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TOXICITY TEST OF ETHANOL EXTRACT 70% JACKFRUITS SEEDS (*Artocarpus heterophyllus* Lam.) IN MICE (*Mus muscullus*)

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Abstract

Jackfruit is one of the plants used as traditional medicine. In a previous study, jackfruit seed extract has an effect as anti-hyperglycemia. The use of herbal medicines as anti-hyperglycemia is usually used for a long period of time. The aim of this study was to determined the toxic effects caused after repeated administration of jackfruit seed extract. The dosage used is 250 mg/kgBW, 500 mg/kgBW and 1000 mg/kgBW given orally for 30 days. Observations that have been made include observation of toxic symptoms, histology observation and examination the value of SGOT, SGPT and creatinine levels did not show any toxic symptoms and organ damage seen from the diameter of the central vein and organ damage seen from the diameter of glomerulus and the distance between glomerulus and bowman capsules.. These results indicate that the use of jackfruit seed ethanol extract in one month does not cause toxic. These results indicate that the use of 70% ethanol extract of jackfruit seeds in one month did not cause toxic effects.

Keyword : Subacute Toxicity, Jackfruit Seeds, SGPT, SGOT, Creatinine Level, Histolgy.

INTRODUCTION

One of the plants in Indonesia is jackfruit (Artocarpus heterophyllus Lam.). Jackfruit has many benefits, in a study conducted by [1], namely the fraction of jackfruit bark has antibacterial activity. Further efficacy of jackfruit leaves can be used as a facilitator of breast milk, treating wounds, fevers, skin diseases, antidiarrheals, analgesics and immunmodulators [2]. Research conducted by [3] also shows that jackfruit leaf extract has a hypoglycemic effect in diabetic rats induced by streptozotosin. Based on research conducted by [4] that ethanol extract of jackfruit seeds (*Artocarpus heterophyllus* Lam.) has the ability to reduce the level of hyperglycemia of mice at a dose of 50 mg / kg with a percentage of 32.3%. Based on these studies, the ethanol extract of jackfruit seeds has medicinal properties.

Based on these studies, the ethanol extract of jackfruit seeds has medicinal properties. Pharmacological research on jackfruit seeds has been carried out, therefore jackfruit seed extract needs to be supported by safety testing. Safety test of a natural material used as a treatment is carried out to determine the potential toxicity of the natural material. Toxicity tests are divided into three categories, namely acute toxicity tests, short-term toxicity tests, and long-term toxicity tests.

Subacute toxicity test is a test to determine the target organ or workplace. In this study using 3 doses, namely the largest dose (the dose that causes toxic effects but does not cause death), the smallest dose (the dose that does not provide toxic effects), and the middle dose (the dose between the largest dose and the smallest dose) [5]. The choice of liver as a target organ is because the liver is the largest parenchymal organ and has an important role in the body's metabolic processes. The liver also modifies drugs and toxins to be inactive or water-soluble, form plasma proteins such as albumin and globulin, produce bile, and as Kuppfer cells. The choice of kidney organ was chosen because the main pathway for excretion is that most of the toxins occur in the kidney through the process of urinalysis. As a result the kidney has a high volume of blood flow, concentrates the toxin in the filtrate carries the toxin through tubular cells, and activates certain toxins [6].

This study was conducted acute, subacute toxicity tests and teratogenic test to determine the toxic effects on the use of jackfruit seeds in a longer period of time and one of the pre-clinical trials designed to see the effect of repeated exposure to a substance with a non-lethal dose.

MATERIALS AND METHODS

Plant Sample Collection and Identification

The plant of *Artocarpus heterophyllus* Lam. ware collected from Balitro (Balai Penelitian tanaman Rempah dan Obat) Bogor, West Java-Indonesia. The botanically identified at Herbarium Bogoriense, Bogor Indonesia.

Acute Toxicity Study

Before conducting the experiment, the animals were randomly selected and grouped into five group and then kept in their cage for 7 days prior to dosing to allow acclimatization to the laboratory conditions. All groups of the mice (*Mus musculus*) fasted overnight prior to

administration. Following the fasting period, all animals were weighed, and the doses were calculated based on their body weight. The extracts were prepared in NaCMC 0.5%.

The 70% ethanol extract of *Artocarpus heterophyllus* Lam. was then administered orally at the doses of 250 mg/kg (dose I), 500 mg/kg (dose II), 1000 mg/kg (dose III) and 2000 mg/kg (dose IV) body weight of mice in the test groups. Control group (group V) received NaCMC 0.5%. These doses were selected based on the previous efficacy studies. After administering the plant materials, the animals were kept under close observation continuously for 1 hour and intermittently for 4 hours and thereafter once every 24 hours for the next 14 days.

During this study period, clinical observations were made for mortality, behavioral, neurological, and any other abnormalities and their weight was measured weekly. Finally, on the 15th day, their final weights were measured, and gross physical examinations were carried out. The rats were then anesthetized under diethyl ether. After sacrificing the rats, gross pathological observation was carried out on vital organs.

Subacute Toxicity Study

The subacute toxicity study was conducted for 30 days to examine the toxicity of the extract on some blood parameters and histopathology of the liver and kidneys [7]. For this study healthy adult mice of both sexes were used. Forty mice were randomly distributed into four groups (I, II, III and IV) each consisting of six mice (five female and five male) per group. Groups I orally administered with solution NaCMC 0,5%, groups II, II, and IV orally administered with 70% ethanol extract of Jackfruit at doses of 250, 500 and 1000 mg/kg body weight per day, respectively, for 30 days. Clinical observation was carried out for 30 days and their weight was measured weekly for four weeks. On the 31st day the final weight of the mice was measured and then they were anesthetized with ketamine injection and blood samples were collected from each animal by sinus orbital.

Blood samples in the test tubes containing EDTA were used to determine SGPT, SGOT and clearen creatinine using Automated Hematology Analyzer. After collection of blood samples, the mice were sacrificed by cervical dislocation and parts of the liver and the kidney were dissected out; and gross pathological observation was performed on liver and kidney to check histology.

Teratogenic Study

The study began with acclimatization in the experimental room for 10 days in order to be able to adapt to the new environment, during which the estrus phase and body weight were observed every day. Animal mating is done by inserting male animals into the cages of animals that have estrus [8]. Animals mixed with a ratio of 1 male mice with 3 female mice. Test animals that have proven to be pregnant are kept in individual cages and are grouped randomly with numbering so that the mice used can represent the population.

The extract used as a treatment for diabetes is given to pregnant mice during the organogenesis. The extract is administered orally every day from the 6th day until the 15th day of pregnancy, on the 18th day of pregnancy a laparotomy is performed and then dissected to take the fetus. Results of laparotomy obtained quantitative data in the form of: fetal weight, number of fetuses, dead fetuses, deformed fetuses, thromboembolism, and fetal physiology abnormalities.

Statistical Analysis

The data obtained were statistically analyzed, initially tested for normality and homogeneity. After that, an analysis of variance (ANOVA) test was carried out in one direction with a significance level of 95% ($p < 0.05$). Then see whether there are significant differences, if there are significant differences then proceed with the Tukey test [9].

RESULTS AND DISCUSSION

Acute Toxicity Study

Table 1. Dose Orientation of Acute Toxicity Study

GROUP	Dose (mg/kgBW)	%Mortality
Orientation 1	250	0
Orientation 2	500	0
Orientation 3	1000	0
Orientation 4	2000	0

There was no death orientation, so the dosage was 1000 mg/KgBW and 2000 mg/KgBW. Then the observations were made until the 14th day. Observations were made on the behavior of clinical symptoms, body weight and measurement of SGOT and SGPT levels on the 15th day. Observations were made on the behavior of clinical symptoms and body weight for 14 days with the aim to determine the changes that occurred in the treatment group after administration of the test substance with a normal control group. In the normal treatment group after

administration of 0.5% NaC CMC, the test animals appeared weak, still and started to move, but on the 2nd day the test animals returned to normal until the 14th day. Observations show weight gain and active movement.

Subacute Study

Table 2. SGOT Level Checking Results (UI/L)

Mice	Control Normal	Dose 250 mg/kgBW	Dose 500 mg/kgBW	Dose 1000 mg/kgBW
1	24.00	27.00	27.00	27.00
2	33.00	33.00	41.00	32.00
3	29.00	30.00	41.00	40.00
4	27.00	28.00	24.00	35.00
5	27.00	29.00	28.00	30.00
6	24.00	28.00	33.00	33.00
7	27.00	31.00	30.00	30.00
8	29.00	32.00	31.00	35.00
9	30.00	31.00	33.00	32.00
10	32.00	30.00	32.00	31.00
Mean	28.20	29.90	32.00	32.50
SD	3.01	1.92	5.52	3.57

Table 3. SGPT Level Checking Results (UI/L)

Mice	Control Normal	Dose 250 mg/kgBW	Dose 500 mg/kgBW	Dose 1000 mg/kgBW
1	9.00	8.00	9.00	10.00
2	9.00	10.00	12.00	12.00
3	10.00	12.00	18.00	15.00
4	14.00	8.00	10.00	12.00
5	12.00	14.00	17.00	18.00
6	4.00	4.00	5.00	6.00
7	5.00	5.00	6.00	6.00
8	4.00	4.00	5.00	6.00
9	7.00	8.00	7.00	9.00
10	4.00	6.00	7.00	7.00
Mean	**7.70**	**7.80**	**9.50**	**9.90**
SD	**3.69**	**3.49**	**4.84**	**4.41**

Based on the measurement of SGOT and SGPT levels, the results of SGOT and SGPT levels were obtained. The results of SGOT and SGPT levels were performed statistical tests. The first statistical test is done with the normality test using Kolmogorov-Smirnov. Based on the data obtained normality test results for levels of SGOT ($\alpha = 0.628$) and SGPT ($\alpha = 689$). The results of one-way ANOVA between the test group and the normal group showed a significant value (α) greater than 0.05 so it can be concluded that the use of jackfruit seed ethanol extract for a long time did not affect the levels of SGOT and SGPT.

Table 4. Results of creatinine levels (mg / dl)

Mice	Control Normal	Dose 250 mg/kgBW	Dose 500 mg/kgBW	Dose 1000 mg/kgBW
1	0.27	0.35	0.65	0.52
2	0.35	0.36	0.35	0.59
3	0,36	0.38	0.65	0.27
4	0.39	0.39	0.65	0.39
5	0.46	0.46	0.55	0.58
6	0.52	0.59	0.67	0.35
7	0.55	0.27	0.55	0.66
8	0.54	0.64	0.36	0.54
9	0.56	0.65	0.55	0.56
10	0.58	0.67	0.33	0.66
Mean	**0.47**	**0.48**	**0.53**	**0.51**
SD	**0.11**	**0.15**	**0.13**	**0.13**

Teratogenic Study

Table 5. The Number and Weight of Fetus

GROUP	TOTAL FETUS	BODY WEIGHT OF FETUS (g)
NORMAL	41	2.07
POSITIVE	35	2.11
NEGATIVE	38	2.06
DOSE 1	36	2.05
DOSE 2	34	2.15
DOSE 3	31	2.22

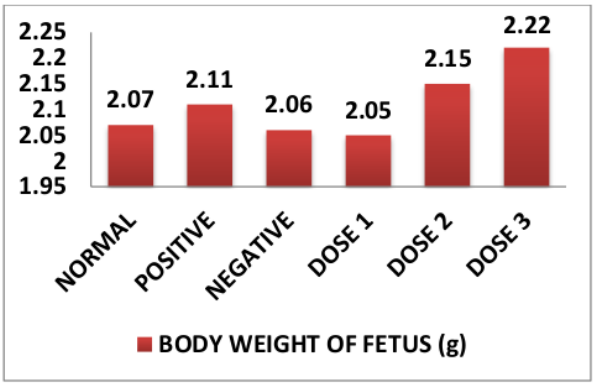

Figure 1. Graph of Weight Fetus

Table 6. Percentage of Malformation Organ in alive Fetuses

GROUP	TOTAL FETUS	Percentage of fetuses with malformated organ (%)					
		Eyes	Cleftpalate	Tail	Forelegs	Hind legs	Tromboemboli
NORMAL	41	0	0	0	0	0	0
POSITIVE	35	0	0	0	0	0	0
NEGATIVE	38	0	0	0	0	0	0
DOSE 1	36	0	0	0	0	0	0
DOSE 2	34	0	0	0	0	0	0
DOSE 3	31	0	0	0	0	0	0

The results of research on the development of rat fetus by giving 70% ethanol extract of jackfruit seeds were carried out during organogenesis, the 6th day to the 15th day of pregnancy. The observations in the form of rat fetus which were seen morphologically and fixed were carried out for 14 days of observation (Table 6), besides the number of live fetuses, body weight (table 5) and length of the fetus were also observed.

The results of observations of defects in the head, ears, eyelids, front-back toes, and tail showed that giving 70% ethanol extract of jackfruit seeds did not cause defects. Observations were made by comparing the normal group with the treatment group.

CONCLUSIONS

The acute toxicity study of the 70% ethanol extract of (*Artocarpus heterophyllus* Lam.) The results of the orientation up to the largest dose of 2000 mg / kgBB did not cause death. Meanwhile, subacute toxicity study of the 70% ethanol extract of Jackfruit did not adversely

affect the body weight and hematological and biochemical parameters of tested doses. There were no signs of toxicity observed in the kidney and liver sections of treated rats.

General teratogen test results, administration of Jackfruit seeds extract did not cause any abnormalities on developed fetuses. However, through the observation in which it may contribute to undeveloped fetus, women should be aware to take it during pregnancy or in women who desire a pregnancy.

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