Acute and Repeated Dose 28-Day Oral Toxicity Study of *Garcinia mangostana* Linn. Rind Extract

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ABSTRACT

The ethanolic rind extract of Garcinia mangostana L. (Guttiferae) is composed of mangostin which can be used as an antibacterial agent for the treatment of sorethroat. This study examined acute oral toxicity (OECD 420) and repeated dose 28-day oral toxicity study in rats (OECD 407) for safety in human use. In acute toxicity test, oral administration of 2, 3 and 5 g/kg BW of crude extract showed no toxic signs, no mortalities and no effect on growth rate in both control and experiment groups. In addition, none of them showed gross pathological changes at necropsy. In subacute toxicity test, Sprag-Dawley rats, (13 of each sex in each group) were gavaged with suspension of G. mangostana L. rind extract at the dose of 0, 50, 500 and 1,000 mg/kg BW/day for 28 consecutive days. Rats in the satellite group were given the test material at the dose of 1000 mg/kg/BW for 28 days and observed thereafter for 14 days in order to study the reversibility of adverse effects. Results of the study showed that there were no significant effects on average body weight, relative organ weight, histopathology of organs, clinical biochemistry and hematological parameters of treated rats. In conclusion, the ethanol extract from the rind of G. mangostana at tested dose and time duration did not cause acute or subacute oral toxicity in rats.

Key words: Acute toxicity, Subacute toxicity, OECD 420, OECD 407, Mangosteen rind extract

INTRODUCTION

Mangosteen, *Garcinia mangostana* Linn. (Guttiferae), the rinds of which have been used as a traditional medicine in Thailand for the treatment of trauma, diarrhea and skin infections (Nakatani et al., 2002). The xanthones, α and β mangostins, are major bioactive compounds found in the fruit hulls of the mangosteen (Jinsart et al., 1992;Chairungsrilerd et al., 1996a,b,c). The biological activities of α mangostin have been confirmed to consist of a competitive antagonism of the histamine H1 receptor (Chairungsrilerd et al., 1996a; Iikubo et al., 2002), antibacterial activity

against *Helicobacter pylori*, anti-inflammatory activities, inhibition of oxidative damage by human low-density lipoproteins (LDL) (Iikubo et al., 2002), antimicrobial activity against methicillin-resistant *Staphylococcus aureus* (Iinuma et al., 1996) and weak antioxidant activity (Chairungsrilerd et al., 1996a). Moreover, α and γ mangostins can inhibit both human immunodeficiency virus (HIV) infection (Chen et al., 1996; Vlietinck et al., 1998) and topoisomerases I and II (Tosa et al., 1997). The mangosteen has long been widely used as an antiinflammatory, antidiarrhea, and anti-ulcer agent in Southeast Asia (Harborne and Baxter, 1993; Lu et al., 1998).

Toxic effects of mangostin were previously examined in toxicity study in mice . Sornprasit et al. (1987) performed toxicity study in mice via intraperitoneal routh with dose 200 mg/kg BW and found that the activities of serum glutamic oxaloacetic (SGOT) and serum glutamic pyruvic tranaminase (SGPT) enzymes increased and reached the maximal level after 12 hours of injection. The activities of these two enzymes increased with dose of mangostin. The mangostin which was forced-fed to rat 1.5 g/kg BW was also compared with paracetamol. It was found that paracetamol increased the activities of SGOT and SGPT much more than did mangostin and the amount of total liver protein of paracetamol-treated rats significantly decreased whereas that of mangostin-treated rats did not change. There has not been much study on acute and subacute toxicity of mangosteen crude extract in spite of the fact that there are many products on sale in Thailand from mangosteen rind crude extract, such as throat spray for the treatment of pharyngitis (Kongchanmitkul, 2002) gel for the treatment of periodontitis (Maungmingsook, 2003), hence, its toxicological data are still lacking. In our acute and subacute toxicity, we therefore determined potential toxic effects and evaluated the safety of this extract for pharmaceutical preparation to be used in human.

MATERIALS AND METHODS

Preparation of plant extract

The mangosteen fruits were purchased from local market in Chiang Mai province, Thailand. The rinds were washed, cut, dried at 50-60°C and powdered to 100 mesh. The extraction was performed by macerating 1.13 kg of mangosteen rind powder with 6.78 kg of 95% ethanol at room temperature for 5 days. After suction-filtering through a buchner funnel, the ethanolic filtrates were evaporated by a rotary evaporator at 40-60°C. Extracts isolated from *G. mangostana* rind were kept at 4°C until testing. The crude extract was analyzed by HPLC, it composed of mangostin 11.45% w/w.

Laboratory animals

Adult Sprague-Dawley rats of either sex, aged 6-8 weeks with a weight of 200-250 g were purchased from the National Laboratory Animal Center, Salaya, Mahidol University, Nakorn Pathom, Thailand. The animals were kept in an animal room where the temperature was maintained at 25±1°C under a 12-hours light dark

cycle. They were provided with food and water for 1 week to acclimatize them before starting the experiment. This study had been approved by Faculty of Pharmacy's Ethical Committee.

Acute toxicity studies

The acute toxicity study of the extracts of *G. mangostana* rind was performed as described by OECD 420. Ethanolic extract suspended in 25% ethanol in water was administered in a single oral dose with 2, 3 and 5 g/kg BW by gavages using a feeding needle. The control group received an equal volume of 25% ethanol in water vehicle. Six females and six males were used for each dosage level. They were deprived of food, but not water 12-14 h prior to the administration of the test suspension. Observations on toxic symptoms were made and recorded systematically at 1, 2, 4 and 6 hours after administration. Finally, the number of survivors was noted after 24 hours and these animals were then maintained for further 14 days with observations made daily. At the conclusion of the experiment, all surviving animals were sacrificed with an injection of pentobarbital and their organs such as liver, lung, heart, spleen, adrenals, kidney, testes and ovaries were excised and weighed. The pathological observations of these tissues were performed on gross anatomical change.

Repeated Dose 28-Day Oral Toxicity Studies

The animals were divided into five groups of thirteen females and thirteen males, totalling 130 rats. Ethanolic extract of *G. mangostana* rind suspended in 10% ethanol in water was administered orally by gavaging at 0, 50, 500 and 1000 mg/kg BW of the extract daily for a period of 28 days. The control group received an equal volume of 10% ethanol in water. In order to assess reversibility, an alcoholic extract of *G. mangostana* rind was administered to a group of rats at 1000 mg/kg BW daily for 28 days, with no treatment for the following 14 days. All rats were weighed and observed daily for physiological and behavioral changes. Any rat that died during the test period was tested pathologically, and all animals were examined at the end of the test period

Blood analysis

All surviving animals fasted overnight and were anesthetized afterwards for blood collection from a common carotid artery. Blood samples were collected into heparinized and dry non-heparinized centrifuge tubes. A blood analysis (both hematology and chemistry) was carried out. The heparinized blood was used for a hematological study which included WBC and differential leukocyte counts, platelet, hematocrit and hemoglobin estimation. The non-heparinized blood was allowed to coagulate before being centrifuged and the serum was separated. The serum was assayed for glucose, creatinine, blood urea nitrogen (BUN), aspartate transaminase (AST), alanine transaminase (ALT), alkaline phosphatases (Alk-P), total protein and albumin

Tissue analysis

After blood collection, the animals were sacrificed for tissue examinations. The following tissues and organs were weighed, examined, and then fixed in 10% buffered formaldehyde solution : heart, lung, thymus, liver, kidney, spleen, adrenals, small intestine, stomach and duodenum, muscle with sciatic nerve, thoracic spines, brain, sex organs, uterus and epididymis. The fixed organs from all animals were examined by histological method.

Statistical analysis

Results were expressed as mean ± standard deviation. (S.D.) In acute and subacute toxicity, statistical significance was determined by one-way analysis of variance (ANOVA) and pos hoc Duncan's multiple range (DMR) test. P values less than 0.05 were considered significant.

RESULTS AND DISCUSSION

Acute toxicity

In the acute toxicity test at the highest dose of 5 g/kg/BW, all rats did not exhibit signs of toxicity and mortality after a single oral administration of 95% ethanol extract from the rinds of *G. mangostana* Linn. The animals did not show any change in general behavior or other physiological activities. The body weight gain and internal organ weight were next observed since a decrease in both parameters would indicate the presence of toxicity. The body weight gain of male rats which received the extract at dose 5 g/kg/BW was slightly lower than control group and other treatment group but the difference was not significant (Table1). The pathological examinations of the tissues on a gross indicated that there were no detectable abnormalities. The organs of both control and treated groups were unremarkable and comparable to each sex. No further evidence of histopathological changes was observed. According to the OECD 420 guideline for testing of chemical, the results of acute toxicity suggested that 95% ethanol extract from the rind of *G. mangostana* Linn. is fairly nontoxic.

Subacute toxicity

Results of the subacute toxicity test showed that administration of the ethanol extract from the rind of *G mangostana* Linn. at dose of 0, 50, 500 or 1000 mg/kg/BW daily for 28 days did not cause mortality. As shown in Table 2 and 3, no statistical difference from the control was also detected on the body weight. The animals did not show any changes in general behavior or other physiological activities. There were no significant differences in the body and organ weights between control and treated animals of both sexes. The weights of internal organs of both male and female rats in the satellite group were not found to be statistically different from those of the treated and the control group.

Table 1. Body weights of rats in acute toxicity of the ethanol extract from the rind of *G. mangostana* Linn.

Sex	Group (n = 6)	Body weight(g)		Weight gain (g) on day 14
		Day 0	Day 14	
Female rats	control	179.2±8.6	205.0±6.3	25.8±4.9
	2 g /kg /BW	187.5±9.9	210.8±5.8	23.3±7.5
	3 g /kg /BW	184.2±13.2	205.8±12.8	21.6±2.6
	5g /kg /BW	185.8±18.5	210.0±15.1	24.2±3.8
Male rats	control	239.2±11.2	309.2±8.6	70.0±4.5
	2 g /kg /BW	240.0±8.9	312.5±8.2	72.5±5.2
	3 g /kg /BW	244.2±8.6	322.5±9.3	78.3±4.0
	5g /kg /BW	237.5±7.5	293.4±5.2	55.9±4.9

Values are expressed as mean ± S.D., n = 6, there were no significant differences ($P > 0.05$)

Table 2. Body and organ weights (g) of male rats treated with ethanolic extract of *G. mangostana* rind in a Repeated Dose 28-Day Oral Toxicity.

Weight	Control (10% ethanol)	*G. mangostana* rind extract (mg/kg BW/day)			
		50	500	1000	1000*
Body weight male (g)					
Initial	309±50	307±54	307±52	312±57	302±50
Final	389±28	394±20	390±52	378±28	363±34
Increased (%)	25.9	28.3	27	21.2	20.2
Organ weight male (g/100 g BW)					
Lung	0.63±0.26	0.58±0.30	0.61±0.38	0.52±0.10	0.82±0.30
Heart	0.36±0.02	0.36±0.02	0.35±0.02	0.35±0.02	0.36±0.03
Liver	3.26±0.40	3.08±0.41	3.22±0.41	3.1±0.43	3.18±0.48
Spleen	0.22± 0.02	0.23± 0.03	0.23±0.03	0.22±0.02	0.27±0.05
Adrenal-left	0.006±0.001	0.006±0.001	0.007±0.001	0.007±0.001	0.007±0.001
Adrenal-right	0.006±0.001	0.006±0.001	0.007±0.001	0.007±0.001	0.007±0.001
Kidney-left	0.39±0.04	0.39±0.03	0.39±0.02	0.38±0.03	0.41±0.04
Kidney-right	0.4±0.04	0.37±0.08	0.39±0.02	0.38±0.03	0.42±0.04
Testis-left	0.481±0.038	0.474±0.057	0.506±0.056	0.525±0.042	0.547±0.069
Testis-right	0.491±0.035	0.493±0.038	0.509±0.079	0.53±0.037	0.545±0.067

Data are expressed as mean ± S.D., n = 13

*A separate group was administered at 1,000 mg/kg BW daily for 28 days followed by no treatment for 14 days.

No significant differences from the control ($P > 0.05$).

Table 3. Body and organ weights (g) of female rats treated with ethanolic extract of *G. mangostana* rind in a Repeated Dose 28-Day Oral Toxicity.

Weight	Control (10% ethanol)	G. mangostana rind extract (mg/kg BW/day)			
		50	500	1000	1000*
Body weight female (g)					
Initial	213±25	210±23	217±24	215±18	212±23
Final	258±20	261±16	262±24	262±24	268±28
Increased (%)	21.1	24.3	20.7	21.9	26.4
Organ weight female g/kg BW/day					
Lung	0.6±0.22	0.58±0.22	0.58±0.25	0.74±0.45	0.67±0.22
Heart	0.38±0.02	0.36±0.02	0.37±0.02	0.37±0.03	0.38±0.01
Liver	3.19±0.40	3.22±0.29	2.98±0.31	3.17±0.31	2.98±0.33
Spleen	0.26±0.02	0.25±0.02	0.25±0.02	0.27?0.04	0.26±0.05
Adrenal-left	0.011±0.001	0.011±0.001	0.012±0.001	0.012±0.001	0.011±0.001
Adrenal-right	0.011±0.001	0.011±0.001	0.012±0.001	0.012±0.001	0.013±0.001
Kidney-left	0.37±0.03	0.36±0.02	0.36±0.02	0.36±0.02	0.37±0.03
kidney-right	0.37±0.02	0.36±0.02	0.36±0.02	0.37±0.02	0.38±0.03
Ovary-left	0.021±0.004	0.024±0.003	0.024±0.005	0.026±0.008	0.031±0.003
Ovary-right	0.021±0.003	0.023±0.005	0.025±0.004	0.022±0.004	0.038±0.005

Data are expressed as mean ± S.D., n = 13

* A separate group was administered at 1,000 mg/kg BW daily for 28 days followed by no treatment for 14 days.

No significant differences from the control ($P > 0.05$).

Hematological and biochemical observations

Histological examination is needed to confirm the characteristic of all the tissues. Hematological parameters provide vital information regarding the status of bone marrow activity and intravascular effect such as hemolysis. The hematological analysis (Table 4) showed no significant differences in any of the parameters examined in either the control or treated groups of both sexes. Blood chemistry analysis (Table 5) revealed no significant changes in any of the parameters examined in either the control or treated groups of both sexes.

Table 4. Hematological values of rats treated with ethanolic extract of *G. mangostana* rind in Repeated Dose 28-Day Oral Toxicity.

Weight (g/100 g body weight)	Control (10% ethanol)	*G. mangostana* rind extract (mg/kg BW/day)			
		50	500	1000	1000*
Male					
WBC X(x106 /ml)	3.01±1.55	3.02±1.59	3.71±2.05	2.71±1.43	2.93±1.07
HGB g/dl	16.37±0.89	16.61±1.59	16.58±2.05	17.28±1.43	15.93±1.07
HCT (%)	48.1±2.8	48.6±3.5	49.1±5.4	49.8±2.9	48.8±3.5
PLT(x 10^6/ml)	818±140.3	833.5±96.5	820.1±113.5	864.8±85.9	808.1±156.0
PMN (%)	11.23±7.73	14.31±8.85	8.54±4.05	11.69±4.05	9.83±4.94
Lymphocyte (%)	86.77±8.87	84.77±8.42	90.31±4.09	87.0±4.93	88.33±4.73
Female					
WBC X (x106 /ml)	1.21±1.2	0.86±0.52	0.94±0.35	1.48±1.05	1.44±0.77
HGB g/dl	15.15±0.82	14.68±1.42	15.34±0.65	15.24±0.94	14.8±0.83
HCT (%)	42.9±2.9	41.3±3.9	42.9±1.7	43.3±4.0	43.3±3.4
PLT (x 10^6 /ml)	665.1±74.0	648.1±85.6	669.9±72.8	680.8±99.7	780.4±109.9
PMN (%)	8.42±4.70	838±4.09	8.92±4.29	11.0±3.87	12±4.63
Lymphocyte (%)	89.83±5.95	90.08±3.45	90.38±4.79	88.15±4.06	87.15±4.28

Data are expressed as mean ± S.D., n = 13

* A separate group was administered at 1,000 mg/kg BW daily for 28 days followed by no treatment for 14 days

No significant differences from the control (P >0.05)

Table 5. Blood chemistry values of rats treated with ethanolic extract of G. mangostana in Repeated Dose 28-Days Oral Toxicity.

Wetght (g/100 g body weight)	Control (10 % ethanol)	Garcinia mangostana rind extract (mg/kg BW/day)			
		50	500	1000	1000*
Male- Glucose (mg/dl)	139.1±44.6	126.7±22.4	127.1±20.9	130.1±15.3	154.3±45.6
BUN (mg/dl)	17.2±2.4	17.36±3.8	16.9±2.2	16.4±1.9	19.5±1.7
Creatinin (mg/dl)	0.87±0.08	0.81±0.13	0.78±0.08	0.81±0.13	0.86±0.10
AST (S.F. unit)	135.5±26.8	137.4±23.8	116.5±19.2	117.9±31.6	119.7±22.1
ALT (S.F. unit)	96.2±13.4	67.0±11.5	60.5±13.2	62.0±16.3	86.4±25.9
Alk-P (B.L.B.unit/l)	209.4±27.5	217.6±53.3	204.5±53.5	213.9±39.7	271.9±55
Protein (g/dl)	6.87±0.46	6.68±0.57	7.03±0.96	7.11±0.52	7.72±0.54
Albumin (g/dl)	3.95±0.25	3.92±0.29	3.68±0.43	3.83±0.32	3.74±0.23
Female- Glucose (mg/dl)	126.6±14.9	127.6±25.8	120.7±11.2	120±28.9	140.4±14.7
BUN (mg/dl)	18.8±2.2	18.6±3.0	17.7±2.2	17.1±2.8	17.9±2.0
Creatinin (mg/dl)	0.8±0.09	0.84±0.10	0.8±0.11	0.89±0.14	0.87±0.09
AST (S.F. unit)	122.3±19.2	122.9±20.5	122.1±19.6	113.0±20.1	122.2±26.8

Table 5. Blood chemistry values of rats treated with ethanolic extract of G. mangostana in Repeated Dose 28-Days Oral Toxicity. (Continue).

ALT (S.F. unit)	44.4±11.7	53.5±7.9	45.9±6.7	45.7±7.1	62.3±29.1
Alk-P (B.L.B. unit/l)	144.3±29.0	158.6±38.0	129.9±28.8	148.1±45.5	151.9±56.9
Protein (g/dl)	6.44±0.43	6.48±0.54	6.63±0.32	6.45±0.44	6.87±0.38
Albumin (g/dl)	4.21±0.36	4.29±0.30	4.33±0.27	4.25±0.23	4.1±0.25

Data are expressed as mean ± S.D., n = 13

*A separate group was administered at 1,000 mg/kg BW daily for 28 days followed by no treatment for 14 days

No significant differences from the control ($P > 0.05$)

Tissues analysis

The histological examinations of the liver, kidney, lung, heart, spleen, adrenal grand, thymus, stomach and duodenum, small intestine, ovary, uterus, testis, epididymis muscle and nerve, thoracic spine, eye and brain were normal in both the control and treated groups.

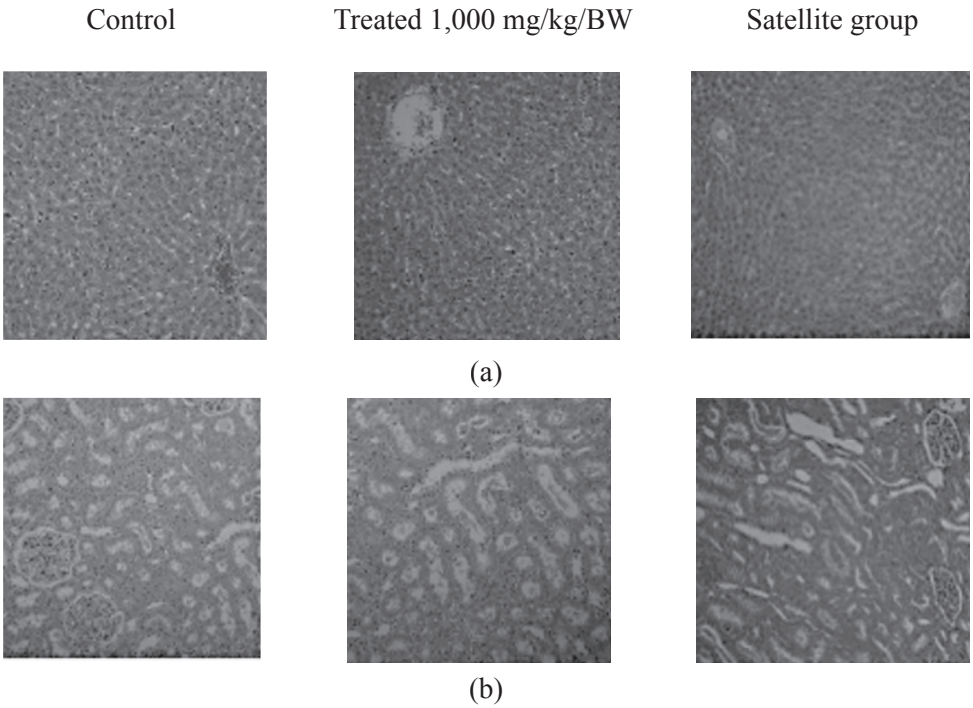

Figure 1.The histology of liver (a) kidney (b) No significant damage was detected in any treatment group.

CONCLUSION

Acute toxicity at doses lower than 5 g/kg BW and 28-days oral toxicity at the doses 50, 500 and 1,000 mg/kg BW/day of mangosteen rind extract did not produce any significant dose-related change of hematological parameters, serum biochemistry or histopathology of any internal organ. Therefore, it is concluded that *G. mangostana* rind extract at the given dose did not produce any significant toxic effect in rats during the period of treatment for 28 days. An additional study on chronic toxicity evaluation is needed to determine the long-term safety of the extract.

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