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

Ruqiah Ganda Putri Panjaitan,*, Titin1, Yohanes Gatot Sutapa Yuliana2

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.

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.4 They also have lower side effects compared to synthetic drugs.4,5

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.6,7 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.,1 pasak bumi/Eurycoma longifolia jack,2 kratom/Mitragyna speciosa Korth.,3 cawat anuman/Bauhinia sp.,4 red dragon fruit/Hylocereus polyrhizus,11 dayak onions/Eleutherine bulbosa (Müll.) Urb.,12 roselle flower/Hibiscus sabdariffa L.13,15 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, aloemodin, 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 anti-diabetic,20 anti-inflammatory,21 antimicrobial,24 antitumor,22,23 antioxidant,22,23 antimalarials,36 the roots also have the potential of being used as antioxidants,26,30 antimicrobial,28 as well as to treat jaundice.1 Furthermore, its flower has antifungal effects,31 while the seeds can be used as antitumor,27 antioxidant,27 and antimalarial.26 The roots also have the potential of being used as antioxidants,26,30 antimicrobial,30 as well as to treat jaundice.1 Furthermore, its flower has antifungal effects,31 while the seeds can be used as antitumor,27 antioxidant,27 and antimalarial.26 The roots also have the potential of being used as antioxidants,26,30 antimicrobial,30 as well as to treat jaundice.1 Furthermore, its flower has antifungal effects,31 while the seeds can be used as antitumor,27 antioxidant,27 and antimalarial.26 The roots also have the potential of being used as antioxidants,26,30 antimicrobial,30 as well as to treat jaundice.1 Furthermore, its flower has antifungal effects,31 while the seeds can be used as antitumor,27 antioxidant,27 and antimalarial.26 The roots also have the potential of being used as antioxidants,26,30 antimicrobial,30 as well as to treat jaundice.1 Furthermore, its flower has antifungal effects,31 while the seeds can be used as antitumor,27 antioxidant,27 and antimalarial.26 The roots also have the potential of being used as antioxidants,26,30 antimicrobial,30 as well as to treat jaundice.1

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.42,43 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.42,43 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.
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.\(^4,5,6\) It can also be used to obtain initial information on the dose level required for further toxicity tests.\(^6\) Soeksmanto et al. (2010)\(^7\) 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.\(^8\) 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.\(^9\) 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.\(^10\) 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.\(^11\)

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 method**

The root of ketepeng was extracted using the method proposed by Harborne (1987).\(^12\) 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 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/1/2021.

**Determination of toxicity and observation of accompanying toxic symptoms**

A toxicity test was carried out using the method proposed by Weil (1952)\(^13\), 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.\(^14,15\) These tests can be used to evaluate their activity, appropriate dosage of use as well as the possible side effects.\(^16\) Toxicity evaluations were also carried out to determine the safety and adverse effects arising from their consumption.\(^17,18,19\) One of the medicinal plants is ketepeng, especially its roots, which is used by people in West Kalimantan to treat jaundice.\(^20\)

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.\(^21,22,23,24\) 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.\(^25\) The acute toxicity potential value as measured by the lethal dose 50 (LD\(_{50}\)) is the parameter used for the test.\(^26,27\) 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.\(^28\) 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 activity, 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)\(^29\) and Ngatidjan (2006)\(^30\) 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)\(^31\) 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.\(^32\) 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>
<thead>
<tr>
<th>No.</th>
<th>Treatment group</th>
<th>Number of experimental animals</th>
<th>Number of dead animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dosage 0.56 mg/20 g body weight</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Dosage 5.6 mg/20 g body weight</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Dosage 56 mg/20 g body weight</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Dosage 560 mg/20 g body weight</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>
Table 2: Description of the experimental animals’ condition observed for 24 hours after administration of *ketepeng* roots ethanol.

<table>
<thead>
<tr>
<th>No.</th>
<th>Observation time</th>
<th>Description</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>0 hour&lt;br&gt;On March 1, 2022, at 07:00 am</td>
<td>Administering <em>ketepeng</em> root extract preparations to the animals.</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>2</td>
<td>1 hour&lt;br&gt;On March 1, 2022, at 08:03 am</td>
<td>All the animals are actively moving. Mice in group P2, P3, and P4 looked restless, and often aim for drinking water.</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>3</td>
<td>2 hours&lt;br&gt;On March 1, 2022, at 09:00 am</td>
<td>The samples were trying to sleep with no activity.</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>4</td>
<td>3 hours&lt;br&gt;On March 1, 2022, at 10:03 am</td>
<td>The samples were trying to sleep with no activity.</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>5</td>
<td>7 hours&lt;br&gt;On March 1, 2022, at 2:30 pm</td>
<td>All mice in group P1 were agile, eating, and actively moving, while one sample in P2 was weak and had diarrhea with bloody discharge.</td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
<tr>
<td>6</td>
<td>8 hours&lt;br&gt;On March 1, 2022, at 3:00 pm</td>
<td>Two mice in group P1 and one mouse in P2 were sleeping.</td>
<td><img src="image6.png" alt="Image" /></td>
</tr>
<tr>
<td>7</td>
<td>9 hours&lt;br&gt;On March 1, 2022, at 4:00 pm</td>
<td>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.</td>
<td><img src="image7.png" alt="Image" /></td>
</tr>
<tr>
<td>8</td>
<td>10 hours&lt;br&gt;On March 1, 2022, at 05:06 pm</td>
<td>The animals in P1 were actively moving, agile, and eating, while one sample in P2 was weak and had diarrhea with blood discharge.</td>
<td><img src="image8.png" alt="Image" /></td>
</tr>
<tr>
<td>9</td>
<td>23 hours&lt;br&gt;On March 2, 2022, at 06:00 am</td>
<td>One sample in group P2 died.</td>
<td><img src="image9.png" alt="Image" /></td>
</tr>
<tr>
<td></td>
<td></td>
<td>All animals in P1 were fresh, agile, eating, and actively moving.</td>
<td><img src="image10.png" alt="Image" /></td>
</tr>
</tbody>
</table>
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.

<table>
<thead>
<tr>
<th>No.</th>
<th>Observation time</th>
<th>Description</th>
<th>Figure</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24 hours</td>
<td>All experimental animals in group P1 were fresh, agile, eating, and moving normally.</td>
<td><img src="image1.png" alt="Image" /></td>
</tr>
<tr>
<td>2</td>
<td>25 hours</td>
<td>All experimental animals in group P1 were fresh, agile, eating, and moving normally.</td>
<td><img src="image2.png" alt="Image" /></td>
</tr>
<tr>
<td>3</td>
<td>26 hours</td>
<td>All experimental animals in group P1 were fresh, agile, eating, and moving normally.</td>
<td><img src="image3.png" alt="Image" /></td>
</tr>
<tr>
<td>4</td>
<td>30 hours</td>
<td>All experimental animals in group P1 were fresh, agile, eating, and moving normally.</td>
<td><img src="image4.png" alt="Image" /></td>
</tr>
<tr>
<td>5</td>
<td>31 hours</td>
<td>All experimental animals in group P1 were fresh, agile, eating, and moving normally.</td>
<td><img src="image5.png" alt="Image" /></td>
</tr>
</tbody>
</table>
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.
All experimental animals in group P1 were fresh, agile, eating, and moving normally.

On March 4, 2022, 06:43 am

All experimental animals in group P1 were fresh, agile, eating, and moving normally.

On March 4, 2022, 08:00 am

All experimental animals in group P1 were fresh, agile, eating and moving normally.

On March 4, 2022, 01:03 pm

All experimental animals in group P1 were fresh, agile, eating, and moving normally.

On March 4, 2022, 02:30 pm

The animals were asleep with no activity.

On March 4, 2022, 04:00 pm

The animals were asleep with no activity.
paralysis, teratogenicity, arrhythmia, and sudden death. Furthermore, anthraquinones are complex aromatic carbons present in some herbs and plants, where they can be consumed as either anthers or bianthrone. Excessive consumption of the compound causes stomach cramps, digestive discomfort, vomiting, dermatitis, nausea, bloody diarrhea, and dizziness. The alkaloids present in the ketepeng root include phsysin, ω-hydroxyemodin, ziganein, apigenin, and trans- resveratrol, while the anthraquinones consist of aloe-emodin, rhein, emodin, and chrysophanol. 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.

ACKNOWLEDGMENTS

The authors are grateful to the Indonesia Ministry of Research and Technology/National Research as well as the Innovation Agency for funding this study. The authors also appreciate all informants for sharing knowledge about plants that are efficacious in treating jaundice as well as the regional heads for connecting the informants.

CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

REFERENCES

42. Ibrahim MA, Anvar, Yusuf NI. Uji lethal dose 50% (LD50) polihedral (Curcuma xanthorrhiza, Kleinovia hospita, Nigella sativa, Arcangelisia flava, and Ophiocephalus striatus) pada hepamin® terhadap mencit (Mus musculus). Jakarta: Royal Medika. 2012.
50. Well C. Tables for Convenient Calculation of Median Effective Dose (LD50 or ED50) and Instruction in Their Use. Biometrics. 1952;8:249-263.
52. Lu FC. Toksikologi dasar asas organ sasaran dan penilaian risiko. Leskonfi Lembaga Studi dan Konsultasi Farmakologi; 2010.
54. Well C. Tables for Convenient Calculation of Median Effective Dose (LD50 or ED50) and Instruction in Their Use. Biometrics. 1952;8:249-263.
GRAPHICAL ABSTRACT

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.

<table>
<thead>
<tr>
<th>No.</th>
<th>Treatment group</th>
<th>Number of experimental animals</th>
<th>Number of dead animals</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Dosage 0.56 mg/20 g body weight</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>Dosage 5.6 mg/20 g body weight</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Dosage 56 mg/20 g body weight</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>4</td>
<td>Dosage 560 mg/20 g body weight</td>
<td>2</td>
<td>2</td>
</tr>
</tbody>
</table>

ABOUT AUTHORS

Ruqiah Ganda Putri Panjaitan is graduated from the Department of Biology, FMIPA Andalas University, Padang. She got her masters and doctorates from the Department of Biology FMIPA Bogor Agricultural University. Her field of interest is the study of medicinal plant activity. Now, she is working as a lecturer in Tanjungpura University.

Titin is graduated from Department of Biology Education, Yogyakarta State University in 2006. She got her master from the Department of Science Education, Sebelas Maret University in 2012. Now, she is working as a Biology Education Lecturer in Tanjungpura University. Her field of expertise is a Science Education. She teaches Teaching and Learning, Plant Morphology, and Plant Taxonomy.

Yohanes Gatot Sutapa Yuliana is a lecturer of Undergraduate and Master Study Program of English Language Education of Tanjungpura University. He wrote his doctoral dissertation on English teaching material development for vocational schools. He also attended sandwich-like for visiting scholar program in University of Illinois at Urbana USA 2010, and Community Development Program in Kansas State University 2019. On his professional duties, he teaches Research Approaches, TEFL Assessment, TEFL Methodologies, Academic Writing and Presentation, and Research Paper, besides doing research on localized folklore.

Cite this article: Panjaitan RGP, Titin, Yuliana YGS. Description of Acute Toxicity of Ketepeng Root Extract (*Senna alata* (L.) Roxb.) Pharmacogn J. 2022;14(4):.