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ACUTE TOXICITY WATER EXTRACT OF *Meretrix meretrix* Linnaeus IN VIVO ON SPRAGUE DAWLEY RATS

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ABSTRACT

Meretrix meretrix empirically has been widely believed by the public to a wide variety of health benefits. It is necessary to do an analysis of the level of toxicity of *Meretrix meretrix* extract. The experiment was carried out extraction sample with water (1;4) (w/v), analysis of acute toxicity *Meretrix meretrix* extract with the OECD method 403:2009. Based on the results of water extract of Meretrix meretrix no effect on physical observations of test animals with LD_{50}> 15 g/kg BW. Histopathological observation on the liver and kidneys, there is necrosis of the liver cells and some cell degeneration in the kidneys, but on the whole network under normal conditions appropriate control group.

Keywords: Histopatology, Kidney, Liver, *Meretrix meretrix*, Water extract

I. INTRODUCTION

Empirically this aquatic biota has been widely used by the community to treat various diseases. One example of marine biota that has the potential to be developed into natural medicinal ingredients is shellfish lamis. Embroidery shell empirically believed by the community to cure various diseases such as jaundice, hypertension can even increase stamina [1]

Previous research has shown that these shells have potential as anti hyperlipidemic, antineoplastik and antioxidant activity [2-5], increase immune system [6-10], antitumor dan anticancer [10], hypertensy activity [2,11-12].

Various studies on the health effects of these biota have been done, but studies on the toxicity of these shells have only been analyzed for subcronic toxicity with no interference or negative effect on blood chemistry and animal metabolism [1]. The research aim to analyze the acute toxicity to see the toxicity level of lamis shell extract in the short term as well as its effect on histopat liver and kidney extract water *Meretrix meretrix*.

2. MATERIAL AND METHODS

Material and Tools

The main material used in this research are lamis shell water extract, *Sprague Dawley* male gender rats with average weight 150 g which can be obtained from Bogor Veterinary Research Center.

Tools used include cages, feeding and drinking mice, digital scales OHAUS PAJ1003, syringe, microtom VIC-Science VCM-3658, LEICA microscope, freeze dryer Christ brand, Shaker JOANLAB, rotary vacuum epavorator Cole-Parmer EW-28615- 01.

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Methods

This research was conducted in several stages, namely, making of lamis shell extract, acute toxicity test where physical observation, growth and consumption of feed, liver and kidney histopathology observation and LD_{50} observation were observed.

Extraction

The extraction process is carried out using the maceration method referred to as Quinn (1988). 50 g shell samples that have been mashed and then macerated with 200 ml (w/v) of water for 24 hours and shaking with JOANLAB Shaker with 150 rpm. The result of maceration in the form of solution is then filtered to clear with Whatman 42 filter paper to obtain filtrate and residue. The extract filtrate was evaporated with a rotary vacuum evaporator Cole-Parmer EW-28615-01 at a temperature of 50 ºC to obtain a water extract in the paste form.

Acute Toxicity Test (OECD 403: 2009)

Acute toxicity testing is performed by following the steps as follows

- Rats were divided into 5 groups, in which the first group control, the second group was given extract with a dose of 2 g/Kg BW, third 4 g/Kg BW, fourth 6 g/Kg BW and fifth 15 g/Kg BW.
- The giving of lamis shell extract is done orally using syringe. .
- Before being given the extract, the test animal is fasted for 4 hours.
- Control mice only given distilled water.
- Treatment is only done once on the first day.

Observations were performed on days 1, 2, 3, 5, 10, and 14. Observations were made on physical parameters and LD_{50}, growth, feed consumption and histopathology of rats and liver.

3. RESULT AND DISCUSSION

Physical Rats Parameter

Physical parameters are one of the test indicators to look at the effect of lam shell extract on the test mice. The following can be seen the results of the physical parameter parameters in Table 1.

Table 1. Physical parameters of rats

No	Organ system	Parameters	Indicators	Water Extract Day- (%)						conclusion	death
				1	2	3	5	10	14		
1	Central system	behavior	Anxious	0	0	0	0	0	0	-	0
			Stomach wrinkles	0	0	0	0	0	0	-	0
			Seizures	0	0	0	0	0	0	-	0
		Activeness to stimuli		100	100	100	100	100	100	Normal	0
2	Breathing	Respiratory rate	Out of breath	0	0	0	0	0	0	-	0
3	Digestive tract	Feces	Diarrhea	0	0	0	0	0	0	-	0
			Shape of feces	100	100	100	100	100	100	Normal	0
			Feces color	100	100	100	100	100	100	Normal	0
4	Skin and fur	Color and wholeness	Color of fur	100	100	100	100	100	100	Normal	0
			wholeness	100	100	100	100	100	100	-	0
5	Eye	Feathers and eyeball	feathers	100	100	100	100	100	100	-	0
			Eye color	100	100	100	100	100	100	Normal	0
			Clarity	100	100	100	100	100	100	Normal	0
6	Mouth	bleeding	bleeding	0	0	0	0	0	0	-	0
			Swelling	0	0	0	0	0	0	-	0
7	nose	bleeding	bleeding	0	0	0	0	0	0	-	0
			Swelling	0	0	0	0	0	0	-	0
8	Genitourinary	Mammary glands	Swelling	0	0	0	0	0	0	-	0
		Penis	Swelling	0	0	0	0	0	0	-	0

Based on the observation of physical parameters from the test rats indicating that, giving lam shell extract with doses of 2, 4, 6 and 15 g/Kg BW did not cause any

signs of poisoning in the test rats. Observation of central nervous system, no change during 14 day observation and no change of activity level of test rat. Observation of respiratory tract also did not show out of breath or 100% sample of rats given shell extract in normal condition. The rat digestive tract is also in normal condition. It can be observed from the normal color and shape of the feces and the absence of a mice that diarrhea. It is presumed, that shell extract does not contain substances or compounds that can interfere with digestion. Observation of the mouth, nose and genitourinary showed no signs of poisoning such as swelling and bleeding.

Based on the result of observation, there were no deaths in the animal test for 14 days observation with lamis dosage extract dose 2, 4, 6 and 15 g / Kg BW. Based on these results it can be seen the degree of toxicity for water extract and methanol shellfish according to the relative toxicity classification of Lu (1995) is practically non toxic with LD50 value of this extract above 15 g/Kg BW. This is because no mice were found dead at that dose.

The value of LD50 is not an absolute biological constant, but only one of the indications of acute toxicity [13]. According to [14], if a number of substances given to a test animal with high doses and no dead test animals, it is considered that all dangerous acute toxicities can be ignored. Acute toxicity observation results are influenced by several factors including species, individual diversity, gender, age, body weight, mode of delivery, animal health and environment [15].

Growth

Observation of the growth of this rat is one of the parameters of toxic effects. According to [14], reduced weight gain is a simple but sensitive toxic effect index. Here we can see the results of observation of the growth of rats for 14 days in Figure 1.

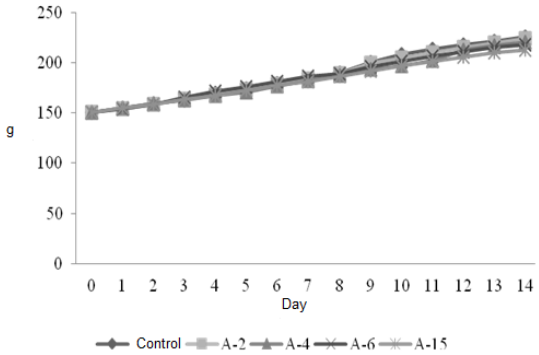

Figure 1. Growth of experiment rats
Information: A2 (dose 2 g/Kg BW), A4 (dose 4 g/Kg BW), A6 (dose 6 g/Kg BW), A15 (dose 15 g/Kg BW).

Based on the observation, there is an increase of average body weight of rat test during observation with increasing range of 3-4,5 gram per day or equal to 1,75-2,8%. [16] stated that the average normal mouse growth is 1.5-3% of the initial weight, this is when the nutrients are well suited and the mice are still under 5 months old. Based on these results, it was concluded that the experimental. This indicates that the lamis shell extract does not contain any compounds that can cause disturbance to the absorption of nutrients, so that the body of test animals can utilize the nutrients well.

Feed Consumption

The growth of rats is closely related to the amount of feed consumed by these mice. Feed serves as a source of energy and growth of rats. The following shows the data of feed consumption of experimental mice for 14 days in Figure 2

Based on the results of observation the amount of feed consumption in each mouse experiment experienced fluctuations and reduction of the amount of feed consumed. The greater the dose of the extract given, is also directly proportional

to the feed consumption pattern of the mice. It also has a direct impact on rat growth.

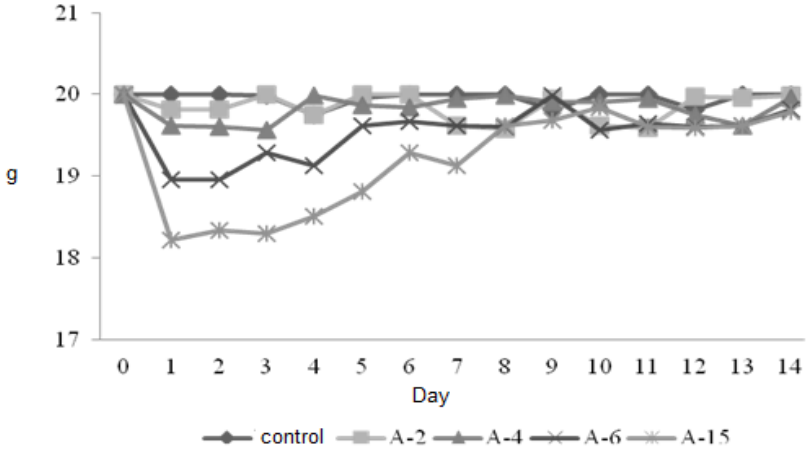

Figure 2. Feed comsumption rats

Information: A2 (dose 2 g/Kg BW), A4 (dose 4 g/Kg BW), A6 (dose 6 g/Kg BW), A15 (dose 15 g/Kg BW)

The consumption of mouse feed during observation fluctuated between 18 - 20 g/head/day. it is still in the category of normal because it does not affect the growth and metabolism of the test rats.

Histopathology of the Liver and Kidney

The most important part of toxicity testing is histopathology of liver and kidney organ. The following histopathologic outcomes can be seen in Figure 3 and 4.

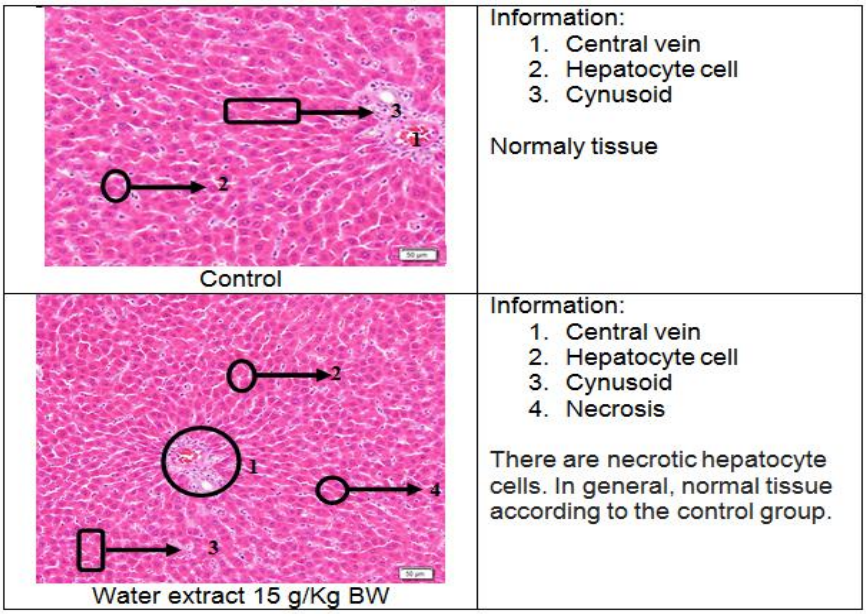

Figure 3. Histopathology of rat liver organ

The liver is the largest organ and has an important role in the physiology of the body that is the function of metabolism and detoxification [17]. The results of histopathologic analysis of liver organ in **Figure 3.** showed no microscopic change in control and treatment of water extract 15

g/Kg BW. However, in the treatment, there are several cells that have necrosis.

[18] states that, necrosis is the occurrence of cell and tissue death in live animals. [19] mentions that, a common death after cells exposed to exogenous stimuli such as chemical stimuli is the occurrence of cell swelling. Next cell rupture, denaturation occurs, cytoplasmic cell coagulation and cell organel.

Treatment giving of water extracts at a dose of 15 g / Kg BW causes necrosis in some hepatocyte cells, although in general the liver condition is normal. The occurrence of necrosis, allegedly due to the active compound saponin which owned shellfish.

[20] states that, saponins are divided into two groups namely triterpenoids and steroids. According to [21], the two compounds are natural glycosides, where there is a complex bond between sugar and aglycone (sapogenin). When hydrolysis occurs either by acid, base or enzyme or physical causes saponin glycoside will decompose into sugar and sapogenin as aglikon, toxicity caused by aglikon. According to [22], saponins have also been known to have activity in stimulating apoptosis, but not yet known where the catch point is. [23] reported that, high levels of saponins in excess of 150 ppm would be toxic and cause pathological conditions of liver organ with the emergence of degeneration and hepatic cell necrosis of the liver.

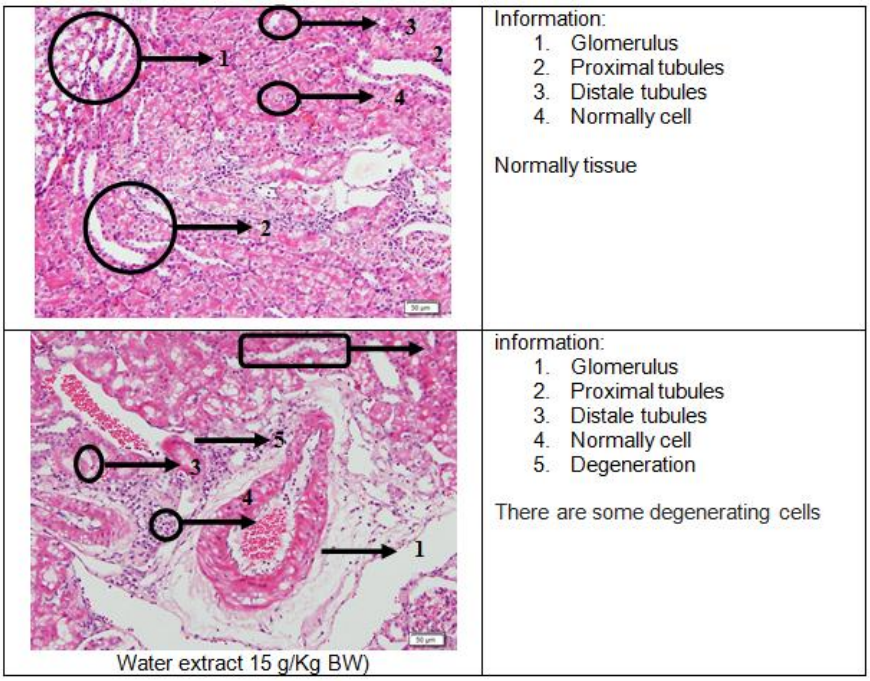

Figure 4. histopathology of renal kidney organs

[24] stated that, the mammalian animal's kidneys are very complex anatomically and functionally. The main function of the kidneys is to remove waste from detoxification. Failure of renal function may occur if the organ is damaged by a substance that is toxic.

Based on observations of histopathologic renal animal tests did not show any microscopic changes in control and treatment. However, there are some degenerated cells. [25] suggest that cell degeneration is a change in cell size, loss of structure and progressive cell function

changes, but not related to neoplasia or inflammation.

Administration of lamis shellfish extract, toxic to liver, but not toxic to kidney organ. Causes of liver cell poisoning suspected as a result of saponin content does not occur in kidney cells. This is presumably because saponin (sapogenin) has been detoxified by the liver so it relieves for the renal glomerulus to perform filtration and tubules in the treatment process for subsequent removal through the urine.

4. CONCLUSION

Based on the results of this study can be concluded that water extract *Meretrix meretrix* did not cause any effect on the physical animal test with LD50 value above 15 g/Kg body weight. Histopathologic observation of the liver and kidney, there was necrosis in some liver cells and degeneration in renal cells, however it can be stated that overally all the tissues were in normal conditions.

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