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Description of Acute Toxicity of Zuriat Fruit Seed Extract (*Hyphaene thebaica* (L.) Mart.) in Mice

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Abstract

This study aims to determine the acute toxicity of administering Zuriat seed extract. This study used 20 male mice, divided randomly into 4 groups, namely the group given Zuriat seed extract at a dose of 0.252 mg/20 g body weight; 2.52 mg/20 g body weight; 25.2 mg/20 g body weight; and 252 mg/20 g body weight. After being given a single dose, observations were carried out for 24 hours to see the number of deaths and toxic symptoms, and further observations were carried out for 7 consecutive days on mice that were still alive. Then the relative weights of mice's stomach, lungs, heart, liver, and kidneys were calculated and the LD_{50} dose categories were analysed. The results of the study showed that within 24 hours, death occurred in the 2.52 mg/20 g body weight group of 3 mice; 25.2 mg/20 g body weight for 4 mice; and a dose of 252 mg/20 g body weight for 5 mice. Before they died, the mice experienced toxic symptoms. Follow-up observations for 7 days showed no toxic symptoms in each group. In measuring the relative weight of organs, it was found that the dose of Zuriat seed extract did not affect the relative weight of the organs of the stomach, lungs, heart, liver, and kidneys of mice. The LD_{50} value category for Zuriat seed extract is moderately toxic. It concluded that administration of Zuriat seed extract at a dose of more than 0.252 mg/20 g body weight is moderately toxic to the animals.

Keywords: $LD_{50,}$ Organs, Symptoms, Toxicity, Zuriat Seeds

1. Introduction

For many years, traditional medicine has been widely used in Indonesia to prevent and treat disease, however, there are several advantages and disadvantages. Considering developments in science, people often prefer medical treatment because its efficacy has been clinically tested compared to traditional ones[1,2]. Even though health facilities already exist, this does not affect the role of plants as medicine because people still adhere to traditions and consider traditional medicines to be cheaper and easier to obtain[1]. Knowledge about traditional medicine is also growing, including activity testing to determine the effectiveness of medicinal plants, testing to determine doses that are efficacious in treatment, and even toxicity tests to determine the safety and possible side effects that arise from consuming plants that are efficacious in traditional medicine[3]. There are various plants whose activity has been tested in the laboratory, including red dragon fruit (*Hylocereus polyrhizus*)[4], Dayak onions (*Eleutherine bulbosa* (Mill.) Urb.)[5], rosella flowers (*Hibiscus sabdariffa* L.)[6], Pasak Bumi (*Eurycoma longifolia* Jack.)[7], and Zuriat (*Hyphaene thebaica* (L.) Mart.)[8,9].

Zuriat is a plant that comes from the Arecaceae family. The leaves and flowers of male Zuriat are reported to contain saponin, flavonoid, phenolic, anthocyanin, tannin,

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and carbohydrate compounds. Zuriat fruit is reported to contain saponin compounds[10-12], flavonoids, tannins, carbohydrates[11,12], phenolics, anthocyanins[11], cardiac glycosides, steroids, terpenoids, and anthraquinones[12]. Zuriat seeds are reported to contain alkaloid and flavonoid compounds[10]. Several research results show that Zuriat root can treat bilharzias, diabetes, and hypertension[9]. Zuriat bark extract can increase immunity, fight infections, and increase the number of red blood cells[13]. Zuriat fruit can be used as a food ingredient[10], overcomes malnutrition[9,14], has good fibre value[8,14], has potential as antioxidant[8,15], antihypertensive[16], anticancer[15], and antidiabetic[17]. Zuriat seeds can be boiled and used together with the fruit as a food ingredient[10].

When using Zuriat seeds as a food ingredient, it must be supported by research and safety testing[18]. Evaluation of the safety of substances used as food or medicine is necessary to evaluate dangerous effects that occur immediately after exposure to a single substance or combination of substances once or several times in a short time also called acute toxicity[19]. Acute toxicity tests are routine tests used by scientists to check the safety of natural or synthetic compounds. The acute toxicity test is also considered the limit for determining the lethal dose to kill 50% of animals (LD_{50}), the negative side of the targeted compound, and thereafter administering a single dose over a certain period of time[20]. The acute toxicity test is intended to obtain information about the symptoms of poisoning, the cause of death, the sequence of the death process, and the range of doses that kill the test animal in a short time[21].

Considering the lack of information regarding the potential of Zuriat seeds and their use as a food ingredient, it is necessary to test the acute toxicity of Zuriat seeds. It is hoped that the results of this research can provide information and enrich knowledge about the safety of Zuriat seeds themselves.

2. Materials and Methods

2.1 Extraction Process

The Zuriat seed extraction process refers to Panjaitan *et al.*[22] a total of 3.400 g of Zuriat seeds were cleaned, cut into small pieces, and dried with a dry weight of 2.970 g. Next, the dried samples were macerated at room temperature using 96% ethanol solvent. Soaking was carried out three times by adding new ethanol for each repetition. Next, the filtrate from Zuriat seeds was concentrated using a vacuum evaporator, and a yield of 131.03 g was obtained.

2.2 Preparation of Test Animals

The test animals used were male albino mice (*Mus musculus*), weighing 20-30 g, in healthy condition, with an age range of 2-3 months. Before the experiment was carried out, all test animals were acclimatised for 5 days by providing standard food and drink ad libitum. The testing procedure has received a statement letter from the Health Research Ethics Commission, Faculty of Health Sciences, Respati University Yogyakarta No. 012.3/FIKES/PL/III/2022.

2.3 Determination of LD50 Values, Observation of Accompanying Toxic Symptoms and Determination of Relative Organ Weights in Mice

A total of 20 mice as test animals were divided into 4 groups, each group consisting of 5 mice. Each test animal was given Zuriat seed extract using a gastric probe. Each test group was given a different test dose with a 10-fold dose interval, namely 0.252 mg/20 g body weight; 2.52 mg/20 g body weight; 25.2 mg/20 g body weight; and 252 mg/20 g body weight. The LD_{50} value was determined by calculating the number of deaths of test animals within the first 24 hours after administering a single dose of extract to each group studied for 7 days[21]. After administering the test material, toxic symptoms such as diarrhoea, urination, changes in breathing, aggressive behaviour, and decreased movement activity are observed[23]. For animals that died within 7 days and animals sacrificed on day 7, surgery was performed and the relative weight of the mice organs, namely the stomach, lungs, heart, liver, and kidneys, was determined using the formula:

$$BRO = \frac{BO}{BB}$$

Where:
BRO: Relative weight of organs
BO : Mice organ weight
BB : Mice body weight

Next, the LD_{50} value is calculated using the Weil[24] method with the formula:

$$\text{Log } m = \text{Log } D + d\ (f + 1)$$

Where:

m : The value of LD_{50}

D : The smallest dose used

d : Log of dose multiples

f : A value in the Weil table, because a certain death rate (r)

3. Result

The acute toxicity test of Zuriat seed extract in this study was tested on test animals in the form of male white mice. The acute toxicity parameters observed in this study were the condition of the mice for 7 days after administration of the test material, the number of deaths of mice within 24 hours, the LD_{50} calculation, and the relative weight of the mice's organs, which included the lungs, liver, kidneys, heart, and stomach. The results of observations for 24 hours can be seen in Table 1 and the results of observations for 7 days can be seen in Table 2. The number of deaths of test animals during 24 hours (5^{th} to 6^{th} hours) dan the LD_{50} value is calculated using the Weil[24] method can be seen in Table 3.

Table 1. Description of the condition of the test animals during the first 24 hours after administration of Zuriat seed extract

Observation Hours	Group of Mice	Description	Figure
0 hour	0.252 mg/20 g body weight	Administration of a single dose of Zuriat seed extract	
	2.52 mg/20 g body weight		
	25.2 mg/20 g body weight		
	252 mg/20 g body weight		
1st hour	0.252 mg/20 g body weight	All mice in each group are fresh and actively moving	
	2.52 mg/20 g body weight		
	25.2 mg/20 g body weight		
	252 mg/20 g body weight		
2nd hours	0.252 mg/20 g body weight	Fresh, actively moving	
	2.52 mg/20 g body weight	Sleeping, much urine	
	25.2 mg/20 g body weight	Fresh, actively moving, much urine	
	252 mg/20 g body weight	Sleeping, bristling fur, diarrhoea	
3rd hours	0.252 mg/20 g body weight	Actively moving, much urine	
	2.52 mg/20 g body weight	Actively moving, much urine	
	25.2 mg/20 g body weight	Sleeping, breathing weakens	
	252 mg/20 g body weight	Sleeping, breathing weakens, diarrhoea	

Table 1. Continued...

Observation Hours	Group of Mice	Description	Figure
4th hours	0.252 mg/20 g body weight	Fresh, actively moving	
	2.52 mg/20 g body weight	Moves a little, breath weakens, fur bristles	
	25.2 mg/20 g body weight	Sleeping, much urine, weak breathing, bristling feathers	
	252 mg/20 g body weight	Sleep, diarrhoea, weakened breathing	
5th to 6th hours	0.252 mg/20 g body weight	Fresh, actively moving	
	2.52 mg/20 g body weight	Three mice died. Two mice are fresh and actively moving	
	25.2 mg/20 g body weight	Four mice died. One mouse moved little, its hind legs were paralyzed, breathing was weak	
	252 mg/20 g body weight	All of the mice died	
7th to 8th hours	0.252 mg/20 g body weight	Fresh, actively moving	
	2.52 mg/20 g body weight	Fresh, actively moving	

Table 1. Continued...

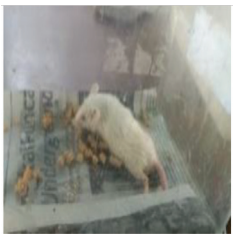
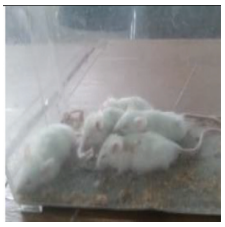
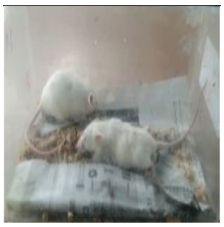
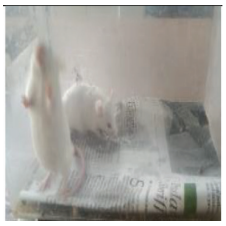
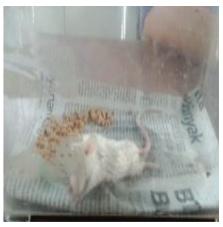
Observation Hours	Group of Mice	Description	Figure
	25.2 mg/20 g body weight	Moves little, breathing weakens, hind legs paralyzed	
9th to 10th hours	0.252 mg/20 g body weight	Sleeping	
	2.52 mg/20 g body weight	Sleeping	
	25.2 mg/20 g body weight	Moves little, breathing weakens, hind legs paralyzed	
23th to 24th hours	0.252 mg/20 g body weight	Fresh, actively moving	
	2.52 mg/20 g body weight	Fresh, actively moving	
	25.2 mg/20 g body weight	Moves a little, hind legs are paralyzed	

Table 2. Description of the condition of the test animals from the 24th hour to the 168th hour

Observation Hours	Group of Mice	Description
24th hours to 48th	0.252 mg/20 g body weight	Fresh, actively moving
	2.52 mg/20 g body weight	Fresh, actively moving
	25.2 mg/20 g body weight	Little movement, weak breathing, hind legs paralyzed, fur bristling
48th hours to 72nd	0.252 mg/20 g body weight	Fresh, actively moving
	2.52 mg/20 g body weight	Fresh, actively moving
	25.2 mg/20 g body weight	Little movement, weak breathing, hind legs paralyzed, fur bristling
72nd hours to 96th	0.252 mg/20 g body weight	Fresh, actively moving
	2.52 mg/20 g body weight	Fresh, actively moving
	25.2 mg/20 g body weight	Little movement, weak breathing, hind legs paralyzed, fur bristling
96th hours to 120th	0.252 mg/20 g body weight	Fresh, actively moving
	2.52 mg/20 g body weight	Fresh, actively moving
	25.2 mg/20 g body weight	Little movement, weak breathing, hind legs paralyzed, fur bristling
120th hours to 144th	0.252 mg/20 g body weight	Fresh, actively moving
	2.52 mg/20 g body weight	Fresh, actively moving
	25.2 mg/20 g body weight	Little movement, weak breathing, hind legs paralyzed, fur bristling
144th hours to 168th	0.252 mg/20 g body weight	Fresh, actively moving
	2.52 mg/20 g body weight	Fresh, actively moving
	25.2 mg/20 g body weight	Little movement, weak breathing, hind legs paralyzed, fur bristling

Table 3. Number of deaths of test animals during 24 hours (5th to 6th hours)

Group of mice	Dose (mg/20 g body weight)	Number of Test Animals	Number of Deaths of Test Animals	Death Percentage
1	0.252	5	0	0%
2	2.52	5	3	60%
3	25.2	5	4	80%
4	252	5	5	100%

Calculation of LD_{50} using the Weil Method:

D = 0.252
d = 10
r = 0, 3, 4, 5
f = 0.100
log m = log D + log d (f + 1)
= -0.60 + 1 (0.100 + 1)
= 0.4 (1.100)
= 1.5
LD_{50} = anti log m
= anti log 1.5
= 31.62 mg/20 g body weight
= 1.58 g/kg body weight
Category = Moderately toxic

4. Discussion

An acute toxicity test is a test carried out to find the lethal dose range which is usually expressed as LD_{50} and the symptoms of poisoning. In acute toxicity testing, the number of test animal deaths is used as a measure of the toxic effect of a substance on a group of test animals[21]. Furthermore, acute toxicity tests can be carried out to determine the lethal dose that kills 50% of animals (LD_{50}), the negative effects of certain compounds, and the effect of administering a single dose over a certain period of time[20]. In the acute toxicity test, the LD_{50} is determined by calculating the number of deaths of test animals that occur in the first 24 hours after administering a single

dose until the 7th day. After administering a single dose of the studied substance, toxic symptoms such as diarrhoea, urination, changes in breathing, aggressive behaviour, and decreased motor activity were observed[23]. Observations were carried out for 24 hours to find out which test animals died and to see the toxic symptoms that occurred[23,25,26] and continued with observations for 7 days to see delayed toxic symptoms[21,23,27].

The results of observations in this study showed that within 0-24 hours after administration of Zuriat seed extract there were no deaths (0%) in the first group at a dose of 0.252 mg/20 g body weight. On the other hand, in the 5th to 6th hour, death occurred in the second group at a dose of 2.52 mg/20 g body weight, 3 mice (60%), in the third group at a dose of 25.2 mg/20 g body weight, 4 mice (80%), and the fourth group had a dose of 252 mg/20 g body weight of 5 mice (100%) (Table 3). According to Jumain *et al.*[27] and Segerstrom and Miller[28] test animal deaths were caused by direct dose effects of the drug. There are 2 possibilities for the test animal to die, namely, it could be caused by stress which can reduce the immune system. Tuti *et al.*[29] and Yusuf[30] also stated that the death of test animals could be caused by the high dose given so there was a toxicity effect on the test animals which resulted in death.

The results of observations in this study showed that oral administration of Zuriat seed extract caused toxic symptoms. Toxic symptoms that appeared in mice before death in the second group at a dose of 2.52 mg/20 g body weight were large amounts of urine, weak breathing, decreased activity, and bristling fur. In the third group with a dose of 25.2 mg/20 g body weight, the toxic symptoms that appeared were large amounts of urine, weak breathing, decreased activity, and bristling feathers. In the fourth group at a dose of 252 mg/20 g body weight, the toxic symptoms that appeared were diarrhoea, weak breathing, decreased activity, and bristling feathers (Table 1). As is known, according to Nonci *et al.*[23], acute toxicity observations were carried out on the appearance of symptoms of toxic effects that occurred. The symptoms shown are excessive salivation, diarrhoea, urination, changes in breathing, aggressive behaviour, and decreased movement activity. Mustapa *et al.*[31] stated that test animals showed symptoms of toxicity in the respiratory system, changes in activity, heart activity, and paralysis. According to Tuti *et al.*[29] administering the test material causes the active substance contained in the test material to be absorbed in the digestive tract. The active substance then undergoes a distribution and metabolism process. Toxic metabolic products work as enzyme inhibitors for the next metabolic stage, reactions between active substances and receptors in effector organs cause toxic symptoms to appear.

Diarrhoea and excessive urination are caused by stimulation of the parasympathetic nerves. Diarrhoea occurs due to stimulation of the activation of the digestive tract and intestinal peristalsis which causes Diarrhoea[27,32], while urination occurs due to stimulation of contraction of the bladder with the ureters which has the effect of facilitating the flow of urine (urination)[27]. Weak breathing is a general sign of toxicity to the cardiovascular system so cardiac output and heart rate are reduced which may be caused by the active compounds in Zuriat seed extract which have a negative ionotropic effect on the heart[33]. Decreased movement activity in test animals is a symptom of acute toxicity[34] and is closely related to central nervous system depression and muscle relaxation[35]. Curly animal fur is a common sign of toxicity that affects the integrity of the fur. The active compounds contained in the test material cause feather loss so that the feathers appear sparser[33].

Death in test animals can occur after the first 24 hours because the poisoning process can be slow. Toxic symptoms that appear after 24 hours indicate that the drug or substance has a catch point of action at a lower level so that toxic symptoms or death appear to be delayed. Therefore, toxic symptoms need to be observed for up to 7 days[21]. The results of observations in this study showed that after the first 24 hours until day 7, all mice in the first group with a dose of 0.252 mg/20 g body weight and the remaining 2 mice in the second group with a dose of 2.52 mg/20 g body weight looked fresh, agile, and active. Meanwhile, the remaining 1 mice in the third group with a dose of 25.2 mg/20 g body weight appeared less active, had weak breathing, paralyzed, and weak hind legs, and its fur was bristling. Apart from that, there were no deaths in mice in each group from the first 24 hours to day 7 (Table 2). Paralyzed hind legs can occur due to disturbances in the nervous system that controls movements such as the hands and feet, which then fails to control the muscles so that the mice become paralyzed[31]. This is because Zuriat seeds contain flavonoids[10] which according to Sandhar[36] flavonoids have a way of disrupting the nervous system.

Ngatidjan[21] states that the lowest dose in toxicity testing is the dose that does not cause any effects or symptoms of poisoning, and the highest dose is the dose that causes the

death of all (100%) of the test animals. The effect observed is death, so if each member in a population of test animals is given a substance (chemical) at a certain dose, then two types of effects will emerge, namely, some individuals will die and some will not die at that dose. According to Lu[18], to determine the LD_{50} correctly, it is necessary to choose a dose that will kill about half of the test animals, another dose that will kill more than half, and a third dose that will kill less than half of the test animals. Four or more doses are often used with the hope that at least three of them will be within the desired dose range.

In this study, after 7 days of observing toxic symptoms, the remaining living mice were sacrificed for macroscopic research, measuring the relative weight of the organs being observed, namely the stomach, lungs, heart, liver, and kidneys. According to Rajeh *et al.*[37] relative organ weight is a basic diagnosis of whether an exposed organ is damaged or not. Vaghasiya *et al.*[38] stated that the stomach, lungs, heart, liver, and kidneys are the main organs affected by metabolic reactions caused by toxicants. According to Oktavia *et al.*[39] the stomach, lungs, heart, liver, and kidneys can be damaged by various types of chemicals that work directly on the target organs or indirectly, namely through the central nervous system or blood vessels. Organ weight ratios are usually a very sensitive indicator of toxic effects.

The stomach is one of the digestive organs and is located between the end of the oesophagus and the beginning of the small intestine. Administration of drugs/food via the oral route enters the gastrointestinal tract which allows exposure to harmful substances in the stomach[40]. The lungs as a respiratory organ are vulnerable to toxins and have a big risk of suffering from poisoning, especially by volatile toxic substances. As an organ that is in direct contact with the outside world, the lungs have defence mechanisms in the form of cilia, mucus, and enzymes that can metabolize certain toxins[21]. The heart is an organ that is easily susceptible to abnormalities caused by chemical compounds because the mitochondria found in the heart muscle in relatively large numbers are more often the target of cardiotoxicity[18]. The liver is an organ that can recover from enormous cell damage. The liver has the cytochrome p-450 enzyme which can metabolize foreign substances in the body, by making some toxicants less toxic and more easily soluble in water[41]. The kidneys are the main target organ for toxic effects because of their role in concentrating toxicants in the filtrate, carrying toxicants through tubular cells, and activating certain toxicants[18]. Statistical results using one-way ANOVA in this study showed that there was no significant difference in the relative weight of the stomach, lungs, heart, liver, and kidneys for each administration group ($p>0.05$). It can be concluded that there is no influence on the relative weight of the stomach, lungs, heart, liver, and kidneys in male white mice.

Based on the results of calculating the LD_{50} value using the Weil method (Table 3), the LD_{50} value of Zuriat seed extract is a moderate toxic category because it is in the range of 0.5-5 g/kg body weight. According to Priyanto[19], generally the smaller the LD_{50} value, the more toxic the compound is, and vice versa, the greater the LD_{50} value, the lower the toxicity. The response of test animals varies at certain doses due to differences in the level of sensitivity of each animal.

5. Conclusions

It was concluded that administration of Zuriat seed extract at a dose of more than 0.252 mg/20 g body weight caused toxic symptoms, but administration of Zuriat seed extract from four groups did not affect the relative weight of the stomach, lungs, heart, liver, and kidneys of mice, as well as the category The LD_{50} value of Zuriat seed extract is moderately toxic.

6. References

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