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α-Mangostin from *Cratoxylum arborescens*: An *in vitro* and *in vivo* toxicological evaluation

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KEYWORDS

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Abstract α-Mangostin (AM) is believed to be beneficial for health due to its versatile biological activities such as antioxidant, antiviral and anticancer properties. In this study, the air-dried stem bark of *Cratoxylum arborescens* was extracted consecutively with hexane, chloroform and methanol. The hexane extract was chromatographed, fractionated and purified to yield AM. The toxic effects of AM have not been completely investigated; therefore, the current study investigates its *in vitro* cytotoxicity on WRL-68 normal cells and *in vivo* effects on renal and hepatic histobiochemical parameters, relative organ weight, lipid profile, peroxidation and reduced glutathione of ICR female and male mice. AM was fed orally at single doses of 0 (as normal group), 100, 500 and 1000 mg/kg body

Abbreviations: AM, alpha mangostin; ALP, alkaline phosphatase; ALT, alanine aminotransferase; AST, aspartate aminotransferase; GGT, gamma-glutamyl transpeptidase; BUN, blood urea nitrogen; OECD, Organization for Economic Cooperation and Development; MDA, malondialdehyde; GSH, glutathione; H&E, hematoxylin and eosin; ANOVA, analysis of variance; SD, standard deviation.

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weight. The results showed that AM did not show adverse effects on body weight, organ weight, serum biochemistry, histopathology and oxidative stress biomarkers. On the other hand, this natural compound showed low cytotoxic activities against normal liver cells (WRL-68) with IC_{50} = 65 μg/ml. To our knowledge, this paper is the first to report the toxicity effects of AM in rodents.

1. Introduction

Plants have been harnessed as medicines since the early centuries. Remedies were initially taken in the form of crude drugs such as tinctures, elixirs, compresses, powders and other herbal formulations (Alsarhan et al., 2012). Statistics about the origin of drugs that were launched in the last twenty five years revealed that both natural products and semi-synthetic compounds, derived from natural origin, comprised 34% of all new chemical entities, while 18% of them were synthetic mimics of natural compounds (Gurib-Fakim, 2006). However, many of these herbs and natural supplements have not been thoroughly evaluated, and their safety as well as effectiveness may not yet have been proven (Dunnick and Nyska, 2013). Pharmacological interest in the efficacy and safety of the herbal medicines has grown during the past decade because of the realization that many people are self-medicated using these agents (Calixto, 2005; Firenzuoli and Gori, 2007). However, the use of herbal products should be based on scientific origin; otherwise, they would be useless and unsafe (Mir et al., 2013). Furthermore, the irrational use of these herbal products may cause serious toxicity in humans (Parthasarathi and Olsson, 2004). Unfortunately, many people underestimate the toxicity of natural products and do not realize that these agents could be as toxic as, if not more than, synthetic drugs. A typical example for a toxic herbal product is the leaves of *Atropa belladonna* and *Digitalis purpurea*, which show severe systemic toxicity if taken orally (Mir et al., 2013).

In this regard, *Cratoxylum arborescens* Blume Guttiferae family (Fig. 1) is a traditional medicine and its natural range of distribution includes Malaysia, South Burma, Sumatra and Borneo. Due to its geographically wide-ranging natural habitat, it is often referred to by several Malay colloquial names depending on the region or locality where it is found. In Sabah, Serangan is synonymous with Geronggang. In Sarawak, it is locally called by different names depending on the community (Jensen and Zwieniecki, 2013). This plant is used traditionally as a cure for fever, cough, diarrhea and other ailments (Srithi et al., 2009). Phytochemicals which have been reported to be found in *Cratoxylum* are xanthones (Iinuma et al., 1996a; José et al., 1998), and some of these xanthones have been shown to exhibit significant pharmacological activities (Jiang et al., 2004). α-Mangostin (AM) (Fig. 2) is one of the major xanthones isolated from the stem bark of the plant (El-Seedi et al., 2009). AM has been reported to possess a wide spectrum of biological activities such as anti-inflammatory (Chairungsrilerd et al., 1996; Shankaranarayan et al., 1979; Tewtrakul et al., 2009), cardioprotective (Devi Sampath and Vijayaraghavan, 2007), anti-tumor (Akao et al., 2008; Chitchumroonchokchai et al., 2013), antidiabetic (Ryu et al., 2011), antibacterial (Iinuma et al., 1996b; Negi et al., 2008; Sundaram et al., 1983), antifungal (Kaomongkolgit et al., 2009), antioxidant (Jung et al., 2006; Márquez-Valadez et al., 2009), antiparasitic (Obolskiy et al., 2009) and can also act as well as anti-obesity (Jiang et al., 2010; Quan et al., 2012) agents. Therefore, the purpose of this study is to investigate the safety of the pure compound of AM and to evaluate its effect on the extent of tissue damages in mouse liver and kidney.

2. Materials and methods

2.1. Extraction and isolation of AM from C. arborescens

The stem bark of *C. arborescens* was collected from wild trees growing in Sarawak, Malaysia in June 2009. A voucher

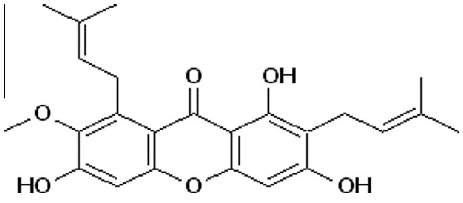

Figure 2 Chemical structure of AM. *Source:* www.chemfaces.com/natural/alpha-Mangostin-CFN97050.html.

Figure 1 *Cratoxylum arborescens* (Guttiferae); (A) the appearance of overall tree. (B) The flowers and leaves. *Source:* www.asian-plant.net/Hypericaceae/Cratoxylum_arborescens.htm.

specimen was deposited at the Herbarium, Department of Biology, University Putra Malaysia, Serdang, Malaysia. The finely ground air-dried stem bark of *C. arborescens* (1.0 kg) was extracted consecutively with hexane, chloroform and methanol to produce 6.12, 28.18 and 40.27 g of dark, viscous semisolid material on solvent removal, respectively. The hexane extract was chromatographed over a vacuum column (COV): silica gel 60 (150 g, particle size 0.040–0.063 mm, *Merck*) and eluted with a solvent of gradually increasing polarity to obtain 26 fractions of 200 ml each. After extensive fractionation and purification, fractions 14–20 yielded AM (Fig. 2).

2.2. Identification of AM by nuclear magnetic resonance (NMR)

The melting point of AM is 178–180 °C (Shankaranarayan et al., 1979), m.p. 181–182 °C). UV MeOH λ_{max} nm (log ε): 390 (2.41), 358 (3.99), 316 (3.99) and 238 (2.65). IR ν_{max} cm^{-1} (KBr): 3369 (OH), 2934 (CH), 1608 (C═C), 1462 and 1286. EIMS *m/z* (% intensity): 410 (43.06), 395 (6.14), 379 (1.61), 354 (25.77), 339 (100.00), 311 (32.57), 296 (12.89), 285 (18.90), 257 (6.46) and 162 (14.16). ^{1}H-NMR (500 MHz, acetone-d6): δ 13.79 (OH-1), 9.62 (OH-6), 9.52 (OH-3), 6.81 (s, 1H, H-5), 6.38 (s, 1H, H-4), 5.26 (t, J = 6.85 Hz, 2H, H-12 and H-17), 4.12 (d, J = 6.85 Hz, 2H, H-11), 3.78 (OMe-7), 3.35 (d, J = 8.00 Hz, 2H, H-16), 1.82 (s, 3H, Me-14), 1.71 (s, 3H, Me-19), 1.64 (s, 6H, Me-15 and Me 20). ^{13}C NMR (125 MHz, acetone-d6): δ 182.0 (C-9), 162.1 (C-4a), 160.9 (C-1), 156.6 (C-10a), 155.4 (C-6), 154.9 (C-3), 143.6 (C-7), 137.3 (C-8), 130.6 (C-18 and C-13), 123.9 (C-12), 122.6 (C-17), 111.2 (C-8a), 110.2 (C-2), 102.8 (C-9a), 101.9 (C-5), 92.3 (C-4), 62.5 (OMe-7), 26.1 (C-11), 25.1 (C-15 and C-20), 21.1 (C-16), 17.5 (C-14) and 17.1 (C-19).

2.3. Chemicals and reagents

All chemicals were obtained from Sigma Chemical Co., St. Louis, MO, USA. All reagents, solvents and chemicals used in this study are of analytical grade. The chemical structure and purity of AM were checked and confirmed using HPLC and LC/MS.

2.4. 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide cell viability assay on human normal hepatic cells (WRL-68)

This colorimetric assay is based on the conversion of the yellow tetrazolium bromide (MTT) to the purple formazan derivatives by mitochondrial succinate dehydrogenase in viable cells (Mosmann, 1983), and it was used to determine any potential cytotoxicity. Human Normal hepatic cells (WRL-68) were obtained from the American Type Cell Collection (ATCC), maintained in a 37 °C incubator with 5% CO_2 saturation and also maintained in Dulbecco's modified Eagle's medium (DMEM). The medium was supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin and 0.1 mg/ml streptomycin. For the measurement of cell viability, cells were seeded at 1×10^5 cells/ml density in a 96-well plate and incubated for 24 h at 37 °C and 5% CO_2. Cells were treated with AM and incubated for 24 h. After 24 h, MTT solution at 2 mg/ml was added for 1 h. Absorbance was measured at 570 nm. Results were expressed as a percentage of control giving percentage cell viability after a 24-hour exposure to the test agent. The potency of cell growth inhibition for α-mangostin was expressed as an IC_{50} value, defined as the concentration that caused a 50% loss of cell growth. Viability was defined as the ratio (expressed as a percentage) of absorbance of treated cells to untreated cells.

2.5. Animals

ICR mice (both genders) of 25–30 g weight were obtained from the Animal House Unit, Faculty of Medicine, University of Malaya (UM). Animals were maintained at 20 ± 2 °C with a 12 h light/dark cycle and relative humidity of 50–60%. Free access to food and water was allowed at all times. Five mice were housed per cage in sterilized plastic cages using homogenized wood shavings as bedding. All experimental protocols held on the animals were done according to the regulations set by the Institutional Animal Care and Use Committee, Faculty of Medicine, UM.

2.6. Experimental design

The single-dose acute oral toxicity design of this study was performed on ICR mice according to OECD 420 (Oecd, 1998). The mice were assigned equally according to their gender into 4 groups (n = 5 per group) labeled as vehicle (distilled water), 100, 500 and 1000 mg/kg body weight of AM pure compound, respectively. The mice were fasted (from food but not water) overnight prior to dosing. The purpose of starving the animals is to eliminate food inside the gastrointestinal tract that may complicate the absorption of the tested substance. Food was withheld for another 3 or 4 h after dosing. The animals were observed for 30 min and 2, 4, 24 and 48 h after the administration for the onset of clinical or toxicological symptoms. Mortality, if any, was observed over a period of 2 weeks. The mice were sacrificed on the 15th day. Serum biochemical and histological (liver and kidney) parameters were determined according to standard methods (Bergmeyer, 1980). Lipid peroxidation was performed on fresh liver and kidney necropsies.

2.7. Assessment of kidney and liver functions

All biochemical assays were performed spectrophotometrically using Hitachi-912 Autoanalyser (Mannheim, Germany) with kits supplied by Roche Diagnostics (Mannheim, Germany). As indicators of kidney function, serum creatinine, blood urea nitrogen, sodium, potassium, chloride, carbon dioxide and anion gap levels were measured. Serum alanine aminotransferase (ALT), aspartate aminotransferase, alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (GGT), total protein, albumin, globulin, total bilirubin and conjugated bilirubin levels were measured to evaluate liver function. In order to obtain data with good sensitivity and validity, serum samples were analyzed blindly and in triplicate.

2.8. Assessment of lipid profile

Total cholesterol and high density lipoprotein (HDL) cholesterol concentrations were estimated using the commercial kits of Span Diagnostics according to the method described by

Wybenga et al. (1970). Triglycerides concentration was assessed by the GPO-PAP end point assay.

2.9. Histopathological examinations

Renal and hepatic tissues were fixed in 10% formalin, and fixed samples were embedded in paraffin, sectioned at 5 μm and stained with hematoxylin-eosin. All sections were examined using a photomicroscope (Olympus BH-2, Japan). Histopathological examinations were performed by an independent histopathologist unaware of the treatment groups.

2.10. Lipid peroxidation

Lipid peroxidation was assessed using malondialdehyde (MDA) as the indicator (Kei, 1978). Briefly, 10% (weight/volume) homogenates of kidney and liver obtained from 0.1 mol/l phosphate buffer were centrifuged at 4 °C and 3500 rpm for 10 min. 0.2 ml supernatant was mixed with 0.67% 2-thiobarbituric acid (TBA) and 20% trichloroacetic acid solution, and then heated in a boiling water bath for 30 min. The pink-colored chromogen formed by the reaction of TBA with MDA was measured at 532 nm. Results were expressed as MDA nmol/mg protein. Contents of protein in the supernatant were measured by the Lowry's method (Lowry et al., 1951).

2.11. Measurement of tissue glutathione

Tissue samples were homogenized in 10 volumes of ice-cold 10% trichloroacetic acid and centrifuged at 1000*g* for 15 min at 4 °C. Supernatant was removed and recentrifuged at 35,000*g* and 4 °C for 8 min. GSH was determined using a spectrophotometric method, which is a modification of the Ellman procedure (Ellman, 1959).

2.12. Statistical analysis

All data were expressed as mean ± SD and analyzed using one-way ANOVA followed by post hoc Tukey HSD multiple comparison tests. The type-1 error level was set at $P < 0.05$ for all tests. All statistical analyses were performed using SPSS software (Chicago, IL, USA) version 19.0 for Microsoft Windows®.

3. Results

3.1. General observations

Ingestion of AM at any tested dose did not cause mortality in mice. There was no abnormal behavior or physical appearance among these animals. In all cases, their feces were dry and dark. The average of daily food and water intake among the groups were similar throughout this study. On the other hand, this natural compound did not show any cytotoxic activities against normal liver cells (WRL-68) (Fig. 3).

3.2. Effect on animal weight and relative organ weight

The measurement of body weight over the whole experimental period found no significant differences between the treated and control groups of the same sex. On average, the male mice grew faster than the females (Table 1). There was no significant difference in relative organ weight among all of the treated groups when compared to the control (Table 2).

3.3. Serum biochemical parameters

Serum biochemistry data are summarized in Table 3. There were no significant changes in the levels of serum total protein, albumin, globulin or BUN. In addition, no statistically significant differences in serum electrolytes such as calcium, potassium, chloride or phosphate were noted. The effects of AM on liver function parameters such as ALT, AST, ALP, conjugated bilirubin and total bilirubin in serum were also investigated. Animals treated with 100, 500 and 1000 mg/kg AM did not exhibit significant differences in these hepatic markers. The effects of AM on triglyceride, high density lipoprotein cholesterol HDL and total cholesterol levels are shown in Table 3. Male mice dosed with 1000 mg/kg showed a significant decrease in triglyceride, HDL cholesterol and total cholesterol levels. Similarly, female mice treated with the same dose also exhibited a decrease in triglycerides, HDL cholesterol and total cholesterol. Similar effects were observed in both sexes at 100 mg/kg exposures.

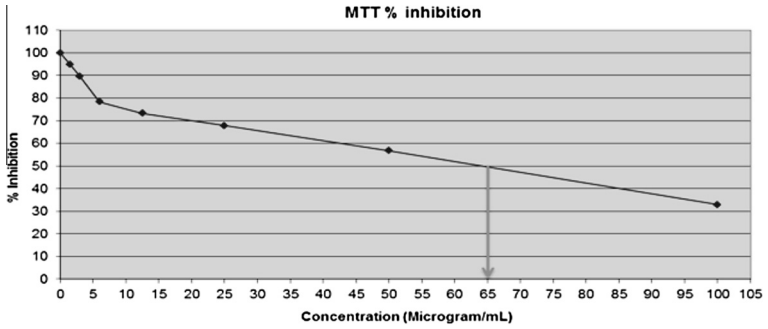

Figure 3 Effect of the AM on WRL-68 cell lines determined by MTT assay. Cells treated with the compound at different concentration (IC_{50} 65 μg/ml).

Table 1 Body weights (grams) of ICR male and female mice in the acute toxicity study of AM.

Days	Day 0	Day 7	Day 15	Weight gained on 15 days
Female				
100 mg AM/kg	28.7 ± 1.5	31.3 ± 1.7	34 ± 1.0	30.3 ± 3.5
500 mg AM/kg	29.2 ± 1.5	31.4 ± 1.2	34 ± 1.0	30.2 ± 2.3
1000 mg AM/kg	26.3 ± 1.5	30.7 ± 2.0	33 ± 2.0	30 ± 1.8
Control	28 ± 2.0	30.7 ± 1.5	32.3 ± 1.5	30.3 ± 1.7
Male				
100 mg AM/kg	29 ± 1.7	31.7 ± 1.52	33 ± 1.0	31.2 ± 0.8
500 mg AM/kg	30 ± 1.7	31.7 ± 1.52	32 ± 1.0	31.2 ± 0.8
1000 mg AM/kg	26.3 ± 1.1	30. 7 ± 2.1	30. 7 ± 2.3	30 ± 1.3
Control	28 ± 1.0	30. 7 ± 2.3	34 ± 1.0	30.9 ± 1.3

Data are expressed as means ± SD of five mice for each group.

Table 2 Weights of organs (in grams) of male and female ICR mice treated with AM.

Organ	Sex	Normal	AM 100 mg/kg (b.w.)	AM 500 mg/kg (b.w.)	AM 1000 mg/kg (b.w.)
Liver	Male	1.35 ± 0.02	1.15 ± 0.05	1.32 ± 0.1	1.33 ± 0.20
	Female	1.35 ± 0.02	1.20 ± 0.05	1.20 ± 0.1	1.21 ± 0.1
Left kidney	Male	0.21 ± 0.02	0.20 ± 0.00	0.21 ± 0.02	0.20 ± 0.02
	Female	0.21 ± 0.02	0.16 ± 0.01	0.16 ± 0.02	0.17 ± 0.02
Right kidney	Male	0.21 ± 0.02	0.22 ± 0.00	0.21 ± 0.02	0.20 ± 0.02
	Female	0.21 ± 0.02	0.16 ± 0.00	0.16 ± 0.01	0.17 ± 0.01
Left lung	Male	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.01
	Female	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.00	0.11 ± 0.00
Right lung	Male	0.12 ± 0.01	0.12 ± 0.02	0.12 ± 0.01	0.13 ± 0.01
	Female	0.12 ± 0.01	0.13 ± 0.02	0.12 ± 0.00	0.11 ± 0.00
Pancreas	Male	0.18 ± 0.03	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01
	Female	0.18 ± 0.03	0.14 ± 0.01	0.14 ± 0.01	0.14 ± 0.01
Heart	Male	0.20 ± 0.04	0.13 ± 0.1	0.13 ± 0.00	0.13 ± 0.00
	Female	0.20 ± 0.04	0.14 ± 0.03	0.12 ± 0.01	0.12 ± 0.00

Data are expressed as means ± SD of five mice for each group.

3.4. Histopathological evaluation

At autopsy, macroscopic examinations of the liver and kidney of the mice did not show any abnormality in gross appearance and weight as a result of the compound consumption (data not shown). In addition, we did not detect any damage in their gastrointestinal tracts, the potential and direct target for toxic effects of ingested foods. Results from the gross examination were also confirmed by histopathological assessment. AM did not produce any significant histological changes in the organ tissues of any of the animals (Fig. 4). Hence, no necrosis, inflammation or cirrhosis was observed.

3.5. Effect on oxidative stress biomarkers

Oxidative stress in the tissues was evaluated quantitatively using measurements of MDA and GSH (Table 4). AM (1000 mg/kg) treated animals showed slightly higher MDA and GSH levels regardless of their gender compared to normal control ($P < 0.05$) while treatment with the dose of 100 mg/kg of AM did not show any abnormal levels of MDA and GSH in male and female treated animals.

4. Discussion

AM, as a natural compound, possesses various biological properties (Pedraza-Chaverri et al., 2008). However, despite several evidences of its pharmacological properties and therapeutic benefits, there have been no reported toxicological evaluations performed on this compound *in vivo*.

In vitro cytotoxicity assay can be used to predict the toxicity of chemical substances (van der Laan et al., 2012; West et al., 2010). Different results have previously been reported, depending on the test agent used and the cytotoxicity assay employed (Weyermann et al., 2005). MTT assay, being the most sensitive in detecting cytotoxic event and causing a cytotoxicity assay based on mitochondrial respiratory activity, would give early signs of toxicity following exposure to a mitochondrial toxicant (Fotakis and Timbrell, 2006).

Cell cultures derived from humans and animals have been used during the past 30 years in determining the cytotoxic effect caused by natural compounds. Permanent cell lines and primary cells have been used as models (Sabaliauskas et al., 2011).

Our study revealed that based on MTT assay, AM low toxic effect to normal liver cells (WRL-68) in a dose-dependent manner with high IC_{50} value 65 μg/ml (Fig. 3) compared to cisplatin (Sigma Aldrich), a known cytotoxic agent as a positive control against WRL-68 with low IC_{50} value 5.1 μg/ml (Abdelwahab et al., 2013).

Toxicologically, an acute toxicity investigation is the initial step toward toxicological analysis of chemical or pharmaceutical materials (Andersen and Krewski, 2009).

Table 3 Serum biochemical data for male and female mice orally administered AM for 14 days.

Parameter	Sex	Normal	AM 100 mg/kg (b.w.)	AM 500 mg/kg (b.w.)	AM 1000 mg/kg (b.w.)
Sodium mmol/L	Male	147.3 ± 0.6	147.± 01.7	148.6 ± 1.1	150.7 ± 3.1
	Female	147.7 ± 1.2	152.7 ± 1.2	150.7 ± 2.5	151.7 ± 1.5
Potassium mmol/L	Male	4.8 ± 0.4	5.1 ± 0.3	5.1 ± 0.1	5.1 ± 0.1
	Female	4.9 ± 0.3	6.1 ± 1.7	4.7 ± 0. 6	4.6 ± 0.6
Chloride mmol/L	Male	113.0 ± 1.7	114.0 ± 2.6	115.0 ± 1.7	116.0 ± 1.7
	Female	112.7 ± 4.0	118.3 ± 2.1	116.7 ± 2.1	117.7 ± 2.1
Carbon dioxide mmol/L	Male	15.5 ± 1.9	16.5 ± 2.0	16.7 ± 2.2	17.9 ± 3.2
	Female	13.2 ± 4.7	10.0 ± 1.5	11.0 ± 3.1	11.0 ± 4.1
Anion gap mmol/L	Male	24.0 ± 1.0	24.7 ± 0.6	26.0 ± 3.6	28.0 ± 3.6
	Female	26.7 ± 1.2	29.7 ± 4.2	26.7 ± 2.5	27.7 ± 3.8
Urea nitrogen mmol/L	Male	14.4 ± 0.2	14.2 ± 1.0	15.3 ± 1.0	15.7 ± 3.0
	Female	9.1 ± 0.5	17.1 ± 3.2	16.2 ± 1.9	15.2 ± 1.8
Creatinine μmol/L	Male	22.7 ± 4.7	17.7 ± 6.5	20.6 ± 1.5	21.7 ± 2.5
	Female	29.7 ± 6.7	31 ± 1.0	28.0 ± 1.2	29.0 ± 4.4
Total protein g/L	Male	63.7 ± 5.5	64.3 ± 7.1	56.3 ± 3.1	58.3 ± 6.1
	Female	59.0 ± 6.1	65.7 ± 5.5	65.1 ± 1.3	65.7 ± 2.3
Albumin g/L	Male	16.7 ± 1.5	13.0 ± 1.7	14.1 ± 0.1	14.3 ± 0.6
	Female	13.3 ± 3.1	16.3 ± 1.2	16.7 ± 1.2	16.7 ± 1.2
Globulin g/L	Male	47.0 ± 4.6	55.3 ± 6.5	45.3 ± 2.5	46.3 ± 3.5
	Female	44.7 ± 2.5	49.3 ± 6.7	49.1 ± 1.0	49.0 ± 2.0
Total bilirubin μmol/L	Male	4.3 ± 0.6	2.3 ± 0.6	2.5 ± 0.6	2.7 ± 0.6
	Female	3.7 ± 0.6	2.7 ± 0.6	2.9 ± 1.0	3.0 ± 1.0
Conjugated bilirubin μmol/L	Male	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0	1.0 ± 0.0
	Female	1.0 ± 0.0	1.0 ± 0.0	1.0 ± .0.0	1.0 ± .0.0
Alkaline phosphatase IU/L	Male	51.3 ± 17.2	55.7 ± 10.0	48.0 ± 6.5	49.0 ± 6.2
	Female	57.3 ± 13.1	65.0 ± 8.5	65.0 ± 13.2	64.0 ± 17.7
Alanine aminotransferase IU/L	Male	47.3 ± 23.3	38.7 ± 4.7	49.0 ± 9.6	51.0 ± 9.6
	Female	56.0 ± 19.1	66.0 ± 14.1	69.1 ± 11.9	69.3 ± 26.1
Ast IU/L	Male	231.7 ± 30.1	232.7 ± 47.1	233.1 ± 43.0	233.7 ± 13.0
	Female	263.0 ± 105.8	271.0 ± 34.8	227.7 ± 69.9	279.7 ± 69.9
G-glutamyal transferase IU/L	Male	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
	Female	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0	3.0 ± 0.0
Triglyceride mmol/L	Male	0.4 ± 0.1	0.3 ± 0.1	0.2 ± 0.2	0.5 ± 0.2
	Female	0.7 ± 0.5	0.6 ± 0.2	0.5 ± 0.0	0.3 ± 0.0
Total cholesterol mmol/L	Male	1.9 ± 0.3	1.7 ± 0.1	1.6 ± 0.3	1.8 ± 0.2
	Female	2.2 ± 0.8	1.9 ± 0.3	1.8 ± 0.5	1.9 ± 0.5
HDL cholesterol mmol/L	Male	1.9 ± 0.3	1.8 ± 0.1	1.7.±0.1	1.8.±0.1
	Female	2.3 ± 0.9	1.8 ± 0.2	1.9 ± 0.4	1.7 ± 0.4

Data are expressed as means ± SD of five mice for each group.

In relation to this, the Environmental Protection Agency Health Effects Test Guidelines, OPPTS 870.3050 (2000) and OECD Guidelines for Testing of Chemicals, Section 407 (1998) as well as the United States have prioritized rats as the standard rodent species. The mouse has been one of the main mammalian species used for preclinical purposes, ranging from pharmacology to safety assessments. The use of rodents as models in safety evaluations is currently required in both pharmaceutical and chemical international guidelines (Suckow et al., 2012). The Food and Drug Administration Guidelines for Toxicological Principles for the Safety Assessment of Food Ingredients, Redbook 2000, Chapter IV.C.3.a (Redbook, 2000) elucidated that short term toxicity studies are conducted with rats and mice. Thus, both male and female mice that are healthy and have not been assigned to previous experimental procedures ought to be utilized in acute toxicity studies.

Results of the present study not only provide scientific evidences to evaluate the safety of AM using an acute toxicity study design in mice; they are also useful in determining the dose range of this pure compound for subsequent studies on mice, such as carcinogenicity, antioxidant, antimicrobial and anti-parasitic studies. Moreover, this is the first time that AM has been investigated in an acute oral toxicity in mice. In addition, the advantages of using mice for research purposes are numerous. Firstly, in spite of their clear physical differences, mice and human genes are approximately 99% identical. In a biological context, genes in the mouse and human function in virtually the same way. Secondly, the genome of mouse is easily manipulated through various genetic engineering technologies for different experiments. Thirdly, mice are relatively small in size and their ease of maintenance reduces research costs. Fourthly, their accelerated lifespan (1 mouse year = 30 human years) allows all life stages to be studied. Fifthly, their short gestation time (3 weeks) and large litter size quickly provide a large sample population and enable rapid genetic and pathophysiologic characterizations. Finally, they can be easily handled with practice (Suckow et al., 2012). Thus, the mouse is an ideal model organism for various experiments.

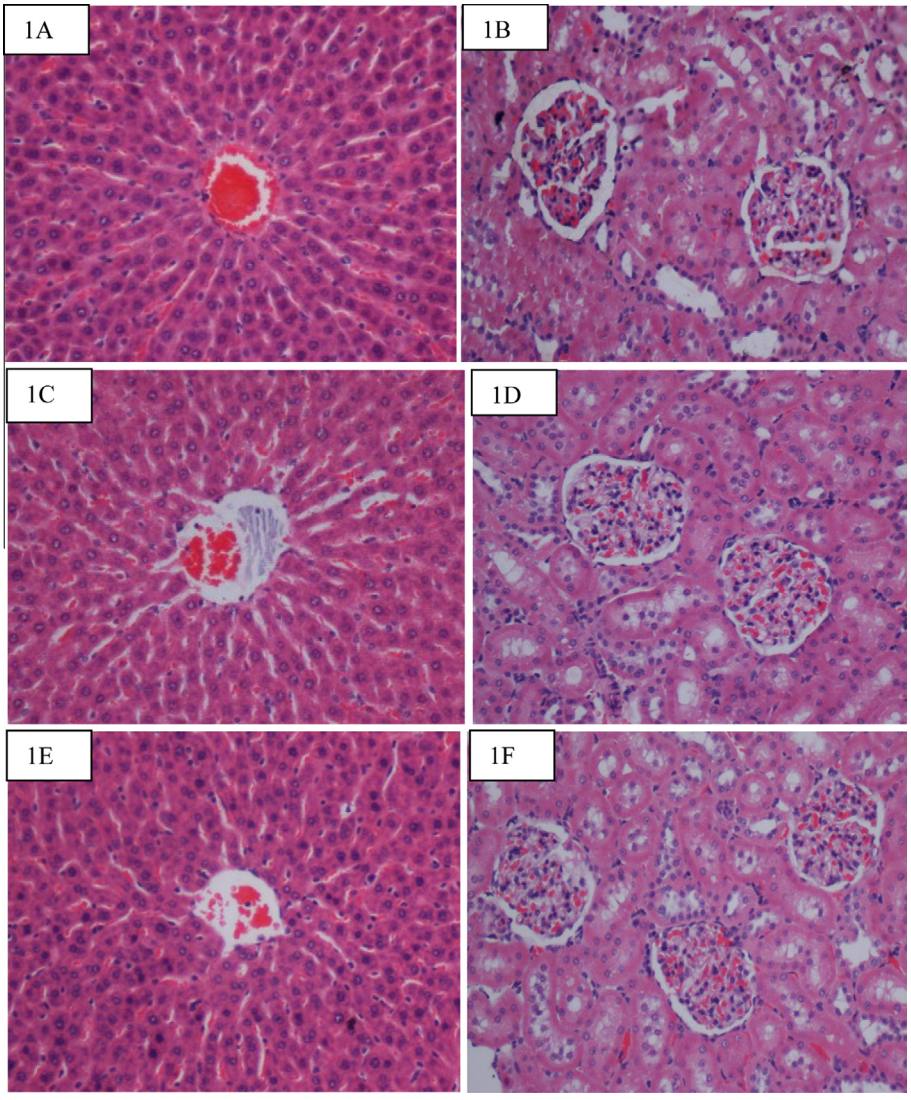

Figure 4 Effect of AM on histological sections of the liver and kidney in mice. (1A and B) Normal control. (1C and D) Mice treated with 100 mg/kg of AM. (1E and F) Mice treated with 1000 mg/kg of AM. There is no significant difference in the architecture of the livers and kidneys between the treated and control groups (Hematoxylin and Eosin stain, 20× magnifications).

Table 4 The males and females tissue malondialdehyde levels and glutathione content of the AM low dose, AM high dose and control.

Treatments	Sex	MDA nmol/g		GSH nmol/g	
		Liver	Kidney	Liver	Kidney
Control	Male	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.04 ± 0.00
	Female	0.05 ± 0.001	0.05 ± 0.000	0.04 ± 0.001	0.04 ± 0.002
100 mg AM/kg (b.w.)	Male	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
	Female	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.002	0.04 ± 0.002
500 mg AM/kg (b.w.)	Male	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.00	0.05 ± 0.00
	Female	0.05 ± 0.00	0.05 ± 0.00	0.04 ± 0.002	0.04 ± 0.002
1000 mg AM/kg (b.w.)	Male	0.06 ± 0.00	0.06 ± 0.00	0.05 ± 0.00	0.05 ± 0.00
	Female	0.07 ± 0.01	0.06 ± 0.00	0.05 ± 0.002	0.05 ± 0.002

Data are expressed as means ± SD of five mice for each group.

The exposure of animals to chemicals toxicologically is divided into four categories: acute, subacute, subchronic and chronic. Acute toxicity is known as the toxicity produced by a pharmaceutical when it is administered in one or more doses during a period not exceeding 24 h. In the case of rodents, they should be monitored for 14 days after pharmaceutical

administration. All clinical signs, mortalities, time of onset, duration, and reversibility of toxicity should be recorded. Gross necropsies should be performed on all animals, including those that were sacrificed moribund, found dead, or terminated at 14 days (Bidlack, 2002). In the present study, ICR mice of both genders were administered oral gavage with AM suspension at single doses of 100, 500 and 1000 mg kg for 14 days. Throughout the study, treatment with AM did not produce any mortality or toxicity in the mice regardless of gender. No treatment-related adverse effects were found on body weight, organ weight, food and water consumption, serum biochemistry and oxidative stress status biomarkers.

Prior to implementing our study design, an analysis done on previous research papers on AM studies in an acute oral toxicity had found a valuable body of literature reported by Hutadilok-Towatana et al. (2010). They reported that mice treated with a single intragastric dose of 2 and 5 g/kg body weight of hydroethanolic extracts from the fruit pericarp of mangosteen upon acute toxicity for 14 days did not show any mortality or toxicity. Moreover, for the purpose of subchronic toxicity study, the mangosteen extracts at 400, 600 and 1200 mg/kg body weight were administered by oral gavage to male and female rats daily for 12 weeks. In all instances, consumption of the extract showed no effect on behavior, food and water intake, growth or health status of these animals. In both sexes, hematology values monitored throughout the study period did not alter from those of the control. After the 12-week period, no significant dose-related differences in blood biochemical parameters were detected among the female groups, whereas in all male groups, dose-variation increases were observed in direct bilirubin compared with the control. However, neither gross necropsy nor histopathological examination of their livers as well as other internal organs revealed any abnormal appearances. However, AM in the present study was isolated from the stem bark of *C. arborescens*. Therefore, the literature reported by Hutadilok-Towatana et al. (2010) has already included a good *in vivo* toxicological design; nevertheless, there is a difference in objective between the literature and our study due to the use of hall extract rather than the pure AM compound.

Single doses effect of AM on the kidney and liver function was carried out to evaluate the nephrotoxicity and hepatotoxicity of this compound on both male and female mice. In the evaluation, the kidney and liver cells were damaged, and creatinine and blood urea nitrogen together with ALT, ALP and GGT enzymes leaked out, causing the blood levels of these chemical components and enzymes to rise. Increased levels of these chemical components and enzymes in blood are considered good indicators for kidney and liver cell damage. In this present study, our results showed no significant changes in the levels of serum electrolytes, total protein, albumin, globulin, creatinine and BUN as well as conjugated bilirubin, total bilirubin ALT, AST and ALP.

In addition, our findings also indicated that there were no nephrocellular and hepatocellular injuries in mice gavage with 100, 500 and 1000 mg/kg AM. This was later confirmed by histological examinations of tissue sections using H&E staining of light microscope and MDA levels in the kidney and liver tissues of the animals. Results from this study suggested that single oral doses of AM at 100–1000 mg/kg have no ill effect on the kidney and liver tissues of both male and female ICR mice.

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