Original Research

Acute toxicity and teratogenicity of α-mangostin in zebrafish embryos

Wannakarn Kittipaspallop[1], Pornnarin Taepavarapruk[2], Chanpen Chanchao[1] and Wittaya Pimtong[3]

[1]Department of Biology, Faculty of Science, Chulalongkorn University, Bangkok 10330, Thailand; [2]Center for Animal Research & Department of Physiology, Faculty of Medical Science, Naresuan University, Pitsanulok 65000, Thailand; [3]Nano Safety and Risk Assessment Laboratory, National Nanotechnology Center, National Science and Technology Development Agency (NSTDA), Pathum Thani 12120, Thailand

Corresponding author: Wittaya Pimtong. Email: wittaya.pimtong@nanotec.or.th

Impact statement

α-Mangostin has been reported to have anticancer properties both *in vitro* and *in vivo* models. Although there are several studies that evaluated the toxicity of the compound in rodent models, we are the first to evaluate the teratogenicity of α-mangostin. In the present work, we found that α-mangostin induced mortality and malformations in zebrafish embryos. In addition, we exhibited that the compound also disrupted the reactive oxygen species and hemoglobin levels. These findings suggest that α-mangostin may possibly cause the same adverse effects on human health. The mechanisms of these toxicological effects of the compound will be further elucidated and the effects found in zebrafish embryos need to be verified in other animal models.

Abstract

α-Mangostin is the most active compound derived from the pericarps of mangosteen. A number of studies have reported its anticancer activity. However, only a few studies have investigated the toxicity of this compound; moreover, the teratogenicity of α-mangostin has not been reported. In this study, we evaluated the effects of α-mangostin on the development of zebrafish embryos. The exposure of zebrafish embryos to α-mangostin for 72 h dose-dependently induced mortality and abnormal development. The derived LC_{50} value of α-mangostin to zebrafish embryos at 72 h was 5.75 ± 0.26 μmol/L. We observed teratogenic effects in α-mangostin-treated embryos, characterized by axis malformation, bent tail, pericardial edema, yolk sac edema, sluggish circulation, and heart malformation. The percentages of the malformed embryos were 78.79% and 100% at 4.5 and 6 μM, respectively. α-Mangostin exposure also caused hemostasis in the ducts of Cuvier and a decreased heart rate in zebrafish embryos at 48 and 72 h post-fertilization. These results indicated that α-mangostin induced cardiac dysfunction in zebrafish embryos. In addition, a sub-lethal concentration and a lethal concentration (3 and 6 μM, respectively) were used to assess the compound effects on oxidative stress and embryonic erythropoiesis. α-Mangostin was found to decrease the level of reactive oxygen species (ROS) in zebrafish embryos. Furthermore, hemoglobin staining revealed a decrease in hemoglobin, which suggested that α-mangostin disrupted embryonic erythropoiesis in zebrafish. To our knowledge, the results of this study, for the first time, demonstrated that α-mangostin was potentially teratogenic and could disrupt embryonic ROS balance and erythropoiesis.

Keywords: α-Mangostin, xanthone, zebrafish, teratogenicity, erythrotoxicity, pharmacology/toxicology

***Experimental Biology and Medicine* 2018; 243: 1212–1219. DOI: 10.1177/1535370218819743**

Introduction

Mangosteen (*Garcinia mangostana*) is a tropical fruit found in Southeast Asia. Due to numerous claims for the improvement of human health, the consumption of mangosteen products has increased; in particular, mangosteen fruit juice has become a popular botanical dietary supplement. The pericarp of mangosteen has been used as a traditional medicine for the treatment of abdominal pain, diarrhea, infected wound, suppuration, and ulcer.[1] α-Mangostin is the most abundant xanthone found in the pericarps of mangosteen fruit. It has diverse biological activities, such as antibacterial, antifungal, antiparasitic, cardioprotective, antioxidant, anti-inflammatory, and antitumor properties.[2] Many previous studies, in both *in vitro* and *in vivo* models,

have reported the anticancer properties of α-mangostin.[3,4] The compound causes cytotoxicity in many cancer cells, such as melanoma, breast cancer, lung cancer, colorectal cancer, prostate cancer, colon cancer, leukemia, and hepatoma cells.[2] *In vivo* studies using rodent models have shown the antitumor activity of α-mangostin to hepatoma, colon, prostate, pancreatic, and mammary cancers.[4] Although α-mangostin has shown potential as an anticancer drug, its toxicity profile should be explored and elucidated. Several *in vivo* evaluations involving the toxicity of mangosteen extracts have been reported, but few studies investigated the toxicological effects of α-mangostin.

Acute toxicity studies of α-mangostin in rats revealed that no toxic symptoms or mortality were observed in doses of up to 2000 mg/kg (oral administration) up to 48 h after administration.[5] Another acute toxicity study of α-mangostin was performed after oral administration to Wistar rats of doses of up to 1250 mg/kg for 48 h. The treated rats did not exhibit any signs or symptoms of toxicity, mortality, and behavioral changes during the study period.[6] On the other hand, in mice, after intraperitoneal administration for 72 h, α-mangostin induced mortality with the median lethal doses of 150 mg/kg.[7] In addition, Gutierrez-Orozco *et al*. have also reported that the inclusion of α-mangostin in mice diet (250 mg/kg, 25 days) induced intestinal dysbiosis.[8] These contradicting results between mouse and rat models could depend on the sensitivity differences between these species, and further studies need to be elucidated. However, in drug development, the study of toxicity using only rodent models was insufficient information to support clinical trials of drugs. For example, approximately 60 years ago, thalidomide was prescribed to pregnant women as a treatment for morning sickness. This led to serious teratogenic effects, such as severe shortening or complete absence of legs and/or arms. No notable toxicity of thalidomide was observed when investigated in the rodent models. However, many studies have subsequently demonstrated that thalidomide could induce limb or fin malformations in monkeys, rabbits, chicks, and zebrafish.[9,10]

Zebrafish (*Danio rerio*) has been established as an effective model organism in several research fields, including developmental biology, toxicology, genetics, and pharmacology, due to their comparable genetics and significant physiological similarities to humans.[11] Approximately 70% of protein-coding human genes are related to genes found in the zebrafish and 84% of human disease-related genes have at least one ortholog in zebrafish.[12] Previous research has demonstrated the ability to build predictive models using zebrafish assays to screen drugs for possible embryotoxic and teratogenic effects.[13–15] The aim of the present study was therefore to evaluate the toxicological effects of α-mangostin on zebrafish embryo development. We evaluated the mortality and morphological malformations of the embryos after exposure to α-mangostin. The effects of the compound on embryonic heart rate were recorded. In addition, hemoglobin and ROS levels in zebrafish embryos were investigated.

Materials and methods

Chemicals

α-Mangostin (≥98%, HPLC) was purchased from Sigma-Aldrich (Chemical Abstracts Service registry No. 6147-11-1; St. Louis, MO). The stock solution of α-mangostin was prepared in dimethyl sulfoxide, DMSO (Sigma-Aldrich).

Zebrafish husbandry

Adult zebrafish (*Danio rerio*) were raised and maintained in accordance with standard breeding protocols (28.5 ± 1°C with a 14/10 h light/dark photoperiod) in a recirculating zebrafish housing system (AAB-074, Yakos65, Taiwan) at the National Nanotechnology Center. Reverse osmosis water was supplied to the system at pH of 6–8 and conductivity of 400–700 μS. All embryos were obtained from natural pairwise mating and kept in E3 medium (1 mM NaCl, 3.4 μM KCl, 6.6 μM $CaCl_2$, and 6.6 μM $MgSO_4$). The experimental procedures were approved by the NSTDA Institutional Animal Care and Use Committee (No. 002-2560).

Zebrafish embryo acute toxicity

The acute toxicity assay was adopted following the Organization for Economic Co-operation and Development guideline 236 (OECD 236, 2013). Briefly, fertilized and normal embryos were identified and collected under a stereomicroscope (Olympus SZX7, Tokyo, Japan) within 4 h post-fertilization (hpf). Thirty embryos per well were transferred to a 12-well culture plate and α-mangostin in DMSO was diluted in E3 medium to final concentrations of 1.5, 3.0, 4.5, 6.0, 7.5, and 9.0 μM in 2 mL; 0.48% DMSO solution was used as a control. Each test solution was vortexed for 20 s before addition to the well. The plates were then incubated at 28.5 ± 1°C with a 14 h light/10 h dark cycle for up to 72 h and the test solutions were refreshed every 24 h. To inhibit pigment formation, the test solutions were prepared in E3 medium with 0.003% (w/v) 1-phenyl 2-thiourea. Experiments with a control mortality of more than 10% were rejected. The percentage mortality was recorded at 72 hpf and SigmaPlot software was used to compute the 50% lethal concentration (LC_{50}). The teratogenic effects on the zebrafish embryos were observed and documented by using a stereomicroscope (SZX16, Olympus, Japan). The control group, one sub-lethal concentration, and one lethal concentration were selected for further investigations.

Heart rate assay

The heart rate of embryos from the teratogenicity test was documented after exposure for 24 and 48 h to α-mangostin. The embryos were anesthetized in 0.016% (w/v) tricaine (ethyl 3-aminobenzoate methanesulfonate, Sigma-Aldrich) before video-recordings of the heart rates of 10 embryos from each group were made by using a stereomicroscope (SZX16) for 1 min. The data were obtained from three independent experiments.

ROS assay

The induction of oxidative stress in zebrafish embryos was analyzed through the assessment of ROS induced by exposure of α-mangostin. The production of ROS in zebrafish embryos was measured in accordance with the method described by Liu *et al.*,[16] with slight modifications. Briefly, after exposure for 48 h, the chorions were removed from 10 embryos administered each treatment. The embryos were then rinsed with DI water and subsequently incubated with 50 μg CM-H_2DCFDA (Life Technologies) in 100 μL DMSO for 1 h at 28.5°C in the dark. After washing, the fluorescence intensity was measured at an excitation wavelength of 495 nm and an emission wavelength of 525 nm using a microplate reader (Spectramax M5, Molecular Devices, USA). The ROS level was reported as relative fluorescence units. Three independent experiments were completed.

o-Dianisidine staining in whole-mount zebrafish larvae

Hemoglobin in zebrafish larvae was stained after exposure for 72 h to α-mangostin, as described previously.[17] Briefly, live larvae were incubated with *o*-dianisidine/hydrogen peroxide (H_2O_2; Sigma) for 15 min at 23–25°C. The larvae were washed three times with phosphate-buffered saline with Tween-20 (8 mM Na_2HPO_4, 150 mM NaCl, 2 mM KH_2PO_4, 3 mM KCl, 0.05% Tween, pH 7.4) and fixed in 4% (w/v) PFA in PBS overnight at 4°C. The larvae, in 50% (v/v) glycerol, were documented on an Olympus SZX16 stereomicroscope equipped with an Olympus DP73 camera and cellSens Standard software. The staining intensity (pixels), representing the number of larvae erythrocytes, was analyzed by using ImageJ software.

Statistical analysis

All data were expressed as the mean ± standard deviation (SD) of three independent determinations for each experiment. We analyzed all the data with the SPSS program (Standard Version 20, SPSS Inc.). The statistical significance between each experimental group was determined by using one-way analysis of variance (ANOVA) followed by Tukey's test to compare the differences between groups. Differences were considered significant at $P < 0.05$.

Results

α-Mangostin induces mortality of zebrafish embryos

To evaluate the toxicity of α-mangostin on zebrafish embryo mortality and development, fertilized eggs of zebrafish were exposed to α-mangostin (0, 1.5, 3.0, 4.5, 6.0, 7.5, and 9.0 μM). After exposure for 72 h, the lower concentrations (≤4.5 μM) did not induce significant mortality (Figure 1). The mortality rates of the 6.0- and 9.0 μM-treated groups were significantly higher than that of the control group. After 7.5 and 9.0 μM treatment, 100% mortality was induced in the zebrafish embryos. The derived LC_{50} value of α-mangostin to zebrafish embryos at 72 h was 5.75 ± 0.26 μM. In order to assure that the mortality did not cause by pH-induced effects, we measured the pH of the test solutions and found that α-mangostin did not affect the pH of the medium (data not shown).

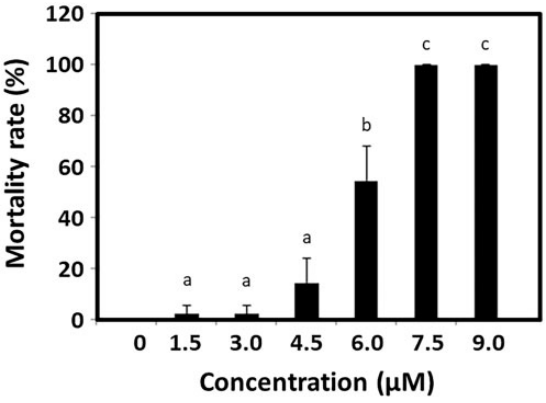

Figure 1. Mortality of zebrafish embryos after exposure to the indicated concentrations of α-mangostin for 72 h. The data are presented as the mean ± SD of three independent experiments. Significant differences between experimental groups are denoted by different letters (one-way ANOVA, followed by Tukey's test, $P < 0.05$).

α-Mangostin causes malformation of zebrafish embryos

Representative zebrafish embryos were documented at 24, 48, and 72 hpf. The control group showed normal morphological development throughout the test period (Figure 2). However, in the α-mangostin-treated group, various malformations of morphological embryonic development were observed, including axis deformity, bent tail, sluggish circulation, pericardial edema, yolk sac edema, and heart malformation (Figure 2). In addition, α-mangostin-treated embryos also exhibited hemostasis in the ducts of Cuvier. However, we found that α-mangostin did not affect the hatching rate (data not shown). There was no significant difference, as measured by the chi-squared test, in the total percentage of malformed embryos at 3 μM (1.19% malformation). At 4.5 and 6 μM, the total percentage of malformed embryos was increased to 78.79% and 100%, respectively. The teratogenic effects of zebrafish embryos treated with different concentrations of α-mangostin are listed in Table 1. We found that the α-mangostin-induced malformations of zebrafish embryos occurred in a dose-dependent manner. The main teratogenic effects were axis malformation, bent tail, and yolk edema.

α-Mangostin treatment reduces the heart rate of zebrafish embryos

In this study, the embryonic heart rates were recorded at 24, 48, and 72 hpf. At 24 hpf, no significant differences in the heart rate were observed between the α-mangostin-treated embryos and the control embryos (Figure 3). However, at 48 and 72 hpf, exposure to 6 μM α-mangostin significantly decreased the embryonic heart rate (23.33% and 18.31%, respectively) compared with the control group (Figure 3).

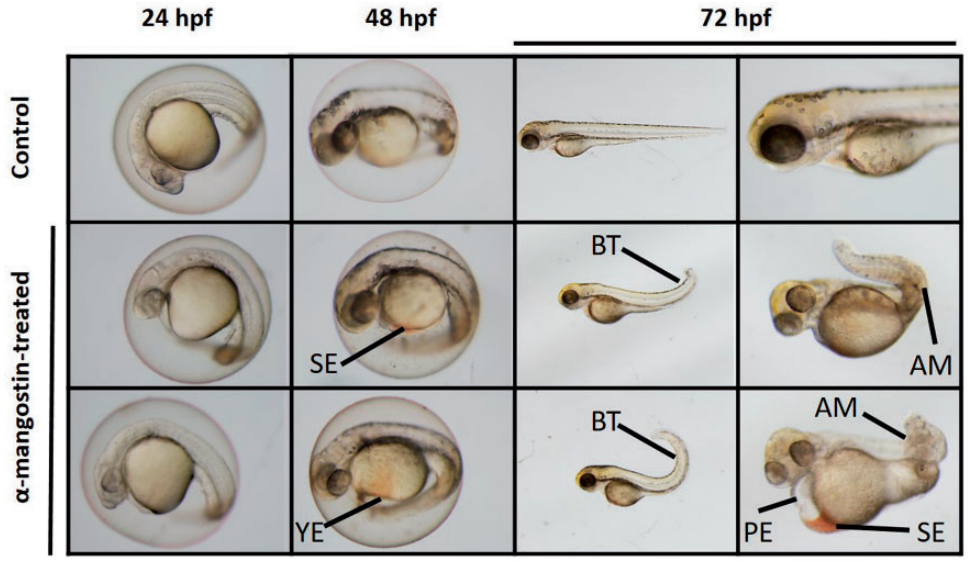

Figure 2. Phenotypes of control- and α-mangostin-treated embryos and larvae. YE: yolk edema; PE: pericardial edema; SE: stasis of erythrocytes in the ducts of Cuvier; AM: axis malformation; BT: bent tail. (A color version of this figure is available in the online journal.)

Table 1. Evaluation of the teratogenic effects in zebrafish embryos induced by exposure to control (0.48% DMSO), 1.5, 3.0, 4.5, and 6.0 μM α-mangostin for 72 h.

Toxic effects	Control	1.5 μM	3.0 μM	4.5 μM	6.0 μM
Axis malformation	–	–	–	+	+
Bent tail	–	+	+	+	+
Yolk edema	–	+	–	+	+
Pericardial edema	–	–	–	+	+
Heart malformation	–	–	–	+	+
Hemostasis	–	–	–	+	+

α-Mangostin treatment resulted in ROS reduction

To gain a closer insight into the mechanisms of α-mangostin on zebrafish embryos, we measured the generation of ROS through the fluorescence intensity of CM-H2DCFDA. The ROS levels in zebrafish embryos after 48 h exposure to α-mangostin are shown in Figure 4. ROS levels in embryos exposed to 3 μM α-mangostin were not significantly different to the control embryos. However, embryos exposed to 6 μM α-mangostin exhibited lower ROS levels than those of the control ($P < 0.05$).

α-Mangostin decreases embryonic hemoglobin level

As shown in Figure 5, the relative number of erythrocytes in the cardiac region were detected by hemoglobin staining with *o*-dianisidine after exposure to α-mangostin for 72 h. After treatment with 6 μM α-mangostin, the relative number of erythrocytes was significantly decreased to 15.1% lower than the control group. This result indicated that the decrease in cardiac output induced by α-mangostin could lead to cardiac dysfunction in zebrafish larvae.

Discussion

Although the biological activities of α-mangostin have been investigated by many researchers, limited information is available on its toxic effects, especially with regard to embryonic development. The use of a zebrafish model emerged as a common model for the investigation of developmental toxicity in drug development.[18–20] The model has many advantages; the major advantages are the use and evaluation of the whole embryo and, owing to the rapid development of zebrafish embryos, the inclusion of all stages of development. Other advantages include transparent embryos, which permit clear observations in the early stages of development, high fecundity, similar development to that of mammals, and their genetic similarities to humans.[12]

In our previous work, we reported the effects of α-mangostin on the embryonic angiogenesis of zebrafish and did not find anti-angiogenic properties of α-mangostin in zebrafish embryos, despite the clear downregulation of the expression of *vegfaa* and *vegfr2* transcripts;[21] however, we did not study other effects on zebrafish embryos than those on angiogenesis. In the present study, we therefore investigated the toxic effects of α-mangostin on embryonic development. After exposure of zebrafish embryos to α-mangostin for 72 h, we found that α-mangostin caused embryonic mortality in a dose-dependent manner. The exposure to low concentrations of α-mangostin (≤4.5 μM) did not significantly affect the survival of zebrafish embryos. It was found that the *in vivo* toxicity of α-mangostin against zebrafish embryos had a derived LC_{50} of 5.75 ± 0.26 μM. This was lower than that found in our previous study, which reported an LC_{50} of 9.4 μM.[21] This may have been a result of the different sources of α-mangostin. In the present study, α-mangostin was purchased from Sigma-Aldrich (purity ≥98%, HPLC grade) while in the previous study the compound was extracted from *T. laeviceps*

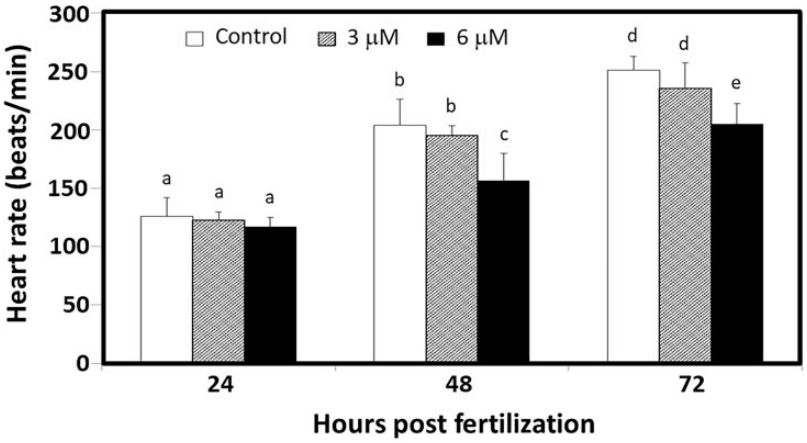

Figure 3. Heart rate of zebrafish embryos exposed to different concentrations of α-mangostin for 24, 48, and 72 h. The data are presented as the mean ± SD of three independent experiments. Significant differences between experimental groups at the same time point are denoted by different letters (one-way ANOVA, followed by Tukey's test, $P < 0.05$).

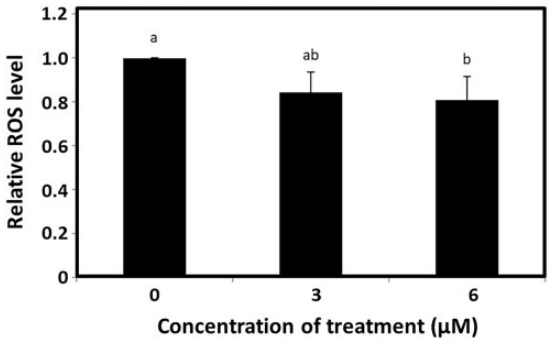

Figure 4. Relative ROS levels in zebrafish larvae exposed to α-mangostin for 72 h. The data are the mean ± SD of independent experiments. Significant differences between experimental groups are denoted by different letters (one-way ANOVA, followed by Tukey's test, $P < 0.05$).

cerumen and the percentage of the compound purity was not reported. Although these median lethal doses of α-mangostin in zebrafish cannot be compared to that in mice ($LC_{50} = 150$ mg/kg),[7] we here first report that α-mangostin could induce mortality in zebrafish embryos. Furthermore, exposure to higher concentrations (≥4.5 μM) induced morphological abnormalities of zebrafish embryos, including yolk and pericardial edema, heart malformation, stasis of erythrocytes in the ducts of Cuvier, axis malformation, and bent tail. These were broadly similar teratogenic effects to those induced by mangosteen leaves and stem-bark lyophilized water extracts.[22] However, the previous study did not report the concentration of α-mangostin in the extracts. To the best of our knowledge, our findings represent the first report on the teratogenic effects of α-mangostin.

In our zebrafish experiments, α-mangostin was used in a range of concentrations of 1.5–9 μM. These concentrations were determined empirically based on the evaluation of LC_{50}. α-Mangostin induced malformed morphology of zebrafish embryos at 4.5 and 6 μM. Chitchumroonchokchai *et al.* studied that an administration of a mangosteen juice contained 78.5 mg of α-mangostin in 60 mL of the product or about 3.19 mM of alpha-mangostin.[23] The concentration of α-mangostin in this product is much higher than the concentrations we used in our study. However, the range of the concentrations we used is in the same range with those concentrations of the pharmaceutical properties of α-mangostin. For example, α-mangostin inhibited the cell growth of the leukemia cell lines (K562, NB4, and U937) at 5–10 μM,[24] 5 μM of the compound protected proximal tubule renal epithelial cells (LLC-PK1) from cisplatin CDDP-induced apoptotic death,[25] and α-mangostin induced cell death effects on PC12 rat pheochromocytoma cells with the EC_{50} value of 4 μM.[26]

As we found hemostasis in the ducts of Cuvier and heart malformation in α-mangostin-treated embryos, we recorded the heart rate of the embryos. Heart rate detection is useful for the assessment of cardiac function, as variations in the heart beat can be an effect of hidden pathological heart conditions. The transparency of zebrafish embryos permits the easy optical observation of heart development and heart rate. Our results indicated that 6 μM of α-mangostin reduced the heart rate of the embryos at 48 and 72 hpf. There is no previous report showing the effect of α-mangostin on heart rate. However, Xie *et al.* reported that there was no significant difference in heart rates of participants before and after consumption of a mangosteen-based drink for 30 days.[27] On the other hand, other studies have reported that ingestion of mangosteen juice blend increased the levels of several pro-inflammatory mediators.[28] Although no adverse events were reported in these trials, we cannot ignore the compound effects found in zebrafish embryos since α-mangostin is a component in these products. Therefore, the mangosteen products require assessment for their potential long-term toxicity.

The cytotoxicity induced by compounds is a result of cellular changes such as oxidative stress and apoptosis. The induction of oxidative stress has been considered as one of the important regulatory factors of a compound cytotoxicity. α-Mangostin is known as an antioxidant compound. Previous studies have been reported that the compound possesses protective effects against oxidative stress in rodent models, such as rats with isoproterenol-induced myocardial infarction,[29] rat brain tissues exposed to

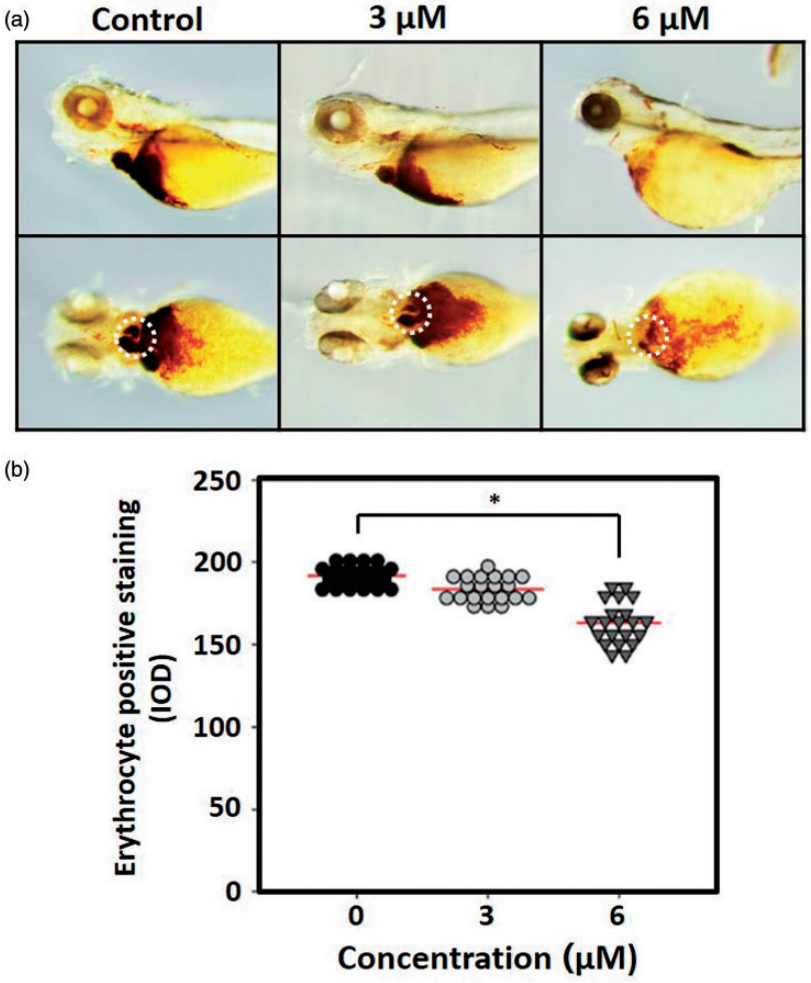

Figure 5. Erythrocyte detection in cardiac sections of α-mangostin-exposed zebrafish. (a) Representative zebrafish larvae showing erythrocyte detections by *o*-dianisidine staining. (b) The relative numbers of erythrocyte was determined by integrated optical density (IOD) analysis. The data are shown as the mean ± SD from independent experiments (*$P < 0.05$). (A color version of this figure is available in the online journal.)

different toxic compounds[30] and mice with light-damaged retina.[31] To evaluate the effects of α-mangostin on oxidative stress, the ROS levels were measured in zebrafish embryos exposed to α-mangostin at lethal and sub-lethal concentrations of 6 and 3 μM, respectively. A reduction in ROS occurred at the lethal concentration. This effect of α-mangostin may be modulated through the alteration of the molecular pathways that regulate the embryonic ROS balance, which also causes the adverse developmental effects in animal models exposed to thalidomide and phenytoin.[32,33] Further studies will explore the mechanisms on the *in vivo* antioxidant interactions with metabolites and the mediation of inflammation pathways.

Furthermore, we explored the effects of α-mangostin on embryonic erythropoiesis. Zebrafish have been used for the investigation and exploration of the molecular mechanisms of hematopoiesis and hematopoietic diseases in humans.[34,35] Zebrafish have all the hematopoietic cells that are characteristic of humans;[36] moreover, zebrafish hematopoietic-related genes and proteins are also conserved in humans.[37] *o*-Dianisidine staining indicated that α-mangostin caused a decrease in hemoglobin level. This was either indicative of (1) lower hemoglobin level in erythrocytes and/or (2) a lower number of erythrocytes in zebrafish larvae. To elucidate this issue, the expression of erythrocyte gene and protein markers should be investigated. However, in a previous study α-mangostin was also found to affect erythrocytes by changing the shape of rat isolated platelets, inhibiting aggregation, and causing cytolysis of the platelets.[38]

In conclusion, we have presented the first demonstration that exposure to α-mangostin induces mortality and the compound is potentially teratogenic in zebrafish embryos. The compound can also cause cardiac dysfunction in zebrafish embryos. In addition, the embryonic ROS balance and erythropoiesis were also disrupted by α-mangostin. Therefore, these findings provide the toxicological profile of α-mangostin in zebrafish embryos, another model besides rodent models. Further molecular mechanisms which lead to toxicity and teratogenicity of α-mangostin are to be explored. The evaluation of α-mangostin teratogenicity in other models is also necessary.

Authors' contributions: WP and CC conceived project; WP designed research; WK and WP performed experiments, analyzed data, prepared figures, and drafted manuscript; PT, CC,

and WP edited and revised manuscript. All authors read and approved the final manuscript.

DECLARATION OF CONFLICTING INTERESTS

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

FUNDING

The author(s) disclosed receipt of the following financial support for the research, authorship, and/or publication of this article: This study was supported by the Institute for the Promotion of Teaching Science and Technology, Thailand (Funding Number: 19/2557, W Pimtong).

ORCID iD

Wittaya Pimtong http://orcid.org/0000-0003-0138-2146

REFERENCES

1. Pedraza-Chaverri J, Cardenas-Rodriguez N, Orozco-Ibarra M, Perez-Rojas JM. Medicinal properties of mangosteen (*Garcinia mangostana*). *Food Chem Toxicol* 2008;**46**:3227–39
2. Ibrahim MY, Hashim NM, Mariod AA, Mohan S, Abdulla MA, Abdelwahab SI, Arbab IA. Alpha-Mangostin from *Garcinia mangostana Linn*: an updated review of its pharmacological properties. *Arab J Chem* 2016;**9**:317–29
3. Brito LC, Berenger ALR, Figueiredo MR. An overview of anticancer activity of Garcinia and Hypericum. *Food Chem Toxicol* 2017;**109**:847–62
4. Zhang KJ, Gu QL, Yang K, Ming XJ, Wang JX. Anticarcinogenic effects of alpha-mangostin: a review. *Planta Med* 2017;**83**:188–202
5. Nelli GB, K AS, Kilari EK. Antidiabetic effect of alpha-mangostin and its protective role in sexual dysfunction of streptozotocin induced diabetic male rats. *Syst Biol Reprod Med* 2013;**59**:319–28
6. Kumar V, Bhatt PC, Kaithwas G, Rashid M, Al-Abbasi FA, Khan JAJ, Anwar F, Verma A. α-Mangostin mediated pharmacological modulation of hepatic carbohydrate metabolism in diabetes induced Wistar rat. *Beni-Seuf Univ J Appl Sci* 2016;**5**:255–76
7. Choi YH, Han SY, Kim YJ, Kim YM, Chin YW. Absorption, tissue distribution, tissue metabolism and safety of alpha-mangostin in mangosteen extract using mouse models. *Food Chem Toxicol* 2014;**66**:140–6
8. Gutierrez-Orozco F, Thomas-Ahner JM, Berman-Booty LD, Galley JD, Chitchumroonchokchai C, Mace T, Suksamrarn S, Bailey MT, Clinton SK, Lesinski GB, Failla ML. Dietary alpha-mangostin, a xanthone from mangosteen fruit, exacerbates experimental colitis and promotes dysbiosis in mice. *Mol Nutr Food Res* 2014;**58**:1226–38
9. Ito T, Ando H, Handa H. Teratogenic effects of thalidomide: molecular mechanisms. *Cell Mol Life Sci* 2011;**68**:1569–79
10. Vargesson N. Thalidomide-induced teratogenesis: history and mechanisms. *Birth Defects Res C Embryo Today* 2015;**105**:140–56
11. Santoriello C, Zon LI. Hooked! Modeling human disease in zebrafish. *J Clin Invest* 2012;**122**:2337–43
12. Howe K, Clark MD, Torroja CF, Torrance J, Berthelot C, Muffato M, Collins JE, Humphray S, McLaren K, Matthews L, McLaren S, Sealy I, Caccamo M, Churcher C, Scott C, Barrett JC, Koch R, Rauch GJ, White S, Chow W, Kilian B, Quintais LT, Guerra-Assunção JA, Zhou Y, Gu Y, Yen J, Vogel JH, Eyre T, Redmond S, Banerjee R, Chi J, Fu B, Langley E, Maguire SF, Laird GK, Lloyd D, Kenyon E, Donaldson S, Sehra H, Almeida-King J, Loveland J, Trevanion S, Jones M, Quail M, Willey D, Hunt A, Burton J, Sims S, McLay K, Plumb B, Davis J, Clee C, Oliver K, Clark R, Riddle C, Elliot D, Threadgold G, Harden G, Ware D, Begum S, Mortimore B, Kerry G, Heath P, Phillimore B, Tracey A, Corby N, Dunn M, Johnson C, Wood J, Clark S, Pelan S, Griffiths G, Smith M, Glithero R, Howden P, Barker N, Lloyd C, Stevens C, Harley J, Holt K, Panagiotidis G, Lovell J, Beasley H, Henderson C, Gordon D, Auger K, Wright D, Collins J, Raisen C, Dyer L, Leung K, Robertson L, Ambridge K, Leongamornlert D, McGuire S, Gilderthorp R, Griffiths C, Manthravadi D, Nichol S, Barker G, Whitehead S, Kay M, Brown J, Murnane C, Gray E, Humphries M, Sycamore N, Barker D, Saunders D, Wallis J, Babbage A, Hammond S, Mashreghi-Mohammadi M, Barr L, Martin S, Wray P, Ellington A, Matthews N, Ellwood M, Woodmansey R, Clark G, Cooper J, Tromans A, Grafham D, Skuce C, Pandian R, Andrews R, Harrison E, Kimberley A, Garnett J, Fosker N, Hall R, Garner P, Kelly D, Bird C, Palmer S, Gehring I, Berger A, Dooley CM, Ersan-Ürün Z, Eser C, Geiger H, Geisler M, Karotki L, Kirn A, Konantz J, Konantz M, Oberländer M, Rudolph-Geiger S, Teucke M, Lanz C, Raddatz G, Osoegawa K, Zhu B, Rapp A, Widaa S, Langford C, Yang F, Schuster SC, Carter NP, Harrow J, Ning Z, Herrero J, Searle SM, Enright A, Geisler R, Plasterk RH, Lee C, Westerfield M, de Jong PJ, Zon LI, Postlethwait JH, Nüsslein-Volhard C, Hubbard TJ, Roest Crollius H, Rogers J, Stemple DL. The zebrafish reference genome sequence and its relationship to the human genome. *Nature* 2013;**496**:498–503
13. Brannen KC, Panzica-Kelly JM, Danberry TL, Augustine-Rauch KA. Development of a zebrafish embryo teratogenicity assay and quantitative prediction model. *Birth Defects Res B Dev Reprod Toxicol* 2010;**89**:66–77
14. McCollum CW, Ducharme NA, Bondesson M, Gustafsson JA. Developmental toxicity screening in zebrafish. *Birth Defects Res C Embryo Today* 2011;**93**:67–114
15. Selderslaghs IW, Blust R, Witters HE. Feasibility study of the zebrafish assay as an alternative method to screen for developmental toxicity and embryotoxicity using a training set of 27 compounds. *Reprod Toxicol* 2012;**33**:142–54
16. Liu H, Sheng N, Zhang W, Dai J. Toxic effects of perfluorononanoic acid on the development of Zebrafish (*Danio rerio*) embryos. *J Environ Sci (China)* 2015;**32**:26–34
17. Pimtong W, Datta M, Ulrich AM, Rhodes J. Drl.3 governs primitive hematopoiesis in zebrafish. *Sci Rep* 2014;**4**:5791
18. Brannen KC, Charlap JH, Lewis EM. Zebrafish teratogenicity testing. *Methods Mol Biol*. 2013;**947**:383–401
19. Raldua D, Pina B. *In vivo* zebrafish assays for analyzing drug toxicity. *Expert Opin Drug Metab Toxicol* 2014;**10**:685–97
20. MacRae CA, Peterson RT. Zebrafish as tools for drug discovery. *Nat Rev Drug Discov* 2015;**14**:721–31
21. Nugitrangson P, Puthong S, Iempridee T, Pimtong W, Pornpakakul S, Chanchao C. *In vitro* and *in vivo* characterization of the anticancer activity of Thai stingless bee (*Tetragonula laeviceps*) cerumen. *Exp Biol Med (Maywood)* 2016;**241**:166–76
22. Jose BV, Dulay RMR, David ES. Toxic and teratogenic assessment of mangosteen (*Garcinia mangostana* L.) leaves and stem-bark lyophilized water extracts in zebrafish (*Danio rerio*) embryos. *Adv Environ Biol* 2016;**10**:96–101
23. Chitchumroonchokchai C, Riedl KM, Suksumrarn S, Clinton SK, Kinghorn AD, Failla ML. Xanthones in mangosteen juice are absorbed and partially conjugated by healthy adults. *J Nutr* 2012;**142**:675–80
24. Matsumoto K, Akao Y, Kobayashi E, Ohguchi K, Ito T, Tanaka T, Iinuma M, Nozawa Y. Induction of apoptosis by xanthones from mangosteen in human leukemia cell lines. *J Nat Prod* 2003;**66**:1124–7
25. Sanchez-Perez Y, Morales-Barcenas R, Garcıa-Cuellar CM, Lopez-Marure R, Calderon-Oliver M, Pedraza-Chaverri J, Chirino YI. The a-mangostin prevention on cisplatin-induced apoptotic death in LLC-PK1 cells is associated to an inhibition of ROS production and p53 induction. *Chem Biol Interact* 2010;**188**:144–50
26. Sato A, Fujiwara H, Oku H, Ishiguro K, Ohizumi Y. Alpha-mangostin inducesCa2+-ATPase-dependent apoptosis via mitochondrial pathway in PC12 cells. *J Pharmacol Sci* 2004;**95**:33–40
27. Xie Z, Sintara M, Chang T, Ou B. Daily consumption of a mangosteen-based drink improves *in vivo* antioxidant and anti-inflammatory biomarkers in healthy adults: a randomized, double-blind, placebo-controlled clinical trial. *Food Sci Nutr* 2015;**3**:342–8
28. Udani JK, Singh BB, Barrett ML, Singh VJ. Evaluation of mangosteen juice blend on biomarkers of inflammation in obese subjects: a pilot, dose finding study. *Nutr J* 2009;**8**:48

29. Devi Sampath P, Vijayaraghavan K. Cardioprotective effect of alpha-mangostin, a xanthone derivative from mangosteen on tissue defense system against isoproterenol-induced myocardial infarction in rats. *J Biochem Mol Toxicol* 2007;**21**:336–9
30. Marquez-Valadez B, Lugo-Huitron R, Valdivia-Cerda V, Miranda-Ramírez LR, Pérez-De La Cruz V, González-Cuahutencos O, Rivero-Cruz I, Mata R, Santamaría A, Pedraza-Chaverrí J. The natural xanthone alpha-mangostin reduces oxidative damage in rat brain tissue. *Nutr Neurosci* 2009;**12**:35–42
31. Fang Y, Su T, Qiu X, Mao P, Xu Y, Hu Z, Zhang Y, Zheng X, Xie P, Liu Q. Protective effect of alpha-mangostin against oxidative stress induced-retinal cell death. *Sci Rep* 2016;**6**:21018
32. Dennery PA. Effects of oxidative stress on embryonic development. *Birth Defects Res C Embryo Today* 2007;**81**:155–62
33. Wells PG, McCallum GP, Chen CS, Henderson JT, Lee CJ, Perstin J, Preston TJ, Wiley MJ, Wong AW. Oxidative stress in developmental origins of disease: teratogenesis, neurodevelopmental deficits, and cancer. *Toxicol Sci* 2009;**108**:4–18
34. Ellett F, Lieschke GJ. Zebrafish as a model for vertebrate hematopoiesis. *Curr Opin Pharmacol* 2010;**10**:563–70
35. Rasighaemi P, Basheer F, Liongue C, Ward AC. Zebrafish as a model for leukemia and other hematopoietic disorders. *J Hematol Oncol* 2015;**8**:29
36. Davidson AJ, Zon LI. The 'definitive' (and 'primitive') guide to zebrafish hematopoiesis. *Oncogene* 2004;**23**:7233–46
37. Song HD, Sun XJ, Deng M, Zhang GW, Zhou Y, Wu XY, Sheng Y, Chen Y, Ruan Z, Jiang CL, Fan HY, Zon LI, Kanki JP, Liu TX, Look AT, Chen Z. Hematopoietic gene expression profile in zebrafish kidney marrow. *Proc Nat Acad Sci U S A* 2004;**101**:16240–5
38. Liu Y, Park JM, Chang KH, Chin YW, Lee MY. Alpha- and gamma-mangostin cause shape changes, inhibit aggregation and induce cytolysis of rat platelets. *Chem Biol Interact* 2015;**240**:240–8

(Received July 17, 2018, Accepted November 26, 2018)