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The Preliminary Study on Safety of Using Mangosteen Peel Extract as Natural Herbs

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Abstract
Background: *The properties of mangosteen skin as anti-inflammatory have been described by many researchers both in vitro and in vivo. Over the past few years, mangosteen peel extract is used as a natural herbal remedy either orally or topically. However, the potential toxicity of extracts and formulations containing mangosteen peels is still a matter of little concern.*
Objective: *To prove that mangosteen skin extract is safe to use as natural herbal medicine.*
Methods: *Experiments with laboratory tests of microbiological tests and acute toxicity tests with samples of 30 Balb / C mice aged 8-10 weeks weight 25-35 g, divided into 6 groups in which 5 groups of intervention (giving mangosteen skin extract) with different dose (5, 50, 300, 2000 and 5000 mg / kgBB) and 1 control group. Parameters observed included weight, motor activity, salivation, respiration, defecation, urination, piloerection every day for 14 days while the observed organ was liver. Behavioral data and organ test results were used to evaluate the toxic effects of mangosteen skin extract. Data was analyzed with ANOVA and Post Hoc Test.*
Results: *Most subjects' mean body weight from day 0 to day 14 tended to stabilize with a slight increase or decrease of less than 1%. The results of observation for 14 days after the intervention to each group there was no death and no effect on the motor system, saliva, respiration, defecation, urination and piloerection and did not happen itch allergies. Based on Post Hoc Test (LSD), there was no significant difference in mean liver weight group with highest dose (5000 mg / kgBB) and control group.*
Conclusion: *Extract of mangosteen skin with dosage ≤ 5000 mg/kg BW is not toxic and safe to use as natural herbal medicine.*
Keywords: *peel of Mangosteen extract, toxicity, natural herbal medicine .*

1. BACKGROUND

Up to this point, herbal medicine plays a major role in the medication especially in the developing countries because of its cost-efficient (Patel et al., 2012). The use of natural herbal medicine is one of the alternative drugs that society needs because the price is more affordable and supports local policies of local governments aiming to maximize the natural cultivation. Mangosteen is an Indonesian origin plant that is easy to find and cheap. The mangosteen peel has been widely used for the treatment and contains phenol derivatives,

xanthones or xanthen-9H-ones (Cui et al. 2010) which have a major content of α-, B-, γ-mangostins. Clinically, mangosteen peel gel can speed recovery in periodontal treatment (Rassameemasmaung et al., 2008). Xanthones are antioxidant, anti-tumor, antibacterial, antiviral, anti-fungal, anti-allergic and anti-inflammatory (Shan et al., 2011) and α-Mangostin able to inhibit hypoxia caused by Reactive Oxygen Species (ROS) (Lei et al., 2014). Furthermore, Xanthones as a natural anti-oxidant are non-toxic, safe for use, effective at low concentrations (0.01-0.02%), available at reasonable prices and resistant to product processing (Ozyurt et al., 2007).

Over the last few years, much research has been done on the use of mangosteen peel extracts containing the main ingredients of xanthone and mangostin as natural herbal remedies either orally or topically. The most commonly found compounds in xanthones in mangosteen peel are α mangostin, and ∂ mangostin (Chaivisuthangkura et al., 2009) and the level of xanthone content per 100 gr of mangosteen peel reaches 1076 ppm (Obolskiy et al., 2009). Efficacy of mangosteen skin extract in the treatment has been proven as the anti-inflammatory and antioxidant.

Nevertheless, there have been several studies suggesting the toxic effects of mangostin. For example, a preliminary survey by (Sornprasit et al., 1987) who conducted toxicity studies in rats through intraperitoneal injection at doses of 200 mg / kg BW, found serum glutamic oxaloacetate (SGOT) enzyme activity and Glutamic Pyruvic Transaminase (SGPT) serum increased and reached its maximum level after 12 hours of injections. Wong and Klemmer, (2008) reported that there were severe cases of lactic acidosis associated with the use of mangosteen juice as a dietary supplement. The potential toxicity of extracts and formulations containing mangosteen peels is still a matter of little concern, and toxicity data is lacking. This way, the researchers want to determine the potential toxic effect of mangosteen peels by evaluating the safety of these extracts that would later be used as natural herbal preparations for human use.

2. MATERIALS AND METHODS

Animal Experimentation

Simple randomized 30 Balb / C mice (8 weeks, 25-35 gram weight) divided into six groups in which five groups of intervention (giving mangosteen skin extract) with different dose (5, 50, 300, 2000 and 5000 mg/kg) and one control group. The animal is first acclimatized for 7 (seven) days and is pre-empted for 4 (four) hours before the provision of the test material, while drinking is still given.

Plant Testing Material

Preparation of plant material obtained by extracting the skin of mangosteen fruit (Figure 1) using the method of maceration (BPOM-RI, 2012):

I. Mangosteen skin is sorted, washed and dried.
II. Soaked with 40% Ethanol solvent for 1 x 24 hours.
III. Filtered with 40% Ethanol solution, evaporated at 600C for 2 hours.

Figure 1. Extract of Mangosteen Feel

Preparation of Acute Toxicity Experimentation of Testing Material

The mangosteen peel extract is suspended in CMC solvent and diluted according to the dose administered. The test material was administered on a per-oral basis with 1 ml volume and 5, 50, 300, 2000 and 5000 mg / kgBB mice, while the control group was given solvent (CMC). The test material with various doses can be seen in Table 1. The ingredients are administered via gastric sonde and are administered only once, while feeding and drinking are given ad libitum during the experiment.

Table 1. Mangrove Skin Extract Testing Material Dose

Groups	Dose	Testing Material
I	5000 mg/kg BB	5000 x 0.025 = 125 mg/mencit
II	2000 mg/kg BB	2000 x 0.025 = 50 mg/mencit
III	300 mg/kg BB	300 x 0.025 = 7.5 mg/mencit
IV	50 mg/kg BB	50 x 0.025 = 1.25 mg/mencit
V	5 mg/kg BB	5 x 0.025 = 0.125 mg/mencit
VI	0 mg/kg BB	CMC

Methods

The research is quantitative analytical research, experiment with randomized controlled group design. Several test steps have been conducted in the laboratory to determine the safety of mangosteen skin extract used in this research.

In the acute toxicity test, the observation of the intentional animal behavior was carried out intensively during the first 6 (six) hours, followed by observation of toxic effects and death every hour for 24 hours and then observed every day for 14 days. Parameters examined include motor activity, salivation, respiration, defecation, urination, and piloerection. The presence of death was observed for 14 days, and immediate necropsy was done to see the possible cause of mortality. The surviving animals are terminated on day 14 and are examined macroscopically by calculating the weight of each mouse. If the animal is dying, the examination of the possibility of organ damage is conducted macroscopically for subsequent microscopic examination of the damaged organ. Weighing animal body weight test is every day for 14 days. Behavioral and organ test results were used to evaluate the spectrum of toxic effects and the data obtained were analyzed descriptively and analytically with ANOVA and Post Hoc Test (LSD) at a 95% confidence level to determine the potential for acute toxicity.

3. RESULTS AND DISCUSSION

A.RESULTS

The results of the analysis of the testing material (mangosteen peel extract) indicated that the mangosteen fruit used was derived from Garcinia mangostana L species was a non-hazardous substance and when identified by HPLC (High Performance Liquid Chromatography) showed that out of 100 gr of mangosteen peel extract positively contained 0.16% xanthones and 0.74% mangostin. The Microbiology Test of Mangosteen Skin Extract is shown in the following table.

Table 2. Microbiological Test Results of Mangosteen Skin Extract

Item	Result	Specification
Total Plate Count	0 x 10^1 cfu/ml	Max 1 x 10^4 cfu/ml
Mold/Yeast	0 x 10^1 cfu/ml	Max 1 x 10^3 cfu/ml
Escherichia coli	Negative/ml	Negative/ml
Pseudomonas aeruginosa	Negative/ml	Negative/ml
Salmonella sp	Negative/ml	Negative/ml
Staphylococcus aureus	Negative/ml	Negative/ml

Table 2 shows the extract of mangosteen peel used as the test material in this study did not contain bacteria of *Escherichia coli*, *Pseudomonas aeruginosa*, *Salmonella sp*, *Staphylococcus aureus* as well as *total plate count* and *mold/yeast* did not exceed the tolerance limit.

The test material was administered on a per-oral basis with 1 ml volume and the dose of 5, 50, 300, 2000 and 5000 mg / kgBB mice while the control group was given solvent (CMC). The mean weight of research subjects up to day 14 is depicted in Table 4.

Table 3. Mean Weight of Research Subject

Group (Doses)	Weight(gr)													
	Days													
	1	2	3	4	5	6	7	8	9	10	11	12	13	14
5 mg	29.02	29.30	29.78	29.70	29.60	29.70	29.86	30.40	30.90	31.20	31.60	31.40	31.06	30.72
50 mg	26.56	27.10	27.70	27.23	26.76	26.67	26.58	26.50	26.58	26.68	26.78	26.38	26.35	26.34
300 mg	26.90	27.30	27.72	26.90	26.06	26.20	26.46	26.50	26.70	26.80	26.82	26.22	26.40	26.76
2000 mg	26.36	26.90	27.68	27.10	26.36	26.20	26.08	16.50	27.50	27.55	27.62	27.10	27.30	27.42
5000 mg	35.22	37.10	38.16	37.50	36.72	36.40	36.22	35.80	35.26	35.10	34.84	34.62	36.60	37.50
Control	30.96	32.50	34.26	32.20	31.18	31.50	31.68	31.90	32.10	32.40	32.66	31.88	32.60	33.38

Figure 2. Body weight up to 14th day

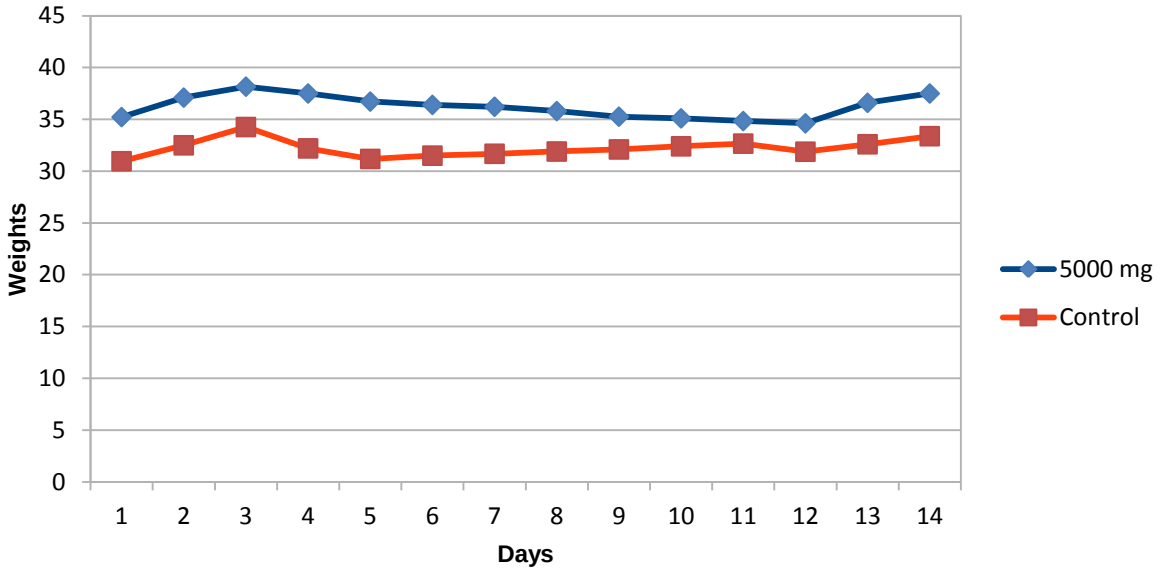

Table 3 and Figure 2 indicated that most of the subjects' mean body weight from day 0 to day 14 had a slight increase ranging from 1.06 to 2.42 gr (0.4-0.8%). The exception was found in the group at doses of 50 mg / kgBW and 300 mg / kg BW tht experienced a slight decrease ranging from 0.14 to 0.22 gr (0.05-0.08%).

The results of intensive observation during the first 6 hours, then every hour for 24 hours and then carried out daily observations for 14 days after giving the test material (mangosteen peel extract) to each group can be seen in Table 4. The result indicated that there was no death and no effect on the motor system, saliva, respiration, defecation, urination, and piloerection as well as no allergies occur.

Table 4. Observation Result of Toxicity Effect of Testing Materials Day 0 to 14

Group (Doses)	Observation Parameter						
	Motoric	Saliva	Respiration	Defecation	Urination	Piloerection	Allergic
5 mg/kgBB	Normal	Normal	Normal	Normal	Normal	Normal	No
50 mg/kgBB	Normal	Normal	Normal	Normal	Normal	Normal	No
300 mg/kgBB	Normal	Normal	Normal	Normal	Normal	Normal	No
2000 mg/kgBB	Normal	Normal	Normal	Normal	Normal	Normal	No
5000 mg/kgBB	Normal	Normal	Normal	Normal	Normal	Normal	No
Kontrol	Normal	Normal	Normal	Normal	Normal	Normal	No

The heart means weight of the study subjects is shown in Table 5.

Table 5. The Mean Heart Weight of Research Subject

Groups		Mean ± SD (gram) ; Median (min-max)	P-Value *
I	5 mg/kgBB	1.94±0.27; 1.97 (1.56-2.32)	
II	50 mg/kgBB	1.92±0.31; 1.90 (1.53-2.40)	
III	300 mg/kgBB	1.70±0.13; 1.70 (1.54-1.84)	
IV	2000 mg/kgBB	1.75±0.31; 1.84 (1.33-2.12)	
V	5000 mg/kgBB	2.32±0.17; 2.30 (2.09-2.55)	> 0.05
VI	Control	2.15±0.34; 2.07 (1.76-2.51)	

*Post Hoc Test (LSD) vs control group

Table 5. shows the mean weight of study subjects ranged from 1.70 to 2.32 grams. Based on the Post Hoc Test (LSD), there was no significant difference in the average group of heart weight in highest dose (5000 mg), and control group as p-value is ≥ 0.05.

B.DISCUSSION

Acute toxicity test on mangosteen peel extract aims to determine the potential toxicity of "Herb," mean lethal doses (LD50). The preliminary information on the symptoms and the possible effects on target organs as well as the sensitivity of species are used to establish dose levels. From this result, the risks of its use or exposure to humans and as a reference for designing subsequent tests of safety and toxicity are estimated. The results of the analysis shows the mangosteen fruit used from the species of Garcinia mangostana L is a safe material and does not contain bacteria pscherichia coli, pseudomonas aeruginosa, Salmonella sp, Staphylococcus aureus. Since total plate count and mold/yeast does not exceed the limit tolerance, the results ensure that the test material used does not contain bacteria and fungi which may affect the acute toxicity test results generated.

HPLC (High-Performance Liquid Chromato-graphy) of 100 gr mangosteen extract positively contains 0.16% Xanthone and 0.74% Mangosteen. These results are consistent with studies of Pedraza-Chaverri et al., (2008); Walker (2007); and Zhao et al., 2016). The compound is commonly found in xanthones and mangosteen rind is α and ∂ mangostin. Most subjects' weight average from day 0 to day 14 tended to be stable with a slight increase or decrease of less than 1%. This result is by the study of Vishnu Priya et al., 2010) stating the weight of the study subjects experienced relatively no significant change during the experiment.

Though the preliminary research by Sornprasit et al., (1987) found the hepatotoxic effects mild form of the enzyme activity of SGOT and SGPT that increased and reached the maximum level after 12 hours of injection, this study found no hepatotoxic effects during Post Hoc Test (LSD) as no significant mean differences in liver weight between the highest dose group (5000 mg / kg) and the control group (p-value ≥ 0:05). There is no death and no effect on the motor system, saliva, breathing, defecation, urination and piloeretion and no allergies occur. These results are consistent with previous studies by Hutadilok-Towatana et al.(2010); Jujun et al., (2008) on the evaluation of acute toxicity of the mangosteen peel extract at a dose of ≤5000 mg. Recent research suggests that the maximum tolerated dose for acute toxicity in rats is 5000 mg (Bunyong et al., 2014).

4. CONCLUSION

Extract of mangosteen peel with doses of ≤ 5000 mg is not toxic and safe to use as natural herbal medicine. Further research is needed to evaluate the chronic toxicity of mangosteen skin extract to determine its long-term safety.

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