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Research Article

Description of Acute Toxicity of *Ketepeng* Root Extract (*Senna alata* (L.) Roxb.)

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ABSTRACT

Introduction: People in Indonesia, especially in the West Kalimantan region often use the root of *ketepeng* as a medicine to treat jaundice, but they lack knowledge regarding the appropriate dosage. Therefore, this study aims to determine the acute toxicity of *ketepeng* root extract. **Methods:** The sample population consists of 8 male mice, which were randomly divided into 4 treatment groups, namely P1, P2, P3, and P4 with dosages of 0.56 mg, 5.6 mg, 56 mg, and 560 mg/20 g body weight, respectively. The extract was administered once, after which the samples were observed for 24 hours to record the number of deaths. Follow-up observations were then carried out for 3 days on the mice that survived the test. **Results:** The results showed that within 24 hours of administration, the samples in P1 were alive, while all animals in the other groups died. Furthermore, the follow-up observations on animals that survived showed that they were in good condition with no toxic symptoms, such as balance disorders, refusal to eat, and lack of physical activity. **Conclusion:** Based on the results, the administration of 0.56 mg/20 g body weight of the extract was relatively safe, while higher doses can cause death. However, further testing must be carried out to complete the toxicity information as well as to determine the exact dosage range to avoid mortality during the treatment.

Key words: Acute toxicity, *Fabaceae*, Roots of *Senna alata* (L.) Roxb.

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INTRODUCTION

Over the years, traditional medicine has been widely used in Indonesia to maintain health as well as to prevent and treat diseases, however, it has several advantages and disadvantages. Considering the development of science, people often prefer medical treatment because it has been clinically tested for its efficacy compared to the traditional variants.[1-3] The availability of health facilities, nevertheless, do not affect the role of plants as an intervention because people still adhere to the native methods and consider them to be cheap and easy to obtain.[2] They also have lower side effects compared to synthetic drugs.[3,4]

Herbal therapy is popular in the community because it is considered an economical treatment that is easy to obtain and has minimal adverse effects. Medicinal herbs are not only obtained from wild plants, they are also widely cultivated.[5,6] This is followed by the fast-growing of knowledge about traditional medicine obtained from the tests carried out to determine their effectiveness, efficacious doses as well as possible side effects.[7] Some of these plants have also been tested for their activities in the laboratory, including *ketepeng*/*Senna alata* (L.) Roxb.,[8] *pasak bumi*/*Eurycoma longifolia Jack.*,[9] *kratom*/*Mitragyna speciosa Korth.*,[6] *cawat anuman*/*Bauhinia* sp.,[10] red dragon fruit/*Hylocereus polyrhizus,*[11] *dayak* onions/*Eleutherine bulbosa* (Mill.) Urb.,[12] roselle flower/*Hibiscus sabdariffa L .*,[13] and *bajakah tampala*/*Spatholobus littoralis Hassk.*[14]

Ketepeng belongs to the *Fabaceae* family,[15] and its leaves contain various compounds including alkaloids, saponins, tannins, steroids, anthraquinones, flavonoids, and carbohydrates.[16] It also contains phenolics, such as rhein, chrysaphanol, kaempferol, aloeemodin, and glycosides as well as fatty acids including oleic, palmitic, and linoleic acids.[17] Furthermore, *ketepeng* flowers contain flavonoid, phenolic, saponin, and tannin compounds,[18] while the roots consist of alkaloid and anthraquinone groups.[19] A previous study reported that the seeds also contain flavonoid group compounds.[19]

Several studies showed that *ketepeng* leaves can be used to produce traditional medicines as antibacterial,[20,21] antidiabetic,[22] anti-inflammatory,[23] antimicrobial,[24] antitumor,[25] antioxidant,[26] antihelmintic,[27] anticancer,[28-30] anti-allergic,[31] antifungal,[32] hepatoprotection,[33] analgesic,[34] antidepressants,[35] and antimalarials.[36] The roots also have the potential of being used as antioxidants,[26,37] antimicrobial,[38] as well as to treat jaundice.[3] Furthermore, its flower has antifungal effects,[39] while the seeds can be used as anticancer[40] and antimicrobial.[41] A previous also showed that the bark of the plant has potential as an antimicrobial agent.[38]

Conducting a toxicity test is one of the ways of developing traditional plants into medicine.[42,43] Toxicity is defined as the capacity of a substance to be harmful to a living organism, and the tests consist of acute, subchronic, chronic, and special types.[43,44] The acute test can be used to obtain information about the symptoms of poisoning, cause of death, sequence of the death process, and the dose range that is lethal to the animal in a short time.[43,45] It can also determine the lethal dose of a substance, its possible mechanism of action, and target organs. It is a test used to determine the potential for acute toxicity.[44] Furthermore, it aims to detect the toxicity

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of a substance, determine the target organ, sensitivity, hazard data after administration by observing the symptoms, the spectrum of toxic effects, and the mechanism of death.[43,44] It can also be used to obtain initial information on the dose level required for further toxicity tests Soeksmanto *et al.* (2010)[46] using the fixed logarithmic multiple. The lowest dose of a substance does not cause any effect or symptom of poisoning, while the highest can cause the death of all test samples.[45] The median lethal dose is defined as the concentration of a substance given once (single) or several times within 24 hours that can statistically kill 50% of the experimental animals.[47] Furthermore, an herbal product is safe when it has been used for 3 generations or its toxicity has been preclinically tested using acute, subchronic, chronic, and mutagenicity tests.[48] Acute toxicity test results are an important part of safety evaluation and they also serve as a prerequisite for pharmacological tests or clinical trials before the drug is used.[47]

Considering the potential of *ketepeng* root as a broad drug, it is necessary to conduct an acute toxicity test. Therefore, this study is expected to increase the knowledge base and provide information about the safety of the plant.

MATERIALS AND METHODS

Extraction process

The root of *ketepeng* was extracted using the method proposed by Harborne (1987).[49] A total of 15.653 kg root sample was cleaned, cut into small pieces, and then dried. Subsequently, it was macerated at room temperature using 96% ethanol as solvent, after which the immersion was repeated by adding new ethanol in each repetition. The filtrate from the roots was then concentrated using a vacuum evaporator and a yield of 135.81 g was obtained.

Preparation of experimental animal

The experimental animals used were 6 healthy white male mice (*Mus musculus*) which were 6-8 weeks old with a weight range of 20-30 g. Before the experiment, the samples were acclimatized for 7 days, where they were administered with standard feed and drinking water ad libitum. Furthermore, this study was approved by the Health Research Ethics Commission, Faculty of Health Sciences, Universitas Respati Yogyakarta with reference number 019.3/FIKES/PL/I/2021.

Determination of toxicity and observation of accompanying toxic symptoms

A toxicity test was carried out using the method proposed by Weil (1952)[50], where eight mice were divided into four groups, namely P1, P2, P3, and P4. Each sample was then given an ethanol extract of *ketepeng* root through oral administration with an interval of 10 times. The dose used for each group was different, where the group with a mark on the head (P1), back (P2), tail (P3) as well as no marking (P4) were administered with 0.56 mg, 5.6 mg, 56 mg, and 560 mg/20g, respectively. To distinguish each animal between the dose levels, a yellow color mark was imprinted on the body, while the individual in each dose group was distinguished with a dot on the tail. This test was carried out by counting the number of deaths that occurred in the first 24 hours after administration. The acute toxicity test was then modified by adding observations for three consecutive days.

RESULTS

Indonesia has a diversity of plant species that are used traditionally for medicine, and are relatively easy to find and cheap. However, the use of plants in the treatment of diseases still requires dose accuracy as well as the determination of consumption period. Several studies have tested the activity of herbs that have traditionally been declared efficacious in medicine.[1,3,5] These tests can be used to evaluate their activity, appropriate dosage of use as well as the possible side effects.[7,46] Toxicity evaluations were also carried out to determine the safety and adverse effects arising from their consumption.[7,44,45] One of the medicinal plants is *ketepeng*, especially its roots, which is used by people in West Kalimantan to treat jaundice.[3]

A preparation is referred to as a drug when it is administered in the right quantity, while an overdose can render it ineffective or cause death.[44,45,47,51] At a certain dose, a compound has a probability of harming the body. An acute toxicity test is one of the toxicological evaluations of herbal drug extracts, which is often carried out before clinical trials.[52] The acute toxicity potential value as measured by the lethal dose 50 (LD_{50}) is the parameter used for the test.[43,53] It is often conducted within a period of 24 hours and conventional studies using experimental animals revealed a series of effects due to exposure to toxicants in various doses. To investigate the effects related to the period of exposure, toxicological studies are usually divided into 3 categories, namely acute, short-term, and long-term toxicity tests.[54] The acute test of *ketepeng* root extract with graded doses is presented in Table 1, Table 2, and Table 3.

DISCUSSION

All the samples were administered with *ketepeng* root ethanol extract orally. Furthermore, they were divided into 4 treatment groups, namely P1, P2, P3, and P4 with different dosages of 0.56 mg, 5.6 mg, 56 mg, and 560 mg/20g body weight, respectively. The test was carried out by counting the number of deaths in the first 24 hours of administration, after which observation was performed for 3 consecutive days on the living samples. The results showed that one animal in group P2 as well as all samples in groups P3 and P4 died within 7 hours after the intervention. The toxic symptoms observed before their death include restlessness, followed by decreased activeness, weakness, after which they died. Furthermore, another animal in group P2 died within 23 hours after the administration of the extract with toxic symptoms, such as inactivity, weakness, and diarrhea with bloody discharge. Priyanto (2010)[47] and Ngatidjan (2006)[45] reported that the toxicity of an extract can be assessed through the appearance of toxic symptoms in the form of cell biochemical, functional, and structural changes.

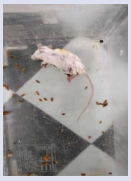
Ngatidjan (2006)[45] stated that the lowest dose in toxicity testing does not cause any effects or poisoning symptoms, while the highest leads to the death of all the experimental animals. The observed effect is death, which indicates that after the administration of a substance, there are 2 possible effects, namely samples that die and others that survive the dose level. The deaths, which occurred in this study after multiple dosing was also associated with the high and low doses administered as well as the number of compounds contained in the extract.

Ketepeng roots contain compounds belonging to the alkaloid and anthraquinone groups.[19] Although alkaloids in plants have been widely used in the field of pharmacology, especially in drug discovery, some of them, such as pyrrolizidine, tropane, piperidine, and indoleizidine have shown toxic effects in humans and animals including itching, nausea, vomiting, mild gastrointestinal disturbances, psychosis,

Table 1: The number of experimental animals' death due to the administration of *ketepeng* roots ethanol extract, which was observed for 24 hours after administration with graded doses.

No.	Treatment group	Number of experimental animals	Number of dead animals
1	Dosage 0.56 mg/20 g body weight	2	0
2	Dosage 5.6 mg/20 g body weight	2	2
3	Dosage 56 mg/20 g body weight	2	2
4	Dosage 560 mg/20 g body weight	2	2

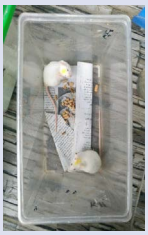
Table 2: Description of the experimental animals' condition observed for 24 hours after administration of *ketepeng* roots ethanol.

No.	Observation time	Description	Figure
1	0 hour On March 1, 2022, at 07:00 am	Administering *ketepeng* root extract preparations to the animals.	
2	1 hour On March 1, 2022, at 08:03 am	All the animals are actively moving. Mice in group P2, P3, and P4 looked restless, and often aim for drinking water.	
3	2 hours On March 1, 2022, at 09:00 am	The samples were trying to sleep with no activity.	
4	3 hours On March 1, 2022, at 10:03 am	The samples were trying to sleep with no activity.	
5	7 hours On March 1, 2022, at 2:30 pm	One sample in P2 (second mice with code back point 2) died along with two animals in P3 and P4. All mice in group P1 were agile, eating, and actively moving, while one sample in P2 was weak and had diarrhea with bloody discharge.	
6	8 hours On March 1, 2022, at 3:00 pm	Two mice in group P1 and one mouse in P2 were sleeping.	
7	9 hours On March 1, 2022, at 4:00 pm	Two animals in group P1 were actively moving, while one sample (back code individual with point 1) in P2 appeared weak and had diarrhea with bloody discharge.	
8	10 hours On March 1, 2022, at 05:06 pm	The animals in P1 were actively moving, agile, and eating, while one sample in P2 was weak and had diarrhea with blood discharge.	
9	23 hours On March 2, 2022, at 06:00 am	One sample in group P2 died. All animals in P1 were fresh, agile, eating, and actively moving.	 

10	24 hours On March 2, 2022, at 07:00 am	All samples in group P1 were fresh, agile, eating, and moving normally.	
		After 24 hours of observation, two mice in P2, P3, and P4 died.	

Table 3: Description of the experimental animals' condition after administration of *ketepeng* roots ethanol extract. Observations were made from the 24th to the 96th hour.

No.	Observation time	Description	Figure
1	24 hours On March 2, 2022, 07:00 am	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
2	25 hours On March 2, 2022, 08:00 am	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
3	26 hours On March 2, 2022, 09:00 am	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
4	30 hours On March 2, 2022, 01:12 pm	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
5	31 hours On March 2, 2022, 02:00 pm	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	

6	32 hours On March 2, 2022, 03.00 pm	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
7	33 hours On March 2, 2022, 4:00 pm	The animals were asleep with no activity.	
8	47 hours On March 3, 2022, 06:38 am	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
9	48 hours On March 3, 2022, 07:30 am	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
10	49 hours On March 3, 2022, 08:00 am	The animals were asleep with no activity.	
11	50 hours On March 3, 2022, 09:00 am	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
12	54 hours On March 3, 2022, 01.28 pm	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	

13	57 hours On March 3, 2022, 04:00 pm	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
14	71 hours On March 4, 2022, 06:43 am	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
15	73 hours On March 4, 2022, 08:00 am	All experimental animals in group P1 were fresh, agile, eating and moving normally.	
16	78 hours On March 4, 2022, 01:03 pm	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
17	79 hours On March 4, 2022, 02:30 pm	The animals were asleep with no activity.	
18	81 hours On March 4, 2022, 04:00 pm	The animals were asleep with no activity.	

19	95 hours On March 5, 2022, 06:00 am	All experimental animals in group P1 were fresh, agile, eating, and moving normally.	
20	96 hours On March 5, 2022, 07:00 am	From the 24th hour to the 96th hour, the samples in the group P1 were fresh, agile, eating, and moving normally. They also sleep regularly.	

paralysis, teratogenicity, arrhythmia, and sudden death.[54] Furthermore, anthraquinones are complex aromatic carbons present in some herbs and plants, where they can be consumed as either anthrones or bianthrones. Excessive consumption of the compound causes stomach cramps, digestive discomfort, vomiting, dermatitis, nausea, bloody diarrhea, and dizziness.[55] The alkaloids present in the *ketepeng* root include physcion, ω-hydroxyemodin, ziganein, apigenin, and trans-resveratrol, while the anthraquinones consist of aloe-emodin, rhein, emodin, and chrysophanol.[19] The results showed that to safely consume the ethanolic extract, caution must be taken in terms of the dose and duration of consumption.

CONCLUSION

It can then be concluded that the administration of less than or equal to 0.56 mg/20 g body weight was relatively safe, while higher doses can cause death. However, further testing is needed to obtain complete toxicity information as well as to determine the exact range of doses that leads to death.

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CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

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GRAPHICAL ABSTRACT

Senna alata (L.) Roxb.

Roots of *Senna alata* (L.) Roxb.

Acute Toxicity Test

The number of experimental animals' death due to the administration of roots of *Senna alata* (L.) Roxb. ethanol extract, which was observed for 24 hours after administration with graded doses.

No.	Treatment group	Number of experimental animals	Number of dead animals
1	Dosage 0.56 mg/20 g body weight	2	0
2	Dosage 5.6 mg/20 g body weight	2	2
3	Dosage 56 mg/20 g body weight	2	2
4	Dosage 560 mg/20 g body weight	2	2

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