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Research Article

Acute and sub-chronic toxicity studies of rambutan (Nephelium lappaceum l.) Fruit peel extract in rats

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Abstract.

Rambutan (Nephelium lappaceum L.) is a plant native to Indonesia that has a variety of potential pharmacological activities. In this study, we evaluated acute and sub-chronic administration of rambutan peel extract. In the acute toxicity study, rambutan peel extract was administered at doses of 400; 1,400; 4,900 and 17,150 mg/Kg BW for 24 hours in order to assess toxic effects that occurred. In subchronic toxicity study, the rambutan peel extract was administered at doses of 3,500 and 6,400mg/kg BW performed for 90 days in wistar rats. The results acute toxicity showed that a high dose of 17,150 mg/kg BW no rats were death after 24 hours oral administration. It could be concluded that extract with LD-50 is categorized as practically non-toxic. Administration of rambutan peel extract at high dose did not induce weight changes significantly. Rambutan peel extract did not affect to urine volume. Liver function at base line and at 45th days based on levels of SGPT and SGOT, dose 3.5 and 6.4 g/kg b.w compared to normal control were not different. While at 90th days dose 3.5 and 6.4 g/kg b.wt SGPT levels were increased. Creatinine and urea levels of groups and normal control were same. Blood parameters such as, hematocrit, hemoglobin, red blood cells, white blood cells, and platelets showed a profile that was almost the same as the control group. Based on data, it could be concluded that oral administration of rambutan peel extract on acute and sub chronic toxicity was safe.

Keywords: Rambutan; toxicity studies; rats

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1. Introduction

In Indonesia, 940 types of natural ingredients are known to have medicinal properties that have been used in traditional medicine from generation to generation by various ethnic groups in Indonesia. The use of natural ingredients as alternative medicine shows a number that continues to increase[1]. Rambutan (*Nephelium lappaceum* L.) is a green tree that is easy to grow in areas with tropical to subtropical climates[2]. The name rambutan comes from the Malay language which means 'hair' because of the many hairy protrusions on the peel of the fruit. Rambutan is included in the *Sapindaceae* family and is a seasonal plant that will bear fruit from December to March[3]. Rambutan is a plant native to Indonesia that has a variety of potential pharmacological activities.Rambutan

has been widely planted in several regions in the world, such as India, Thailand, Indonesia, Costa Rica, Equador, Australia, Guatemala and Mexico[4].

Rambutan peel accounts for 45.7% of the total rambutan fruit. This rambutan peel has not been widely used because it is considered a waste in the food industry[5]. Rambutan fruit peel contains several compounds such as flavonoids, tannins, saponins [6], and *epigallocatechin-3-gallat*e which have antihyperglycemic activity [7]. The ethanol extract of rambutan fruit peel also has high antioxidant activity and gets a higher value than vitamin E [8].

Acute and sub chronic toxicity studies are a method used to evaluate the safety of a chemical and analyze its active mechanism. The acute toxicity test is a test used to observe the symptoms and level of toxicity that can be caused by a single dose given to experimental animals [9]. The subchronic toxicity test is a test carried out on experimental animals by observing the liver because it is the primary site of action [10].

2. Methods

2.1. Materials

Research sites: This research is an experimental research. The research was conducted at the Pharmacology and Toxicology Laboratory of the Pharmacy Biology section of the Faculty of Pharmacy UMS for in vivo testing of pharmacology and acute-subchronic toxicity. **Pharmacological Test:** The materials used for pharmacological tests and acute subchronic toxicity use standard tools commonly used for pharmacological tests and sub-chronic acute toxicity. **Reagent and Chemical Test kit:** The materials used in this study were aspartate aminotransferase (ASAT), alanine aminotransferase (ALAT), creatinine and urea were purchased from BioSystems (Spain). Urine test strips, Labistix, were obtained from Laboratory Diagnostics (Siemens, France). **Plant material:**The plant material used in this research is rambutan peel which is obtained from Solo and its surroundings. The samples obtained were then determined in the Biology laboratory of FKIP UMS.

2.2. Methods

2.2.1. Acute toxicity test

Acute toxicity testing is conducted in accordance with the Organization for Economic Cooperation and Development (OECD) Handbook. Based on the manual, the toxicity test was carried out with a variation of the concentration of 5000 mg/kg, because with this concentration the experimental animals did not experience any real abnormality or symptoms[11]. After adjusting for the *lethal dose* (LD50), the experimental animals were divided into 4 groups of females and 4 groups of males with various doses. Each group contains by 5 rats.

Before the experiment, rat were fasted for 12 hours. Experimental animal groups with various concentrations of 400 mg/kg; 1400 mg/kg; 4900 mg/kg; and 17150 mg/kg. All animals was marked and weighed at the start. Observations were made at 30 minutes, 1 hour, 2 hours and 4 hours after giving the sample. Food intake is given by calculating body weight. Likewise, water intake was recorded daily during the trial period [12]. It was noted that there were abnormality and symptoms that appeared in rat such as death, organ coefficients and others.

2.2.2. Subchronic toxicity test

Body weight, food intake and water intake

The behavior and condition of all experimental animals were observed before and after feeding. Symptoms in mice were noted. Every food intake and water intake given are measured first. The calculation of the body weight of the rats was carried out on the 0, $45^{th}$and 90^{th}day. If any experimental animal dies, record it immediately.

Hematologycal analysis

1 ml of rat venous blood was collected in a vacuum blood tube containing lithium heparin. The observed hematologycal parameters were: leucocytes (WBC), lymphocytes (LYM), lymphocyte percentage (LYM%), monocytes (MON), granulocyte percentage (GRA%), red blood cells (RBC), hemoglobin (HGB), mean corpuscular hemoglobin (MCH), mean corpuscular hemoglobin concentration (MCHC), mean corpuscular volume (MCV), red blood cell volume (HCT), platelets (PLT), platelet pressure (PCT), and mean platelet volume (MPV)[12].

In previous studies showed that the hematologycal parametersvery sensitive when used as an indicator so that it can be used to determineintrusion of toxic substances[13].

Histopathologycal analysis

The liver and kidney fragments were buffered with formalin with a pH of 7.4. The fragments will be dehydrated in absolute ethyl alcohol and immersed in xylol and grown in paraffin [12].

3. Results and Discussion

3.1. Acute toxicity study

The acute toxicity test was conducted to determine the potential toxicity be observed from the Lethal Dose (LD_{50}) value for 24 hours[14]. The LD_{50} value can be used to assess the types of clinical symptoms that can be raised and the mechanisms that cause morbidity in tested animals. The LD_{50} is the dose used as a parameter of mordibity in 50% of the population. The beginning parameters were carried out for 24 hours and if there was no death then the observation was continued for 14 days. This is done to determine the possibility of delayed toxic effects by observing behavior and histopathology.

Based on a literature survey on conventional LD tests, female experimental animals are more sensitive than male experimental animals [15]. In this study, male and female experimental animals were used to compare the results obtained.

Table 1: Percentage of Morbidity of female and male rat.

Groups	N	Dose (mg/Kg BW)	Female rats die	Female rats life	Male rats die	Male rats life
I	5	400	0	5	0	5
II	5	1,400	0	5	0	5
III	5	4,900	0	5	0	5
IV	5	17,150	0	5	0	5

Based on table 1, it can be seen that the four experimental groups did not cause toxicity effects. This is indicated by the absence of death, especially at the highest dose within the maximum volume limit that can be administered. This means that the highest dose of 17,150 mg/KgBW does not cause death for 24 hours running. Because in 24 hours it is non-toxic, it needs to be extended to 14 days. It is known that 2 test animals died on the second day, but this dose was assigned to the pseudo LD_{50} dose.

Administration at doses of 4,900 and 17,150 mg/KgBW showed toxic symptoms after treating the rats eyes slightly glazed. For a dose of 17,150 mg/KgBW often experienced anxiety and increased frequency of vomiting, whereas in other groups it did not occur.

Based on the table, it can be seen that none of the female Wistar rats died. Like male wistars, female wistars died on the second day of observation. However, these results can be said to be non-toxic because at the highest dose of 17,150 mg/KgBW did not raised any death effect during the 24 hours treatment.

Giving a dose of 17,150 mg/KgBW to female rats showed toxic symptoms in the form of glazed eyes whose frequency was more frequent than male rats and anxiety with frequent intensity. The frequency of scratching of experimental animals was rare in the 4,900 mg/kg BW and 17,150 mg/kgBW groups, whereas the other groups not find.

Table 2: Body Weight.

Group	Doses mg/kgWB	Body Weight (g)		
		Before	After 24 hours	After 14 days
I	0	136.04	138.08	149.22
II	400	132.74	135.28	143.20
III	1,400	140.96	144.40	159.86
IV	4,900	151.58	153.80	168.38
V	17,150	135.20	137.13	153.74

Based on the table 2, it can be said that the group of male and female rats experienced an increase in body weight during 24 hours of observation and 14 days. So that it can be ascertained that giving rambutan fruit peel extract does not affect appetite in male and female rats.

3.2. Subchronic toxicity study

Dosage is a factor that can affect the safety of other compounds. In this study, stratified doses were used, namely 3.6 and 6.4% / kgBW. The test was carried out for 90 days on Wistar rats. Some things that were observed were changes in body weight, organ function, blood and urine as well as kidney and liver hispatology.

Based on the table, changes in body weight of the treatment and negative control groups did not show any significant changes. This also occurs in the parameters of food and water intake. So it can be said that the rambutan fruit peel extract does not stimulate changes in body weight, food and water intake, even though on the 45th and 90th days there was a change in food and water intake to be less than before.

The liver is the main organ used for toxic metabolism and the liver coefficient is usually used to evaluate the toxicity of the sample to be tested [15]. SGPT and SGOT activities have a close relationship with liver pathology and decreased enzyme activity

Table 3: Body Weight, Food Intake and Water Intake Parameters.

Observation	Parametric	Group		
		A	B	C
Day 0	Body Weight (g)	183.78	177.12	191.64
	Food intake (g)	16.68	17.02	20.70
	Water intake (mL)	28.0	30.0	30.0
Day 45	Body Weight (g)	262.36	249.14	274.58
	Food intake (g)	13.28	28.66	29.02
	Water intake (mL)	26.00	19.00	22.00
Day 90	Body Weight (g)	288.72	281.26	284.82
	Food intake (g)	15.32	10.36	12,00
	Water intake (mL)	29.0	20.0	23.6

Table 4: Kidneys and liver Function parameters (n=5).

Days observation	Parametric	Group		
		A	B	C
Baseline	Urea	41.8	40.8	36.4
	Creatinin	0.70	0.82	0.94
	SGOT	81.6	81.6	53.4
	SGPT	26.8	23.6	27.6
45^{th}	Urea	25.8	28.6	33.8
	Creatinin	0.60	0.72	0.78
	SGOT	43.8	51.2	52.2
	SGPT	24.6	26.8	28.2
90^{th}	Urea	40.2	37.8	45.6
	Creatinin	40.2	37.8	45.6
	SGOT	0.86	0.76	0,82
	SGPT	61.2	51.0	74.2

indicates an improvement in liver function. SGPT activity at a dose of 6.4% / KgBW on day 90 showed that the activity in that group had increased more than the control group, whereas in the 3.6% / KgBW dose group it showed that the group experienced a decrease compared to the control group. . This shows that in large doses, rats will experience a decrease in liver function while at lower doses there will be an improvement in liver function.

Creatinine is a metabolite of creatine which is excreted in the form of urine through the renal glomerulus. Profiles that do not differ from those of controls indicate good renal function.

3.3. Creatinine Parameters

Urine is the main route of excretion of most toxic compounds, so that the kidneys, which have a high enough blood volume, can concentrate toxins on the filtrate and carry these toxins through tubular cells. In these data, there is no significant difference in the urine profile of male and female rats and controls. The treatment is carried out by checking the pH value.

On the observation of urination on the first day, before and after administration for 90 days continued, the urine volume of all treatment and control groups tended to decrease in volume. The decrease was relatively same in each group. It can be said that the test preparation did not affect the urine of the test animals.

3.4. Blood Parameters

Based on the table, it can be stated that each tested parameter shows a profile that is almost the same as the control group. There was a difference in the number of white blood cells of mice on the 90th day of observation, where all groups, both control and treatment groups, experienced an increase in concentration. This is inconclusive because it occurs in all experimental animals.

4. Conclusions

Based on the results of the acute toxicity study, the rambutan fruit peel extract was declared non-toxic and given a sign that for 24 hours of treatment, no experimental animals died from either male or female rats. The results of the subchronic toxicity test showed that the rambutan peel extract was safe to use. This can be seen from the insignificant changes in each of its parameters such as parameters of body weight, food intake and water intake; kidney and liver parameters; blood parameters; and creatinine parameters. Rambutan peel extract with acute and subchronic toxicity tests did not show any toxicity and was safe to use.

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