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Acute toxicity of red fruits (*Pandanus conoideus* Lamk) oil and the hepatic enzyme level in rat

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ABSTRACT

Red fruit (*Pandanus conoideus* Lamk) empirically has been used Papuapeople, Indonesia as a natural medicine to treat a variety of diseases including cancer, HIV / AIDS, herpes and diabetes. The information about the toxicity of this plant is very important considering this plant is potential as medicine. The aim of this study is to determine acute toxicity of red fruit oil and its effect on hepatic enzyme level (AST and ALT). The oil was extracted by heat extraction method. Acute toxicity testing conducted based on OECD 423 guideline. In acute toxicity study, the oral dose of red fruit oil was administrated to 3 group (300, 2000 and 5000 mg/kg BW) in single dose. The general behaviour, adverse effect and clinical symptom was observed every hour in first 4 hours, 24 hours, 48 hours, and continue to observe for 14 days after administration of red fruit oil. No animals showed toxic symptoms in 300 and 2000 mg/kg dosing group. One animal in 5000 mg/kg BW dosing group had diarrhea one hour after administration. No animal dead in this experiment after 14 days observation. AST and ALT mean value for rats on 300 mg/kg BW, 2000 mg/kg BW, and 5000 mg/kg BW dosing groups are 22.70 ± 1.05 IU/L, 24.15 ± 8.89 IU/L and 24.54 ± 6.26 IU/L and 18.04 ± 0.77 IU / L, 19.69 ± 3.08 IU/L, 16.78 ± 1.60 IU / L, respectively. No statistically significant difference of the value of AST and ALT levels in each treatment group ($p > 0.05$). Based on the 2001 OECD acute toxicity, red fruit can be categorized as Category 5 GHS (Globally Harmonized System for Chemical Classification Substances and Mixtures) as practically non-toxic materials.

Keywords: *Pandanus conoideus* Lamk, Acute toxicity, Red fruits, AST, ALT.

INTRODUCTION

Buah Merah or red fruit (*Pandanus conoideus* Lamk) is an indigenous plant from Papua Island that belongs to genus *Pandanus*. This plant spread in Papua, New Guinea, and began planted in some areas in Indonesia^[1]. People usually utilize red fruits as food and consume directly, as sauce or use the oil for so much purpose. Red fruits oil used by people for traditional medicine^[2, 3]. Local people in Papua extracted the oil from the fruits by heating method and use for some purpose like food and medicine^[4].

Red fruit oil has high level of monounsaturated fatty acid (oleic acid), β -carotene, β -cryptoxanthin, α -tocopherol, phenolic compound and flavonoid that potentially become functional food and medicine. Red fruit oil empirically used by local people as natural medicine for many diseases such as cancer, rheumatoid arthritis and stroke, HIV Aids^[5, 6, 7].

The potential plant as medicine has to be followed by the data of safety level of the product. The substance and chemical compound of plant product may result in chronic toxicity or acute toxicity. Biological testing is important role in toxicity study. Acute toxicity is defined as the effects that may occurs either immediately or at a short time interval after single or multiple administration of such substance within 24 hours^[8, 9]. Acute toxicity testing is determining the effect of a single dose on a particular animal species. Some method was developed to determine acute toxicity such as the fixed dose procedure, the acute toxic category, and the up and down method^[10]. Different method have been developed which may require the use of fewer animals if possible^[11].

The liver is vital organ that performance and regulating homeostasis of the body. Some medicinal agent may injure liver and can cause subclinical injury to liver which can manifest only as abnormal liver enzyme^[12]. Elevation of hepatic AST levels can be attributed to toxic liver injury and increased in ALT

serum levels is more specific for liver damage [13]. There no data available about the effect of administration of high dose red fruit oil.

The aim of this study are to determine acute toxicity of red fruit oil and to identify effect of high dose red fruit oil for alanine transaminase (ALT) and aspartate transaminase (AST) enzyme in rats.

MATERIAL AND METHOD

Preparation of the plant

Plant was collected from Biopharmaca Conservation and Cultivation Station Tropical Biopharmaca Research Center Bogor Agricultural University. The method that been used to extract the oil from red fruit adapted from local Papua people. Red fruit oil extracted using hot extraction method. Ripe red fruit was steamed and blender to get the paste and then heat to form the oil.

Acute Toxicity Testing

Acute toxicity testing conducted in female rats 8 weeks old Sprague Dawley strain, weighing 150 ± 10 g. Dose for acute toxicity test of oil extract of red fruits were 300,2000 and 5000 mg/kg body weight, three animal each dose. Minimum three animals per dose are performed to this test based on OECD 423 Guidelines [14]. Then the animals were observed individually after a given dosage in the first 30 minutes and regularly at 24 hours (every 4 hours) and then observed for 14 days .We observed all condition of the animal such as skin and hair, eyes and mucous membranes, respiratory and circulatory system, the autonomic nervous system, the central nervous system, somatrotroph activity, and its behavior. It should be noted the existence of tremors, convulsions, hyper salivation, diarrhea, lethargy, sleep and coma. If animals suffered euthanasia can be performed and recorded. Body weight was measured before treatment and the end of the treatment All animals, humanly euthanized and a necropsy to assess the gross pathology. If there are any abnormalities, then continue on microscopic examination.

Serum assay for ALT and AST level

The activity of hepatic enzymes ALT (Alanin Transaminase) and AST (Aspartate Transaminase) conducted to determine acute toxic effect on the liver. ALT and AST was performed using Biolabo® reagentand read in 340 nm wavelengths.

RESULT

The mean body weight of rats used in this experiment was 155 g ± 4.06 g . Fourteen days after treatment, all rats showed increased body weight with a mean of 185.8 g ± 7.24 g , there is no difference between the increase in body weight in each dose group (p<0.05). Changes in body weight of rats are presented in Table 1 and Figure 1.

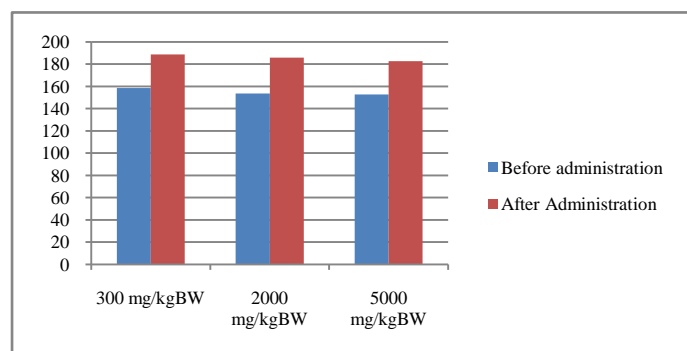


Figure 1: The Average of Body weight before and after administration of red fruit oil

Table 1: The average of Rats body weight before and after administration of red fruit oil

Group	Body weight before administration (g)	Body weight after administration (g)
1 (300 mg/kgBB)	158.67 ± 1.25	188.67 ± 1.25
2 (2000 mg/kgBB)	153.67 ± 3.30	196 ± 7.81
3 (5000 mg/kgBB)	152.67 ± 4.93	182.67 ± 9.84

No clinical symptoms had occurred in 300 and 2000 mg/kg body weight group after administration of red fruit oil, but in group who had 5000 mg/kg dose, one rat was got diarrhea at 60 minutes after red fruit oil administration. The diarrhea is a typical form of mucus red fruit oil but in subsequent observations no other clinical symptoms appear. No animals died in this experiment. Description of animals after treatment is presented in Table 2.

Table 2: Clinical sign and death after treatment of each dose

Dose	Rats / group	Number rat had toxicity symptom	Clinical sign	Number of death rats
300 mg/kgBB	3	-	-	-
2000 mg/kgBB	3	-	-	-
5000 mg/kBB	3	1	Diarrhea	-

There were no abnormalities when the necropsy on the organs of rats at group dose of 300 mg/kg, 2000 mg/kg and 5000 mg/kg of body weight.

Additional analyzes were performed in acute toxicity testing red fruit is an analysis of the activity of the enzymes ALT and AST. Examination of ALT and AST in the blood of rats conducted at the days 14 after administration. Average value of AST were 22.70 ± 1,05 IU / L, 24.15 ± 8,89 IU / L and 24.54 ± 6.26 IU/L for group of 300, 2000 and 5000 mg/kg body weight respectively,. However, there was no statistically significant difference in AST values between groups (p> 0.05).

Average value of ALT were 18.04 ± 0.77 IU / L, 19.69 ± 3:08 IU / L, 16.78 ± 1.60 IU / L for group of 300, 2000 and 5000 mg/kg body weight respectively. No significant difference in ALT values between groups (p> 0.05).

DISCUSSION

The utilization of plant for traditional medicine is very common especially in Indonesia, and become popular universally now days. Buahmerah or red fruit has been use by local people for many years ago and believed has lot of benefit for health. Medicinal plants are believed has less disadvantaged because its bioactive compound is natural. However, there is lack scientific information about the safety and adverse effect of medicinal plant. The method for the acute toxicity testing has developing now days. The use less animal as possible is preferred. The aimed of this study were to evaluated the red fruit oil for acute toxicity study and to identify the range that probably safe to use this plant as medicinal plant source.

The oral acute toxicity of red fruit oil carried out on 8 weeks old Sprague dawley rat at single dose of 300, 2000 and 5000 mg/kg body weight and following observation for first 4 hour and following 14 days after administration. The effect of the administration just occurred in one rat that given dose 5000 mg/kg body weight of red fruit oil. Clinical symptom that shown were diarrhea in first 60 minutes after administration but no further toxicity symptom occurred. Since no animals has dead on this study, red fruit oil seems to be safe at the dose level 5000 mg/kg body weight and the LD50 is considered be more than 5000 mg/kg body weight. This is suggesting that red fruit oil is practically non toxic material. This finding has

similarity with other research by Widowati 2009^[15]. All animal in this study has increase the body weight, scientific evidence confirmed that increase or decrease body weight are accompanied with accumulation of fats and physiological adaptation response to the plant rather than toxic effect of chemical^[16].

There no pathological symptom that occurred in all animal vital organ. However, toxicity of the medicine has been shown in biochemical parameter. The normal value level of aspartate amino transaminase (AST) in rat according to Jawl *et al*, (2008) is 17 to 30.2 IU/L. So in this experiment there was no increased AST activity after administration of high dose red fruit oil. ALT value in this experiment is lower than normal value (38 IU/L) based on normal values^[17]. The clinical implication of low ALT level has less known, but increased blood level of ALT has associated with liver injury. The alteration of AST and ALT level is common happen in hepatic toxic injury whether acute or chronic. Red fruits oil seems not hepatotoxic based on this study.

According to the 2001 OECD (Organization for Economic Cooperation and Development) guideline 423 of acute toxicity, red fruit oil can be categorized as Category 5 GHS (Globally Harmonized System for Chemical Classification Substances and Mixtures) since the dose of 5000 mg/kg body weight no death animal occurred. In this category means red fruits oil are practically non-toxic materials.

CONCLUSION

The acute toxicity of red fruit oil (*Pandanus conoideus* Lamk) was more than 5000 mg/kg body weight. There was no statistically significant on blood level of ALT and AST level in all group administration.

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