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Research Article

Acute Oral Toxicity Testing of Ethyl Acetate Fraction from *Garcinia mangostana* Linn Extract in Sprague-Dawley Rats

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Abstract

Garcinia mangostana-Linn (GM) or commonly known as Manggis by Indonesian, is a tropical tree native to Southeast Asia. It produces a fruit, whose pericarp contains tricyclic isoprenylated polyphenol or xanthones. The toxicity's evaluation of the ethyl acetate fraction from GM pericarp extract was needed before therapeutic use. The purpose of this article is to examine the oral acute toxicity test. The acute toxicity test was conducted in female sprague-dawley rats as per standard protocol. One group of rats administered single dose of 8 mg kg^{-1} b.wt. and the second group with a single dose of 18 mg kg^{-1} b.wt., of ethyl acetate fraction of GM extract, administrated orally and one control group. Body weight, behavioral changes and mortality were observed for 14 days. Dietary and water intake were also noted. At the end of the study, rats were sacrificed by decapitation. The result of this study showed that the Lethal Dose (LD_{50}) was found to be >15.480 mg kg^{-1} b.wt. There was a significant weight increase ($p<0.05$). Neither mortality nor behavioral changes were noted during 14 day study periods. This study demonstrated that there is a wide margin of safety for ethyl acetate of GM extract and there is no significant toxicity effect on the rats. In conclusion, ethyl acetate fraction GM may be considered for the therapeutic use in pharmaceutical formulation.

Key words: *Garcinia mangostana* L., acute oral toxicity, ethyl acetate fraction, sprague-dawley

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Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Strike a note the last 2000 years of the history of medicine in mankind have mainly used plants as the best source of medicine. More than 248,000 species of higher plants have been identified and 12,000 plants known to have medicinal properties. The World Health Organization (WHO) estimated that 80% of people used traditional medicine for primary health care (Sumithra and Arunachalam, 2014). One of the species from the genus Garcinia belongs to the family Clusiaceae, which is popular as mangosteen has been widely used as traditional medicine in Southeast Asia. Mangosteen or *Garcinia mangostana*-Linn (GM) is an indigenous plant of Indonesia and the tropical rain forest of Southeast Asian nation, such as Malaysia, Philippines and Thailand (Al-Massarani *et al*., 2013). The tree can growth up 6-25 m height but slow to grow. It is also known as the queen of fruits due to the best taste of tropical fruits. The fruit is a small round in shape, dark purple or reddish, with white juicy pulp and has a unique flavor, slightly sour and sweet (Gutierrez-Orozco and Failla, 2013; Kosem *et al*., 2013; Pedraza-Chaverri *et al*., 2008).

Several previous phytochemical studies have shown the active compound of mangosteen pericarp. Its pericarp contains a variety of xanthones as secondary metabolites and has been used in traditional medicine in Southeast Asia for centuries (Wang *et al*., 2011; Gutierrez-Orozco and Failla, 2013). People have used GML pericarp to treat diarrhea, antibacteria, antioxidant, inflammation and anticancer (Nguyen and Marquis, 2011; Dharmaratne *et al*., 2013), every drug has to be tested to ensure that the compound is safe for consumption. The determination of the toxic effects of a new drug is a prerequisite to guarantee the safety in use and in accordance with regulations for the use. Acute toxicity is one of the toxicity tests to identify the harmful effect on an organism through a single or short term exposure (Walum, 1998). There are only a few studies on the toxicity of drugs delivered from plants, especially GM, thus present study is proposed to demonstrate the acute toxicity test of the ethyl acetate fraction of the GM pericarp ethanol extract.

MATERIALS AND METHODS

Plant materials and extraction: The GM pericarp powder was extracted with ethanol 75% and macerated for 48 h. The solvent was evaporated with rotary vacuum evaporator at 50°C. After that, the crude extract was chromatographed on a silica gel column, with ethyl acetate (Kaomongkolgit *et al*., 2009; Ee *et al*., 2006).

Experimental animals: Specific-pathogen-free white female sprague-dawley rats were purchased from Indonesia National Agency of Drug and Food Control and acclimatized to the laboratory conditions for seven days. Fifteen female sprague-dawley rats of 3 month-old were chosen for this study divided into 2 groups. They were under observation in cages in a controlled condition, with room temperature 25-27°C. The individual cage with food and water were provided to acclimatize the rats before starting the study. All procedure were conducted in accordance with European Community Guidelines (EEC Directive of 1986;86/609/EEC) and were approved by Animal Ethics Committee of Faculty of Medicine, Universitas Indonesia, Indonesia.

Assignment of animals: Group 1, that served as normal control was administered water and the experimental animals were randomly divided into two groups. Group 2 was administered with 8 mL kg^{-1} b.wt. and Group 3 was administered with 18 mL kg^{-1} b.wt. They were identified by marking their body.

Mode of administration: The ethyl acetate fraction of GM pericarp was administered in a single dose by gavage using specially designed mice oral needles. Tested animal food fasted for 17-20 h prior to dosing but still given water to drink.

Administration dose: Animals were weighted and ethyl acetate fraction of the GM pericarp extract was administered orally at single dose 8 mg kg^{-1} b.wt. (Group 2) and the third group was administered 18 mg kg^{-1} b.wt. (Group 3). The concentration of test substance was 3094 ppm. The administration volume was 1 mL kg^{-1} of animal body wteight, in a single oral dose by gavages using a feeding needle. The acute toxicity test is performed according to the Organization of Economic Co-operation and Development (OECD) guidelines for testing of chemical (OECD., 2001).

Observation periods: Five Sprague-dawley rats were clinically observed in toxic symptoms and recorded systematically at 1, 2 and 4 h after test substance administered. The observation was continued day by day for as long as 14 days. The number of survivors was noted after 24 h and all rats were observed individually during

14 days observation. Appearance and behavioral changes were recorded (Sumithra and Arunachalam, 2014).

Pathological observation: On the last day of observation, all rats were decapitated and examined macroscopically. Anomalies in the internal organs were documented and examined microscopically. After these thorough examinations were done, the remaining rats and tissue were sacrificed and discarded.

Statistical analysis: Statistical analysis of Lethal Dose (LD_{50}) value was performed using Thompson-Weil with 95% confidence interval. The comparison between before and after treatment was analysed with t-paired test. A p-value of 0.05 or less was considered as significant.

RESULTS

Five minutes after administration of 8 and 18 mL kg^{-1} b.wt., the ethyl acetate fraction of the GM pericarp extract orally, the rats were becoming less active. On the second day, the rats were still less active, but on the third day the rats were becoming normal. There were not any behavioral signs of toxicity changes after the rat exposed to the ethyl acetate fraction of GM pericarp.

The animal experimental weight loss had occurred on the first day, but then the body weight increase until 14 days of the observation. On day seven, rat weight was measure and compared with the initial body weight. It showed that there was a significant increase in body weight in the third groups ($p = 0.042$) but there was not a significant increase of body weight in control and the second group at the middle observation period. However, at the end of observation, body weights in all groups were significantly increased (Table 1). There were no abnormal findings from gross pathological examination of all internal organs and no mortality. Based on these results, the oral LD_{50} of the ethyl acetate fraction of GM pericarp is suggested to be greater than 15,480 mg kg^{-1} b.wt., for female rats.

Table 1: Effect of ethyl acetate fractionation of GML pericarp on the weight changes in treated rats

	Body weight (Mean±SD)		
Dose group (mL kg^{-1} b.wt.)	Day 1	Day 7	Day 14
Control	159±1.00	159.20±1.43	165.65±1.77
Group1		(p = 0.5)	(p = 0.00)*
8	159±1.00	159.37±1.36	162.65±1.64
Group 2		(p = 0.271)	(p = 0.001)*
18	156.8±2.28	156.11±1.92	160.00±1.77
Group 3		(p = 0.042)*	(p = 0.000)*

t: Paired test analysis ($p<0.05$), *Significant difference and *Garcinia mangostana* Linn.

DISCUSSION

The GM has been used for hundreds of years in Southeast Asia to treat medical problems, such as, inflammation, diarrhea and skin infection. Recently, several studies have revealed that GM exhibit antimicrobial, antioxidant, antiproliferative, antiallergic and anti-inflammatory properties, due to the GM pericarp contains many secondary metabolites and fifty xanthones have been isolated from the GM pericarp (Pedraza-Chaverri *et al*., 2008).

This study demonstrated the increased of body weight and was different with the previous study using methanolic extract of GM pericarp at the doses of 10, 100, 500 and 1000 mg kg^{-1} day^{-1}, in their chronic toxicity study (Chivapat *et al*., 2011). The results demonstrated that administered the dose of methanolic extract of mangosteen pericarp in Wistar rats for sixth months did not cause any toxic signs or mortality in the animal tested but the body weight measurement indicated the depressed of body weight. The methanolic extract suppressed the food intake of the animal. This study states that the GM pericarp extract does not cause a change in pharmacotoxic signs and abnormalities in hematological values (Chivapat *et al*., 2011). Acute toxicity of methanolic extract of the GM pericarp has also examined at doses 1, 2 and 3 g kg^{-1} in Wistar rats, showed a slight increase in the body weight and did not induce any mortality to the animals.

The determination of the toxic effects of a new drug is a prerequisite in accordance with regulations for the use and for the public health guarantee. Therefore, this acute toxicity examination is necessary. Acute toxicity is adverse changes occurring immediately or a short time following a single ora short period of exposure to a substance or substances or as adverse effects within a short time administration of a single dose of a substance or multiple doses given within 24 h. The term acute oral toxicity is most often used in connection with lethality and LD_{50} determinations (Walum, 1998). The classification of acute systemic toxicity based on oral LD_{50} values according to OECD declared very toxic less than or equal to 5 mg kg^{-1} b.wt., toxic greater than 5 less than or equal to 50 mg kg^{-1}, harmful greater than 50 less than or equal to 500 mg kg^{-1} and no label greater than 500 less than to equal 2000 mg kg^{-1} (OECD., 2001).

There are only a few studies on the toxicity of xanthones and also acute toxicity studies of GML have not been widely publicized. Previous research has analyses acute toxicity of Crude Methanolic Extract (CME) from mangosteen pericarp. It has a LD_{50} value and approximately lethal dose at 1,000 mg kg^{-1}, whereas the suitable dose of short term study

should be less than or equal to 200 mg kg^{-1} (Kosem *et al*., 2013). The acute toxicity analysis of the ethanolic extract of the GM have shown no toxic effect in rats following a single dose of 5,000 mg kg^{-1} b.wt., during 14 days of observation (Hutadilok-Towatana *et al*., 2010; Bunyong *et al*., 2014). Other study also used ethyl acetate fraction of *Sargassum ilicifolium* (Turner) C. Agardh in rodent demonstrated neither mortality nor behavioral changes in acute and subacute examination (Sumithra and Arunachalam, 2014). The solvent used to extract GM pericarp in previous research were very diverse, therefore the resultof determinationon the toxicity of the extract can have different effects on different types of animals. Considering different bioactive component can be originated from the use of different solvents and also the variety of natural environment of the plants would produce different secondary metabolite compositions. However, studies have shown the safety of the GM pericarps extract (Sumithra and Arunachalam, 2014; Bunyong *et al*., 2014) and the result of this study showed the oral LD_{50} was greater than 15,480 mg kg^{-1} b.wt. and there was no label of toxicity to ethyl acetate fractionation of GML pericarp to female sprague-dawley rats. Further research on the effect of subacute/chronic toxicity and identification of active compounds and its bio-activities is necessary conducted for public health safety guarantee.

CONCLUSION

This study concludes that in this acute toxicity study, no mortality or toxicology signs were observed. The fraction was safe up to 15480 mg kg^{-1} b.wt. and also increased body weight of female sprague-dawley rats.

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