ETHANOLIC EXTRACT OF CATAPPA LEAVES (*Terminalia catappa*) LETHAL DOSE (LD$_{50}$) ACUTE TOXICITY TEST IN MICE (*Mus musculus*)

LIM SUK WUN

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ABSTRACT

LIM SUK WUN. Ethanolic Extract of Catappa Leaves (Terminalia Catappa) Lethal Dose (LD$_{50}$) Acute Toxicity Test in Mice (Mus Musculus). Supervised by ANDRIYANTO and TUTIK WRESDIYATI.

Catappa leaves are commonly used as a traditional herb by the local community to treat certain diseases because it contains medicinal properties which include antimicrobial, antiparasitic, antibacterial, antiinflammatory, antidiabetic, antioxidant, hepatoprotective and anticancer properties. Acute toxicity test was done to evaluate the safety level of the catappa leaf extract. This study used 30 male mice (25±5g) which were divided into 6 groups; 1 control group and 5 treatment groups. The control group was treated with aquadest, while the treatment groups were treated with catappa leaf ethanolic extract at doses of 1, 2, 4, 8, and 16 g/kg body weight (BW). The catappa leaf ethanolic extract and aquadest were administered in a single dose orally by using a oral gavage. Mortality rate of the mice was observed at 48 hours post treatment. The primary output is the LD$_{50}$ values of the extract. The value of LD$_{50}$ is 16.3523 g/kg BW, which meant that the extract is practically nontoxic. Histomorphological evaluation showed that some degeneration of cells were observed in the liver and kidney, especially in treatment groups of doses 4, 8, and 16 g/kg BW.

Keywords: histomorphology, LD$_{50}$, Terminalia catappa.

ABSTRAK

LIM SUK WUN. Uji Toxidisitas Akut Lethal Dose (LD$_{50}$) Ekstrak Etanol Daun Ketapang (Terminalia catappa) pada Mencit (Mus musculus). Dibimbing oleh ANDRIYANTO dan TUTIK WRESDIYATI.

Daun ketapang digunakan oleh masyarakat sebagai ramuan tradisional pengobatan beberapa penyakit karena mempunyai aktivitas antimikroba, antiparasit, antibakteri, antiinflamasi, antidiabetik, antioksidan, hepatoprotektif, dan aktivitas antikanker. Uji toxidisitas akut ini dilakukan untuk mengetahui tingkat keamanan penggunaan ekstrak etanol daun ketapang (Terminalia catappa). Penelitian ini menggunakan mencit jantan sebanyak 30 ekor (25±5 g) yang dibagi menjadi 6 kelompok; 1 kelompok kontrol dan 5 kelompok perlakuan. Kelompok kontrol dicekok akuades dan kelompok perlakuan dicekok ekstrak etanol daun ketapang dengan dosis bertingkat sebanyak 1, 2, 4, 8, dan 16 g/kg bobot badan (BB). Pemberian ekstrak etanol daun ketapang dan akuades menggunakan sonde lambung dan diberikan dalam dosis tunggal. Mortalitas didapatkan pada 48 jam setelah perlakuan. Berdasarkan hasil penelitian nilai LD$_{50}$ yang didapat adalah sebesar 16.3523 g/kg BB dan termasuk dalam kategori praktis tidak toksik. Pengamatan histomorfologi menunjukkan adanya degenerasi sel pada hati dan ginjal terutama pada kelompok perlakuan dosis 4, 8, dan 16 g/kg BB.

Kata kunci: histomorfologi, LD$_{50}$, Terminalia catappa
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LIM SUK WUN

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The author is aware that this paper is far from perfect, so critics and suggestions are highly expected for better research results. Thank you to the party who gave criticism and suggestions to the author. The author hopes that this article can provide special benefits for writers and readers.

Bogor, June 2019

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INTRODUCTION

Background

The use of plants as medicinal raw materials is one of the alternatives to meet the needs of the community in the health sector. One of the plants used is *Terminalia catappa*. *Terminalia catappa* comes from the *Combretaceae* family or locally known as ketapang (catappa) in Indonesia. This plant is naturally found in subtropical and tropical regions. It has been proven that this plant extract is rich in phenolic compounds, flavonoids, alkaloids, triterpenoids, tannins and other compounds that allow catappa plants to be used as drugs (El-Rafie & Hamed 2014). The efficacy of catappa plants is antimicrobial, antitumor, antiparasitic, antibacterial, anti-inflammatory, antidiabetic, antioxidant, anticancer and as hepatoprotector (Sri Rahayu et al. 2009; Akharaiyi et al. 2011; Pandya et al. 2013; Anand et al. 2015; Sutraeni et al. 2016).

Every part of the catappa plant can be used as medicine, its leaves can treat several diseases. According to Fan et al. (2004), catappa leaves can reduce edema by more than 50% in mice ears because it has anti-inflammatory properties. According to Khan et al. (2014), catappa leaves made into ointments have antibacterial properties in treating wounds and can accelerate wound healing in mice. Phenolic compounds and flavonoids contained in catappa show antitumor and antioxidant effects in mice. Pandya et al. (2013) reported that mice treated with catappa showed an increase in peritoneal cells compared to control mice due to the antitumor and antioxidant effects of catappa. According to Lin et al. (1997), catappa also showed hepatoprotector activity of carbon tetrachloride (CCl4) induced in mice.

Traditionally, catappa plants are used by society to treat diseases related to the liver, diarrhea, abdominal pain, headaches, skin diseases, as antioxidants and preventing cancer. In addition, catappa leaves are also used to treat dysentery and diarrhea in patients (Lin & Kan 1990; Jaziroh 2008; Chansue & Assawawongkasem 2011). Research by Sumintir et al. (2012) showed that extracts taken from catappa leaves and catappa skin had antimicrobial properties against *Staphylococcus aureus* and *Candida albicans*.

Catappa leaves are used to treat fish infected by parasites, fungi and bacteria. According to Sumino et al. (2013), the concentration of catappa leaf extract of 200 mg/mL had the most optimum results in treating *Aeromonas salmonicida* infections in catfish. Chitmanat et al. (2005) stated that catappa leaf extract concentration of 800 ppm proved effective against *Trichodina alveolate* and other bacteria and fungi infections in *Tilapia*. In addition, catappa leaf extract can inhibit the growth of *Aeromonas hydrophila* in tilapia and is effective in reducing fungal infections in tilapia eggs (Astyva 2014).

Catappa leaves have potential as a cure for several diseases, but little information is known about the level of toxicity and safety. Toxicity tests are an important requirement in determining the level of toxicity and the toxic effects of substances contained in catappa leaves. Toxicity is defined as the concentration of chemicals that can interfere with the physiological function of an organism. One method for testing toxicity is the Lethal Dose Test (LD₅₀) (Supriyono 2007). This
test is used to determine the level of toxicity and unexpected side effects of catappa leaf extract. This study aims to determine the LD$_{50}$ value of the ethanolic extract of catappa leaves in mice. The results are expected to be a base of scientific information for consideration when using plants as a medicinal herb.

**Purpose of Study**

This study aimed to test acute toxicity (LD$_{50}$) of ethanolic extract of catappa leaves in mice strain Dunken Yoken Deutschland (DDY) based on number of deaths through Thomson and Weil method and its effect on histomorphological changes in liver and kidney.

**Benefits of Study**

This study is expected to provide information on acute toxicity LD$_{50}$ of ethanolic extract of catappa leaves in mice. This study is also expected to provide information about the relationship of dosage and toxicity to repeated administration of ethanolic extract of catappa leaves in a certain period of time to liver and kidney function in healthy mice, as a basis in considering the use of the plant as phytopharmaca.

**Hypothesis of Study**

The administration of ethanolic extract of catappa leaves with certain concentrations in mice can cause the death of 50% of the population and cause histomorphological changes in liver and kidney.

**LITERATURE REVIEW**

**Catappa Leaf**

*Terminalia catappa* is a plant commonly known as ketapang in Indonesia or as "Tropical or Indian almond" in other countries such as England. This plant is from the family *Combretaceae* (Dwi 2008). Catappa plants are large trees with a height reaching 40 m and stems up to 1.5 m. This plant is commonly found in Southeast Asia, but is rarely found in Sumatra and Kalimantan. This plant can also be found in northern Australia, Polynesia, India, Pakistan, Madagascar, East Africa, West Africa, Central America and South America. Catappa leaves are cylindrical with a slightly flattened side that thickens at the base. This leaf is slippery on the upper surface and has smooth hairs on the lower side (Thomson & Evans 2006). The scientific classification of *Terminalia catappa* according to Jagessar and Alleyne (2011) is as follows:
Kingdom: Plantae
Phylum: Magnoliophyta
Class: Magnoliopsida
Order: Myrtales
Family: Combretaceae
Genus: Terminalia
Species: Terminalia catappa.

Based on the phytochemical identification of catappa leaves conducted by Akharaiyil et al. (2011), catappa leaves can be used as medicine because they contain many compounds such as hydrolyzed tannins (terflavins A and B), terpallagine, tercatain, punicalagin, punicalin, chebulagic acid, geraniin, granatin B, corilagin, 1-desgalloyleugeniiin, flavonoids (kaemopherol or quercetin), saponins and pitoster (Tanaka et al. 1986; Dwi 2008; Jagessar & Alleyne 2011). The contents of these compounds are more common in young catappa leaves. Based on several studies, almost every parts of the catappa plant can be used as traditional medicine (Zuhrotun & Suganda 2010). In addition, the ethanolic extract of catappa leaves also has a hepatoprotector effect by inhibiting peroxidase activity (Gao et al. 2004; Sri Rahayu et al. 2009).

Catappa plants can be used to treat diseases related to liver, diarrhea, abdominal pain, headaches, skin diseases, antioxidants and in preventing cancer (Jaziroh 2008). Catappa leaves are used as a herb to prevent and treat liver-related diseases such as hepatitis in Taiwan (Lin & Kan 1990). Tea made from catappa leaves is prescribed to patients who experience dysentery and diarrhea in Suriname. Leaves stored in aquariums are said to reduce pH and heavy metal content of water. In addition, catappa leaves also have antibacterial and antifungal properties that can prevent mold formation in fish eggs (Chansue & Assawawongkasem 2011).

Toxicity

Toxicity is defined as the concentration of chemicals that can interfere with the physiological function of an organism. It is a detrimental effect caused by body cells when interacting with toxicants. The interaction between toxics and body cells can be reversible or irreversible (Imono 2001). Reversible or irreversible toxic effects are largely determined by the tissue involved, duration of exposure and dosage given. Reversible toxic effects usually occur because organs can regenerate tissue quickly, such as the liver, intestinal mucosa and blood cells. Irreversible toxic effects usually occur during central nervous system (CNS) damage, carcinogenesis, mutagenesis and teratogenesis.

Toxicity usually occurs due to errors in dosage or errors in the route of administration of chemicals (Siswandono & Bambang 1995). Chemicals at high dosage can cause toxicity. Toxicity usually affects vital organs such as the liver and kidneys, causing adverse effects on humans and animals. Therefore, toxicity tests are needed to measure the toxicity of the compounds tested.

Toxicity tests can be used to evaluate acute, subchronic and chronic toxic effects (Asante-Duah 2017). Acute toxic effects appear after a single exposure to
chemicals and usually react quickly after giving one or several chemicals to an organism in a short time. Subchronic effects appear when chemicals cause effects after several months to years. Chronic effects are effects that appear after repeated exposure to chemicals, usually on repeated or continuous exposure to produce adverse effects. Chronic effects can cause harmful effects over a long period of time and can sometimes last a lifetime in exposed organisms (McGee et al. 1998).

LD$_{50}$ Acute Toxicity

Acute toxicity is the degree of toxic effect of a compound that can cause toxicity in a short time. Acute toxicity is carried out by giving compounds or chemicals that are tested once or several times and observations carried out within 24-48 hours. Acute toxicity test can provide quantitative data on toxicity tests by determining LD$_{50}$ values.

Lethal Dose 50 (LD$_{50}$) is the number of doses obtained from chemicals that can statistically give rise to 50% of deaths from the number of animals tested and observed in a certain period of time (Adamson 2016). Symptoms of poisoning can be determined by the number of animal deaths. Based on the Environmental Protection Agency (EPA 1998), LD$_{50}$ can be measured using multilevel doses in several groups. The group was divided into control groups and several treatment groups with different doses levels. Calculation of LD$_{50}$ can be done based on the number of deaths of experimental animals in each group. Observations were carried out for 24-48 hours. Assessment of LD$_{50}$ has now been used as the main parameter in measuring acute toxicity as well as an initial procedure for general screening of chemical agent toxicity and pharmacological agents. Acute toxicity studies only provide information about LD$_{50}$, therapeutic index and the level of safety of pharmacological agents. According to Handayani et al. (2012), the LD$_{50}$ value is very useful for classifying chemicals according to their relative toxicity. Furthermore, toxicity classification is presented in Table 1.

<table>
<thead>
<tr>
<th>Toxicity</th>
<th>Lethal Dose (LD$_{50}$)</th>
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<tr>
<td>Super toxic</td>
<td>$\leq 5$ mg/kg</td>
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<tr>
<td>Extremely toxic</td>
<td>5–50 mg/kg</td>
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<tr>
<td>Very toxic</td>
<td>50–500 mg/kg</td>
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<tr>
<td>Moderately toxic</td>
<td>0.5–5 g/kg</td>
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<tr>
<td>Slightly toxic</td>
<td>5–15 g/kg</td>
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<tr>
<td>Practically non toxic</td>
<td>$&gt;15$ g/kg</td>
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Table 1 Classification of toxicity according to Handayani et al. (2012)

Mice strain Deutschland Dunken Yoken (DDY)

Mouse (*Mus musculus*) is an experimental animal that is often used in research. These animals are quite economical because they breed quickly, are easily maintained in large quantities, have short pregnancy times and do not require a large space. Mice are widely used for toxicity testing, making it easier to
compare the toxicity of the chemicals tested. The response of various animals to toxicity tests can vary due to differences in progeny, age and body condition. One strain of mice that is often used in research is Deutschland, Denken and Yoken.

Mice strain Deutschland, Denken and Yoken have two different strains, the ddY strain and DDY strain. ddY strain is an outbred strain in a closed colony. DDY strains are inbred strains found in ddY colonies. These strain mice are very good for use in pharmacological, pharmacokinetics and toxicological studies because they have advantages in the field of superior reproduction and growth, as well as their purity and sensitivity for drug testing (Yamazaki et al. 2012).

**Histomorphology**

Histomorphological analysis was carried out to confirm the changes in organ cells structure. Histomorphological examination is a standard for evaluating the toxicity of a substance through morphological changes in tissues and organs due to the toxic effects of catappa leaves. Toxic effects can occur when toxic substances bind to vital organs such as the liver and kidneys.

The liver is an organ which is the main target of acute toxicity because it plays a role in drug metabolism. The liver receives various substances absorbed from the intestine including high concentrations toxic substances thus can cause hepatotoxic. When substances are processed in the body, most substances from this process are excreted outside the body through the kidneys. Product substances directly or indirectly can cause damage to the kidneys. The toxicity of a substance can be determined by looking at the histomorphology of tissue and organ cells from the liver and kidney (Jothy et al. 2011).

**METHOD**

**Time and Place of Research**

The study was conducted from May 2017 to November 2018 at Educational Animal Hospital (RSHP), Faculty of Veterinary Medicine, IPB University (FKH IPB). Phytochemical screening was carried out at the IPB Biopharmaca Study Center Laboratory. Histomorphological examination in liver and kidney tissue samples was carried out in the Histology Laboratory, Department of Anatomy, Physiology and Pharmacology, FKH IPB.

**Apparatus and Materials**

The apparatus used in this study are animal scales (neraca), buckets, plastic bags, wood, funnels, filter cloths, cages made from plastic tubs, metal gavage feeding needle, dry cloth, 1 mL syringes, gloves, scalpels, tissue forceps, microscopes and glass slides. The materials used in this study were DDY strain
mice, catappa leaves (*Terminalia catappa*) from Dramaga, Bogor, 70% ethanol, sterile aquades, catappa leaf ethanolic extract (1%, 2%, 4%, 8% and 16%), alcohol (70%, 80%, 90%, 95%, and 100%), 4% paraformaldehyde, xylene, paraffin, hematoxylin eosin (HE) and mouse feed. The experimental animals used were 30 male mice, aged 2 months old with body weight ranging from 25±5 g obtained from the Laboratory Animal Management Unit (UPLH) FKH IPB.

**Research Procedure**

**Preparation of Crude**

3 kg catappa leaves are collected, washed and dried without direct contact with sunlight. The crude of catappa leaves is put into a blender then grounded so that it is in the form of fine powder.

**Preparation of Extract**

Preparation of catappa leaf extract is done by maceration method. The dried catappa leaves were dissolved in 70% ethanol with a ratio of 70% ethanol: catappa leaves of 10:3 at room temperature for 36 hours. After that, the solution was filtered and evaporated to get catappa leaf extract.

**Phytochemical Screening**

Phytochemical screening is an initial step to detect bioactive compounds in the ethanolic extract of catappa leaves qualitatively. According to Jagessar and Allen (2012), phytochemical results of catappa leaf extract contain flavonoids, phenolics, saponins, steroids/terpenoids, alkaloids, tannins (punicalagin, punicalin, terflavin A and B, tergallagine, tercatain, chebulagic acid, geranin, granatin B, corilagin, isovitexin, vitexin, isoorientin, routine and triterpenoids.

**LD50 Test with Thomson and Weil Method (1952)**

In this study, 30 male mice, DDY strains were used, aged 2 months old, weighing between 25±5g. Mice were divided into 6 groups and each group consisted of 5 mice, namely:

- **Group I**: mice were given aquades (control)
- **Group II**: mice were given 1 g/kg BW ethanolic extract of catappa leaves
- **Group III**: mice were given 2 g/kg BW ethanolic extract of catappa leaves
- **Group IV**: mice were given 4 g/kg BW ethanolic extract of catappa leaves
- **Group V**: mice were given 8 g/kg BW ethanolic extract of catappa leaves
- **Group VI**: mice were given 16 g/kg BW ethanolic extract of catappa leaves

Mice are placed in an environmental condition with 12 hours of bright lighting and 12 hours of darkness, room temperature around 23-25 °C, with constant humidity. Food is given once a day and water is given in *ad libitum*. The mice are acclimatized for two weeks. Food was given to mice approximately one hour after treatment (Jothy *et al*. 2011). Ethanolic extract of catappa leaves will be dissolved with sterile aquades before force-fed. Mice were force-fed according to the treatment group using the metal gavage feeding needle. After that, mice were observed for 48 hours to see post-treatment deaths. The procedure of this study
has been examined and approved by the animal ethics commission FKH IPB with the number 141/KEH/SKE/V/2019.

Retrieval of Liver and Kidney

The number of mice organs taken is based on dead mice. If no dead mouse is found, only 3 mice will be necropsied. If one or more mice are found to be dead, then all the mice in the same dose group will be necropsied. Living mice are euthanized by cervical dislocations. Furthermore, necropsy was performed on experimental mice, then the liver and kidneys were taken for histomorphological examination. All organs from the treatment group with different doses will be compared with the control group.

Making of Liver and Kidney Tissues Preparation

Liver and kidney samples taken from each individual were fixed in 4% paraformaldehyde buffer solution, dehydrated using various levels of alcohol, clearing with xylene and embedding with paraffin. Tissue samples were cut as thick as 4 μm on glass slides and stained with hematoxylin eosin (HE) (Jothy et al. 2011).

Histomorphological Observations of Liver and Kidney Tissues

Histomorphological observation of liver and kidney tissues were carried out using a microscope (Olympus CH20). Observations were made on all parts of the liver and kidney tissue. Observation of liver tissue is carried out against damage to liver cells, such as hydropic degeneration, fat degeneration and necrosis. Observation is also performed on the interstitium against sinusoidal congestion and dilatation. Observation of kidney tissue is carried out on the renal tubules and renal corpusculus. Renal tubules were observed for degeneration and necrosis in renal tubular cells, while renal corpusculus was observed for glomerular and Bowman space damage.

Data Analysis

The data obtained from this study are quantitative and qualitative data. Quantitative data in the form of number of dead mice were then analyzed by probit analysis and processed using the Minitab 15 statistical program to determine the potential for acute toxicity (LD50) of catappa leaf extract. Qualitative data were obtained from histological analysis of liver and kidneys in experimental mice.
RESULTS AND DISCUSSION

Phytochemical Analysis

Phytochemical analysis is the initial stage to detect the active compounds in the ethanolic extract of catappa leaves. Catappa leaves contain active compounds or chemical compounds in the form of secondary metabolites with physiological and pharmacological effects that are beneficial for animals and humans, thus have the potential to be developed into medicinal plants. Therefore, phytochemical analysis on catappa leaf extract needs to be done as a basis in developing its potential as medicines based on its natural compounds. The test results of phytochemical analysis of ethanolic extract of catappa leaves are presented in Table 4.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Results</th>
<th>Analysis Technique</th>
</tr>
</thead>
<tbody>
<tr>
<td>Flavonoid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Alkaloid</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Tannin</td>
<td>+</td>
<td>Colour visualization</td>
</tr>
<tr>
<td>Saponin</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Quinon</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>Steroid</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>Triterpenoid</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

Description: (+) positive results, (-) negative results. Analysis results from Biopharmaca Study Center Laboratory IPB (2018)

The results of the phytochemical analysis test obtained from Biopharmaca Study Center Laboratory IPB showed that the ethanolic extract of catappa leaves contained flavonoids, tannins, saponins and steroids. The active compounds found in medicinal plants are almost always toxic in high doses. Deaths in test animals given the dosage of ethanolic extract of catappa leaves amounting to 16g/kg BW were thought to occur because the dosage of compounds that the body could tolerate was too high. Toxic activity of secondary metabolites, especially flavonoids found in the ethanolic extract of catappa leaves, is thought to be the main cause of animal mortality (Marlinda et al. 2012).

Flavonoid compounds contained in the ethanolic extract of catappa leaves can function as depressants (Saputri 2014). According to Fernández et al. (2006), flavonoid compounds work in depressing the central nervous system so that it can be used as a sedative and anticonvulsant in mice. High flavonoid compounds will depress the central nervous system and depression of the respiratory center which can cause death in experimental mice due to respiratory failure. Respiratory failure will interfere with the exchange between oxygen and carbon dioxide in the body causing the tissue experiences oxygen depletion and an increase in carbon dioxide. Furthermore, the brain that experiences oxygen depletion will experience damage to its tissues and show symptoms of seizures, coma and death.

In addition, the saponin compounds contained in the ethanolic extract of catappa leaves are thought to cause death in mice. This can happen because
according to Diwan et al. (2000), saponin compounds can cause acute hypoglycemia (low glucose levels in the blood). Glucose is the main energy source for the brain. Lack of glucose supply in the body, especially the brain, will cause damage to the brain tissue permanently, thus causing death in mice.

Lethal Dose (LD₅₀) Value

Medicinal plants are becoming popular generally in health care, especially in developing countries. Most people believe that medicinal plants have no side effects or potential health risks because they are made from natural sources. However, knowledge of the level of toxicity and safety in the use of medicinal plants is still low. Therefore, acute toxicity test and lethal dose (LD₅₀) test are needed to find out about the level of toxicity and safety of the use of these plants as phytopharmaca. The test results on the number of mice deaths in 48 hours after administration of the ethanolic extract of catappa leaves are presented in Table 2.

<table>
<thead>
<tr>
<th>Dose (g/kgBW)</th>
<th>Number of experimental mice</th>
<th>Number of mice deaths</th>
<th>Mortality (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>0</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>8</td>
<td>5</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>16</td>
<td>5</td>
<td>2</td>
<td>40</td>
</tr>
</tbody>
</table>

Acute toxicity test aims to determine the degree of toxic effects of a compound that can cause toxicity in a short time of 48 hours. Lethal Dose 50 (LD₅₀) is the number of doses obtained from chemicals statistically that can cause 50% death from the number of animals tested and observed for a certain period of time (Adamson 2016). The LD₅₀ value can be determined from the number of dead mice within 48 hours after multilevel dose exposure in the experimental mice, namely doses 1, 2, 4, 8, and 16 g/kg BW. The greater the animal weight, the greater the dose given. Unhealthy animals can produce different LD₅₀ values.

Based on Table 2, the groups given treatment with control doses of 0, 1, 2, 4, and 8 g/kg did not cause death within 48 hours of observation. The death of mice was found in the group given treatment of 16 g/kg BW in 48 hours of observation after the administration of ethanolic extract of catappa leaves with a mortality rate of 40%. Furthermore, the graph of the probability of death of mice is presented in Figure 1.
Figure 1: Graphic of Probability of Mice Deaths in LD$_{50}$ Test

Based on Figure 1 obtained using the Minitab statistical program with a confidence level of 95%, the LD$_{50}$ value was as high as 16.3523 g/kg BW. According to Handayani et al. (2012), the compounds contained in the ethanolic extract of catappa leaves can be classified as practically non toxic because results show a value of more than 15 g/kgBB. According to Supriyono (2007), each treatment will react differently at certain doses. The difference in reaction is caused by differences of sensitivity of each animal so that it is necessary to know the range of LD$_{50}$. The LD$_{50}$ range of catappa leaf extract was obtained using the Minitab statistical program with a confidence level of 95%. Based on the results obtained, the value of LD$_{50}$ range of catappa leaf extract was 15.05 to 17.65 g/kg BW. The LD$_{50}$ results in the ethanolic extract of catappa leaves can be influenced by several factors such as species, strain, diversity of individuals, sex, age, weight, animal health, diet, environmental and housing factors (Supriyono 2007). These factors are made uniform to ensure that the response produced is only influenced by treatment.

The administration of catappa leaf extract orally at a dose of 2000 mg/kg is not toxic to rat because it does not cause death (Arjariya et al. 2013). Based on these results it can be proven that Terminalia catappa extract is practically non toxic. Similar results were found in the administration of hydroalcohol extract from Terminalia catappa for 42 days at doses of 0.5, 1.0 and 3.0 g/kg BW in rats and proved to be non toxic (Batubo 2018). According to Arjariya et al. (2013), giving catappa leaves to mice did not cause significant changes to the skin, fur, eyes, mucous membranes, respiratory system, circulatory system, somatomotor, autonomic system, central nervous system, motor activity, behavior, seizures and coma. The increase in rat body weight showed that administration of ethanolic extract of catappa leaves had a negligible level of toxicity in animal growth.
Histomorphological Observations of Liver and Kidney

LD₅₀ test is often used in assessing the toxicity of a substance or drug, but the type of experimental animal used will give a different reaction to a particular test. The difference in reaction is caused by differences in the sensitivity of each animal. Thus further testing needs to be done to strengthen the toxicity analysis of a medicinal ingredient. One of the further testings is histomorphological examination of vital organs such as the liver and kidney. Histomorphological examination is a standard test to evaluate the toxicity of a substance through morphological changes in tissues and organs due to the toxic effects of catappa leaves.

The liver is the largest organ and functions in carbohydrate, protein, cholesterol (fat), haemoglobin and drugs metabolism. The liver also functions in excreting metabolites and detoxification drugs so that they often experience damage (Ramadori et al. 2008). The liver receives various substances absorbed from the intestine including high concentrations toxic substances, causing hepatotoxicity in mice. Changes will occur in liver tissue due to toxic exposure to chemical compounds from the ethanolic extract of catappa leaves. Changes such as hydropic degeneration, fat degeneration and necrosis occur in liver tissue, whereas changes such as sinusoidal congestion and dilation occur in the interstitium.

The results of study and observations of the histomorphology of liver cells in mice in the control group showed that there were several inflammatory cells around the central vein and the portal triad but in relatively small amounts. The presence of inflammatory cells in the control group can be caused by various factors such as the condition of the cage, mice stress factors, the effect of substance or disease, immunity and susceptibility of mice (Amalia 2009). The above factors can cause an increase in free radicals in the body of mice which will cause oxidative stress. Oxidative stress is a condition in which the production of free radicals or reactive oxygen compounds exceeds the body's defense system. Non-neutralized free radicals can cause damage to cells (Sinaga 2017; Widayati 2019).
Figure 3 Photomicrograph of liver tissues of mice with hematoxylin eosin (HE) staining. (A) = group given 1 g/kg BW ethanolic extract of catappa leaf. (B) = group given 2 g/kg BW ethanolic extract of catappa leaf. (C) = group given 4 g/kg BW ethanolic extract of catappa leaf. (D) = group given 8 g/kg BW ethanolic extract of catappa leaf. (E) = group given 16 g/kg BW ethanolic extract of catappa leaf. (F) = liver of dead mouse from group given 16 g/kg BW ethanolic extract of catappa leaf. Magnification 400x. Scale 50 µm.

The tissue structure in the treatment group given 1 g/kg BW and 2 g/kg BW ethanolic extract of catappa leaves (Figure 3, A and B) did not show any hepatocyte cell degeneration (hydropic degeneration and fat degeneration), necrosis, dilation sinusoids and congestion, thus can be concluded that the histomorphology of mice liver cells does not show any changes when compared with the liver tissue of the control group mice. In the treatment group given 4 g/kg BW and 8 g/kg BW ethanolic extract of catappa leaves (Figure 3, C and D) showed changes in hepatocytes. Most of the changes found were hydropic degeneration and inflammation while necrosis was not found. In the treatment group given 16 g/kg BW ethanolic extract of catappa leaves (Figure 3, E) showed changes such as hydropic degeneration and inflammation. The dead mice in the group given 16 g/kg BW ethanolic extract of catappa leaves (Figure 3, F) showed changes such as cell swelling before hydropic degeneration occurred and inflammation, but necrosis was not found in hepatocyte cells.
Changes in hepatocyte cells occur due to exposure to high doses of active compounds in medicinal plants (Maulina 2018). Cell changes are characterized by cell degeneration to necrosis. Hepatocyte cell degeneration usually occurs because of a chemical compound that can affect chemical changes in cell membranes and cause cell membrane to rupture. In addition to chemical compounds, cell degeneration can occur due to lack of food, old tissue age, or lack of oxygen in tissues (Zulaeha 2017). Cell degeneration is a condition of loss of normal cell structure before cell death (necrosis) occurs. Fat degeneration and hydropic degeneration are reversible cell degeneration (Maulina 2018). Saponin compounds contained in the ethanolic extract of catappa leaves are thought to cause cellular damage to hepatocytes. According to Nurqolibiah et al. (2016), saponin causes red blood cell hemolysis and gastrointestinal irritation and can disrupt around the extracellular of hepatocyte cells.

Hydropic degeneration is the initial manifestation of hepatocyte damage and is characterized by swelling of cell cytoplasm due to excessive accumulation of fluid thus cells appear larger than normal cells (Saputri 2014). This can be caused by damage to hepatocyte cell membranes due to toxic substances. Toxic substances will cause an increase in the permeability of cell membranes, thus the cell is unable to maintain homeostasis and regulation of fluid (Maulina 2018). Cell swelling occurs because of disruption of regulation of sodium-potassium ions (Na-K). The instability of cells in pumping Na\(^+\) ions out of the cell causes an increase in the amount of fluid entering from the extracellular, thus the cell is unable to pump enough sodium ions. This will cause cell swelling so the cell loses its membrane integrity and experiences death (Saputri 2014). Inflammation shows that hepatocyte cells experience inflammatory cell infiltration. Inflammatory cells can be found in the area around the portal triad, central vein and sinusoid. Inflammation indicates an immune response towards toxic substances that enter the liver.

Kidneys play an important role in filtering and reabsorbing some materials from the blood circulation in the body. Kidneys filter toxic substances that are metabolized from the liver and excreted outside from body through urine. Urine is the main pathway for the excretion of most toxic substances. Products of toxic substances can directly or indirectly cause damage to the kidneys thus causing changes in kidney tissue, especially in the renal tubules and renal corpuscles. The renal corpusculus consists of bowman capsules and glomerular while the renal tubules consist of the proximal tubule, loop of Henle and distal tubule. Renal tubules undergo changes in the form of degeneration and necrosis in the cells of the renal tubules, while the renal corpusculus undergoes changes in the form of glomerular and Bowman space damage.
Based on the results of research and observations, the histomorphology of kidney tissues in mouse of control group showed little degeneration in the renal tubular cells. Cell damage that occurs in the kidneys can be caused by several factors such as toxic substances from the environment contained in food, water and air, less ideal laboratory conditions, stress factors, diseases and other internal factors such as immunity and vulnerability mice (Amalia 2009).

Histomorphological observations of kidney of mice in the treatment group given 1 g/kg BW and 2 g/kg BW ethanolic extract of catappa leaves (Figure 5, A and B) did not show any degeneration, necrosis of renal tubular cells, glomerular damage and bowman capsules when compared to the kidney tissue of the control group mice. The treatment group given 4 g/kg BW and 8 g/kg BW ethanolic extract of catappa leaves (Figure 5, C and D) showed no changes in the renal
tubules, but there were slight changes in the renal corpuscles, which are expansion of Bowman capsule space and atrophy glomerulus.

The kidneys of mice in the treatment group given 16 g/kg BW ethanolic extract of catappa leaves (figure 5, E) showed changes in the renal corpuscles and renal tubules. The Bowman capsule space undergoes expansion which indicates that the glomerulus is experiencing atrophy or size reduction. In addition, the renal tubules experience degeneration. Kidney of dead mice in the treatment group given 16 g/kg BW ethanolic extract of catappa leaves (figure 5, F) showed changes in the renal corpuscles and renal tubules. The Bowman capsule space undergoes expansion and the glomerulus experiences atrophy or size reduction. Renal tubules undergo degeneration of the renal tubules to necrosis which characterized by pyknosis, karyolysis and karyorrhexis of nucleus. Some of the tubular lumen is dilated with protein deposits.

Glomerulus is a complex capillary that functions in filtration. Damage to the glomerulus results in filtration disturbances thus disrupts the peritubular vascular system and cause the flow of toxic substances into the tubules resulting in degeneration of renal tubular cells. Conversely, damage to the tubules will result in increased intraglomerular pressure causing glomerular atrophy. Glomerular atrophy indicates that the Bowman capsule space is expanding (Mardiastuti 2002). The kidneys of mice in the treatment group given 16 g/kg BW ethanolic extract of catappa leaves (figure 5, E) showed a protein deposition in the lumen of the renal tubules. Protein deposits can indicate a leak in the glomerular filtration membrane because of damage to the glomerulus resulting in the entry of proteins that accumulate around the renal tubules. Damages of kidney tissue other than renal corpuscle can also be found in the renal tubules. Damages that can be found in the renal tubules are in the form of degeneration of the renal tubules to necrosis and tubular dilatation. Degeneration is the beginning of cell damage due to toxins and is usually non-fatal. Degeneration is reversible thus the cells can return to normal when the cause is removed. Exposure to toxic substances that are severe or last long enough for cells can cause cell death (necrosis) (Winarsih et al. 2012).

Necrosis is a form of cell death that is irreversible and is an advanced process of degeneration. In the treatment group with dose of 16 g/kg BW (Figure 5, F), there were dead mice. Renal tubules in dead mice undergo degenerate which have led to necrosis. Cells that experience necrosis due to toxic substances will show changes in the nucleus and cytoplasm. Necrosis is characterized by a pyknosis, karyolysis, and karyorrhexis of nucleus. Pyknosis shows nucleus that looks dense and dark due to the condensation of chromatin in nucleus. Karyorrhexis shows a destroyed cell nucleus while karyolysis shows cell nucleus that disappears. Necrosis is usually consists of inflammatory cell infiltration which serves to phagocytes dead cells. But in this study there was no visible inflammatory cell infiltration because the necrosis process occurred in a short time (acute) (Nurjunitar 2016).

Tubular dilatation is a widening or expansion of the tubular structure and usually occurs due to a toxic excretion process that can cause damage to the kidneys. Damage to tubular cells begins with the occurrence of edema in the proximal tubular cells due to the movement of water from extracellular into the cell. Tubular dilatation is also caused by urinary retention or inflammation of the renal interstitium. In this study, tubular dilatation was characterized by an
expansion of the lumen accompanied by a reduction or atrophy of tubular cells. According to Fatonah (2015), dilated tubular cells will experience lysis, hypoxia and death.

The report of Filippich et al. (1991) stated that tannin compounds contained in catappa leaves can cause damage to the kidneys. Tannin compounds are usually present in small amounts but can cause kidney damage at high doses. According to the Batubo (2018), administration of hydroalcohol extract from *Terminalia catappa* for 42 days at doses of 0.5, 1.0 and 3.0 g/kg in Wistar strain white rats had a large safety margin due to kidney function parameters (Na⁺, K⁺, Cl⁻, HCO₃⁻, urea, uric acid and creatinine) did not change significantly (p> 0.05). Parameters of kidney function that do not change significantly are indicative of good kidney function. Based on histomorphological observations of liver and kidney tissues, liver tissue was found to have more cell degeneration than kidney tissue.

**CONCLUSION AND SUGGESTION**

**Conclusion**

Based on the research that has been done, LD₅₀ value of ethanolic extract of catappa leaves (*Terminalia catappa*) in mice given orally was 16.3523 g/kg BW. The value of LD₅₀ range is 15.05 to 17.65 g/kg BW. Based on the toxicity classification shown, the ethanolic extract of catappa leaves is categorized as practically non toxic so it is relatively safe to use. Histomorphological observations showed cell degeneration in the liver and kidneys mainly in mice of 4, 8, and 16 g/kg BW treatment groups. Based on histomorphological observations of liver and kidney tissues, liver tissues was found to have more cell degeneration than kidney tissues.

**Suggestion**

Suggestions proposed based on this research are acute toxicity testing with methods other than Thomson and Weil method. In addition, further testing of subchronic and chronic toxicity needs to be done to determine the impact that will be caused by giving ethanolic extract of catappa leaves. An Effective Dose Test (ED₅₀) needs to be done to determine the therapeutic index of the ethanolic extract of catappa leaves.
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BIOGRAPHY

The author was born on 21st May 1996 in Kota Kinabalu, Sabah, Malaysia. The author was born to Lim Boon Ching and Lee Lee Ling. The author is the third child of 3 siblings. The author graduated from Kota Kinabalu High School (STKK) in 2013 and continued her education at Adroit College in 2014. In 2015, the author passed the selection to continue her education at Bogor Agricultural University (IPB) and was accepted into Faculty of Veterinary Medicine.