showed no noticeable difference ($P>0.05$) in each organ, but in the female animal group, there was a noticeable difference ($p<0.05$) in the dose group of 1250mg/kg body weight, 2500 mg/kg body weight and 5000 mg/kg body weight in the heart organs, a dose of 2500mg/kg body weight in the liver organs, a dose of 1250mg/kg body weight, 2500 mg/kg body weight and 5000 mg/kg body weight in the lung organs, dose 2500 mg/kg body weight in kidney organs, dose 2500mg/kg body weight in gastric organs.

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