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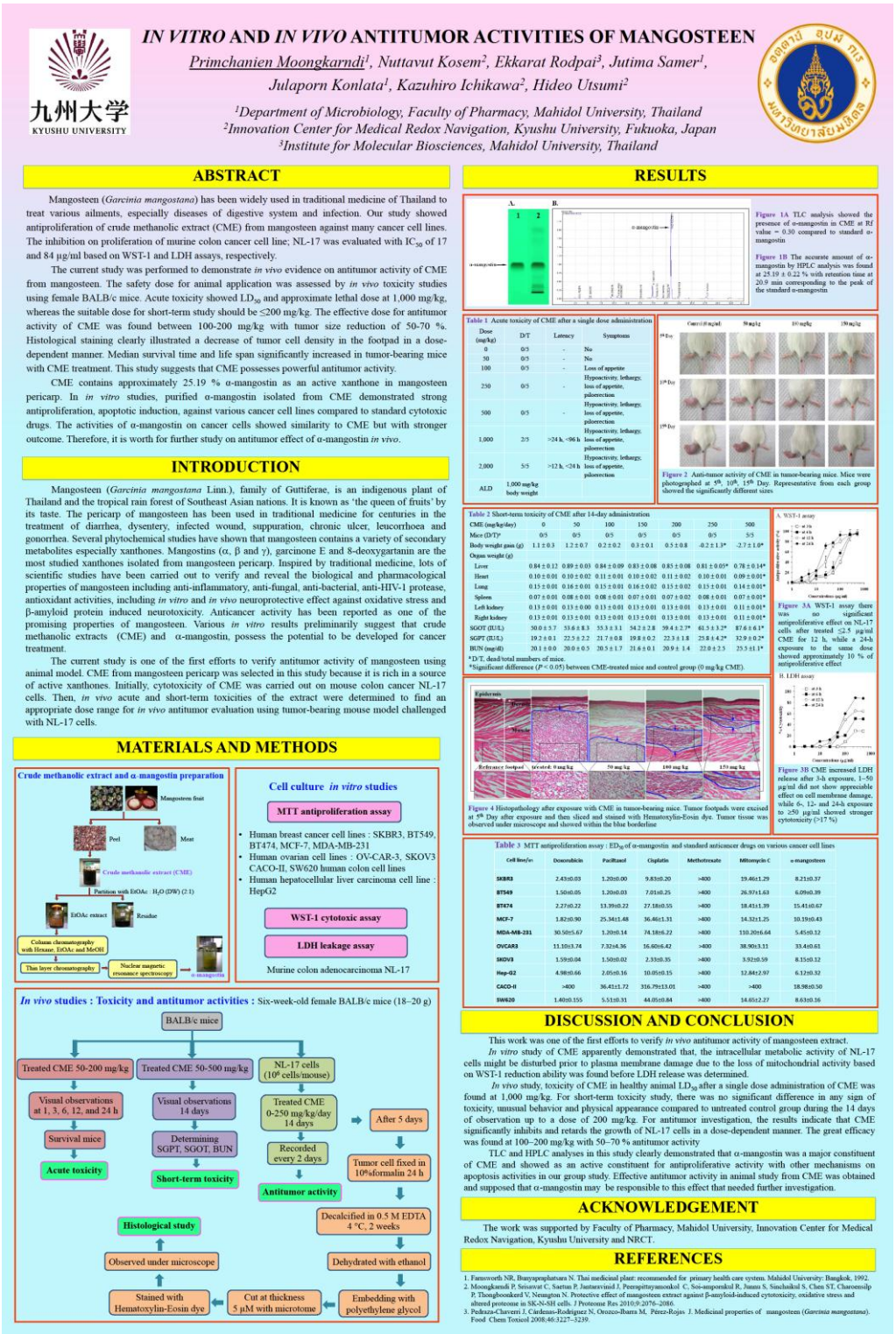

IN VITRO AND IN VIVO ANTITUMOR ACTIVITIES OF MANGOSTEEN

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ABSTRACT

Mangosteen (*Garcinia mangostana*) has been widely used in traditional medicine of Thailand to treat various ailments, especially diseases of digestive system and infection. Our study showed antiproliferation of crude methanolic extract (CME) from mangosteen against many cancer cell lines. The inhibition on proliferation of murine colon cancer cell line; NL-17 was evaluated with IC_{50} of 17 and 84 µg/ml based on WST-1 and LDH assays, respectively.

The current study was performed to demonstrate *in vivo* evidence on antitumor activity of CME from mangosteen. The safety dose for animal application was assessed by *in vivo* toxicity studies using female BALB/c mice. Acute toxicity showed LD_{50} and approximate lethal dose at 1,000 mg/kg, whereas the suitable dose for short-term study should be ≤200 mg/kg. The effective dose for antitumor activity of CME was found between 100-200 mg/kg with tumor size reduction of 50-70 %. Histological staining clearly illustrated a decrease of tumor cell density in the footpad in a dose-dependent manner. Median survival time and life span significantly increased in tumor-bearing mice with CME treatment. This study suggests that CME possesses powerful antitumor activity.

CME contains approximately 25.19 % α-mangostin as an active xanthone in mangosteen pericarp. In *in vitro* studies, purified α-mangostin isolated from CME demonstrated strong antiproliferation, apoptotic induction, against various cancer cell lines compared to standard cytotoxic drugs. The activities of α-mangostin on cancer cells showed similarity to CME but with stronger outcome. Therefore, it is worth for further study on antitumor effect of α-mangostin *in vivo*.

INTRODUCTION

Mangosteen (*Garcinia mangostana* Linn.), family of Guttiferae, is an indigenous plant of Thailand and the tropical rain forest of Southeast Asian nations. It is known as 'the queen of fruits' by its taste. The pericarp of mangosteen has been used in traditional medicine for centuries in the treatment of diarrhea, dysentery, infected wound, suppuration, chronic ulcer, leucorrhoea and gonorrhea. Several phytochemical studies have shown that mangosteen contains a variety of secondary metabolites especially xanthones. Mangostins (α, β and γ), garcinone E and 8-deoxygartanin are the most studied xanthones isolated from mangosteen pericarp. Inspired by traditional medicine, lots of scientific studies have been carried out to verify and reveal the biological and pharmacological properties of mangosteen including anti-inflammatory, anti-fungal, anti-bacterial, anti-HIV-1 protease, antioxidant activities, including *in vitro* and *in vivo* neuroprotective effect against oxidative stress and β-amyloid protein induced neurotoxicity. Anticancer activity has been reported as one of the promising properties of mangosteen. Various *in vitro* results preliminarily suggest that crude methanolic extracts (CME) and α-mangostin, possess the potential to be developed for cancer treatment.

The current study is one of the first efforts to verify antitumor activity of mangosteen using animal model. CME from mangosteen pericarp was selected in this study because it is rich in a source of active xanthones. Initially, cytotoxicity of CME was carried out on mouse colon cancer NL-17 cells. Then, *in vivo* acute and short-term toxicities of the extract were determined to find an appropriate dose range for *in vivo* antitumor evaluation using tumor-bearing mouse model challenged with NL-17 cells.

MATERIALS AND METHODS

Crude methanolic extract and α-mangostin preparation

Mangosteen fruit → Peel, Meat → Crude methanolic extract (CME) → Partition with EtOAc : H_2O (DW) (2:1) → EtOAc extract, Residue → Column chromatography with Hexane, EtOAc and MeOH → Thin layer chromatography → Nuclear magnetic resonance spectroscopy → α-mangostin

Cell culture *in vitro* studies

MTT antiproliferation assay

- Human breast cancer cell lines : SKBR3, BT549, BT474, MCF-7, MDA-MB-231
- Human ovarian cell lines : OV-CAR-3, SKOV3
- CACO-II, SW620 human colon cell lines
- Human hepatocellular liver carcinoma cell line : HepG2

WST-1 cytotoxic assay

LDH leakage assay

Murine colon adenocarcinoma NL-17

***In vivo* studies : Toxicity and antitumor activities :** Six-week-old female BALB/c mice (18–20 g)

BALB/c mice

- Treated CME 50-200 mg/kg → Visual observations at 1, 3, 6, 12, and 24 h → Survival mice → Acute toxicity
- Treated CME 50-500 mg/kg → Visual observations 14 days → Determining SGPT, SGOT, BUN → Short-term toxicity
- NL-17 cells (10^6 cells/mouse) → Treated CME 0-250 mg/kg/day 14 days → Recorded every 2 days → Antitumor activity
  - After 5 days → Tumor cell fixed in 10%formalin 24 h → Decalcified in 0.5 M EDTA 4 °C, 2 weeks → Dehydrated with ethanol → Embedding with polyethylene glycol → Cut at thickness 5 µM with microtome → Stained with Hematoxylin-Eosin dye → Observed under microscope → Histological study

RESULTS

Figure 1A TLC analysis showed the presence of α-mangostin in CME at Rf value = 0.30 compared to standard α-mangostin

Figure 1B The accurate amount of α-mangostin by HPLC analysis was found at 25.19 ± 0.22 % with retention time at 20.9 min corresponding to the peak of the standard α-mangostin

Table 1 Acute toxicity of CME after a single dose administration

Dose (mg/kg)	D/T	Latency	Symptoms
0	0/5	-	No
50	0/5	-	No
100	0/5	-	Loss of appetite
250	0/5	-	Hypoactivity, lethargy, loss of appetite, piloerection
500	0/5	-	Hypoactivity, lethargy, loss of appetite, piloerection
1,000	2/5	>24 h, <96 h	Hypoactivity, lethargy, loss of appetite, piloerection
2,000	5/5	>12 h, <24 h	Hypoactivity, lethargy, loss of appetite, piloerection
ALD	1,000 mg/kg body weight		

Figure 2 Anti-tumor activity of CME in tumor-bearing mice. Mice were photographed at 6th, 10th, 15th Day. Representative from each group showed the significantly different sizes

Table 2 Short-term toxicity of CME after 14-day administration

CME (mg/kg/day)	0	50	100	150	200	250	500
Mice (D/T)*	0/5	0/5	0/5	0/5	0/5	0/5	5/5
Body weight gain (g)	1.1 ± 0.3	1.2 ± 0.7	0.2 ± 0.2	0.3 ± 0.1	0.5 ± 0.8	-0.2 ± 1.3*	-2.7 ± 1.0*
Organ weight (g)							
Liver	0.84 ± 0.12	0.89 ± 0.03	0.84 ± 0.09	0.83 ± 0.08	0.85 ± 0.08	0.81 ± 0.05*	0.78 ± 0.14*
Heart	0.10 ± 0.01	0.10 ± 0.02	0.11 ± 0.01	0.10 ± 0.02	0.11 ± 0.02	0.10 ± 0.01	0.09 ± 0.01*
Lung	0.15 ± 0.01	0.16 ± 0.01	0.15 ± 0.01	0.16 ± 0.02	0.15 ± 0.02	0.13 ± 0.01	0.14 ± 0.01*
Spleen	0.07 ± 0.01	0.08 ± 0.01	0.08 ± 0.01	0.07 ± 0.01	0.07 ± 0.02	0.08 ± 0.01	0.07 ± 0.01*
Left kidney	0.13 ± 0.01	0.13 ± 0.00	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01*
Right kidney	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.13 ± 0.01	0.11 ± 0.01*
SGOT (IU/L)	50.0 ± 3.7	53.6 ± 8.3	53.3 ± 3.1	54.2 ± 2.8	59.4 ± 2.7*	61.3 ± 3.2*	87.6 ± 6.1*
SGPT (IU/L)	19.2 ± 0.1	22.5 ± 2.2	21.7 ± 0.8	19.8 ± 0.2	22.3 ± 1.8	25.8 ± 4.2*	32.9 ± 0.2*
BUN (mg/dl)	20.1 ± 0.0	20.0 ± 0.5	20.5 ± 1.7	21.6 ± 0.1	20.9 ± 1.4	22.0 ± 2.5	25.5 ± 1.1*

*D/T, dead/total numbers of mice.
*Significant difference ($P < 0.05$) between CME-treated mice and control group (0 mg/kg CME).

Figure 3A WST-1 assay there was no significant antiproliferative effect on NL-17 cells after treated ≤2.5 µg/ml CME for 12 h, while a 24-h exposure to the same dose showed approximately 10 % of antiproliferative effect

Figure 3B CME increased LDH release after 3-h exposure, 1–50 µg/ml did not show appreciable effect on cell membrane damage, while 6-, 12- and 24-h exposure to ≥50 µg/ml showed stronger cytotoxicity (>17 %)

Figure 4 Histopathology after exposure with CME in tumor-bearing mice. Tumor footpads were excised at 5th Day after exposure and then sliced and stained with Hematoxylin-Eosin dye. Tumor tissue was observed under microscope and showed within the blue borderline

Table 3 MTT antiproliferation assay : ED_{50} of α-mangostin and standard anticancer drugs on various cancer cell lines

Cell lines/µm	Doxorubicin	Paclitaxel	Cisplatin	Methotrexate	Mitomycin C	α-mangostin
SKBR3	2.43±0.03	1.20±0.00	9.83±0.20	>400	19.46±1.29	8.21±0.37
BT549	1.50±0.05	1.20±0.03	7.01±0.25	>400	26.97±1.63	6.09±0.39
BT474	2.27±0.22	13.39±0.22	27.18±0.55	>400	18.43±1.39	15.41±0.67
MCF-7	1.82±0.90	25.34±1.48	36.46±1.31	>400	14.32±1.25	10.19±0.43
MDA-MB-231	30.50±5.67	1.20±0.14	74.18±6.22	>400	110.20±6.64	5.45±0.12
OVCAR3	11.10±3.74	7.32±4.36	16.60±6.42	>400	38.90±3.11	33.4±0.61
SKOV3	1.59±0.04	1.50±0.02	2.33±0.35	>400	3.92±0.59	8.15±0.12
Hep-G2	4.98±0.66	2.05±0.16	10.05±0.15	>400	12.84±2.97	6.12±0.32
CACO-II	>400	36.41±1.72	316.79±13.01	>400	>400	18.98±0.50
SW620	1.40±0.155	5.51±0.31	44.05±0.84	>400	14.65±2.27	8.63±0.36

DISCUSSION AND CONCLUSION

This work was one of the first efforts to verify *in vivo* antitumor activity of mangosteen extract.

In vitro study of CME apparently demonstrated that, the intracellular metabolic activity of NL-17 cells might be disturbed prior to plasma membrane damage due to the loss of mitochondrial activity based on WST-1 reduction ability was found before LDH release was determined.

In vivo study, toxicity of CME in healthy animal LD_{50} after a single dose administration of CME was found at 1,000 mg/kg. For short-term toxicity study, there was no significant difference in any sign of toxicity, unusual behavior and physical appearance compared to untreated control group during the 14 days of observation up to a dose of 200 mg/kg. For antitumor investigation, the results indicate that CME significantly inhibits and retards the growth of NL-17 cells in a dose-dependent manner. The great efficacy was found at 100–200 mg/kg with 50–70 % antitumor activity.

TLC and HPLC analyses in this study clearly demonstrated that α-mangostin was a major constituent of CME and showed as an active constituent for antiproliferative activity with other mechanisms on apoptosis activities in our group study. Effective antitumor activity in animal study from CME was obtained and supposed that α-mangostin may be responsible to this effect that needed further investigation.

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