Cytotoxic Activities of the Ethanolic Extract of *Kaempferia galanga* Linn. and its Active Component Against Human Cholangiocarcinoma Cell line and PBMC

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Abstract

Kaempferia galanga Linn. (KG) is one of the plants in the Zingiberaceae family. Its rhizome extract has been used traditionally in Southeast Asia for analgesic and anti-inflammatory activities. Cholangiocarcinoma (CCA) is a bile duct tumor which is an important public health problem in the Northeastern region of Thailand. Standard chemotherapeutics for treatment of CCA is currently unsatisfactory. The aim of the study was to investigate cytotoxicity of the ethanolic extract of KG rhizomes including its bioactive compound ethyl-p-methoxycinnamate (EPMC) against the human CCA cell line CL-6 and peripheral blood mononuclear cell (PBMC) by MTT assay. The half maximal inhibitory concentration (IC_{50}) (mean ± SD) values of KG extract, EPMC, and the reference drug 5-fluorouracil (5-FU) in CL-6 cell were 82.56±24.89, 93.63±22.34 and 19.45±12.37 µg/ml, respectively. The corresponding IC_{50} values for the PBMC cell were 227.87±16.54, 96.14±9.82 and 502.14±11.71µg/ml, respectively. Results provide as a first-step, screening information on potential anti-CCA and cytotoxic effects of the KG extract and EPMC.

Keywords: *Kaempferiagalanga Linn., cytotoxicity, cholangiocarcinoma*

1. Introduction

Cholangiocarcinoma (CCA) is a serious health problem of people in the northeastern region of Thailand (Sripa et al., 2015). Standard chemotherapeutics for treatment of CCA is currently unsatisfactory. Attempts have been made to search for new alternative medicines for treatment of CCA (Plengsuriyakarn et al., 2012; Zhang et al., 2016). *Kaempferia galanga* Linn. (KG) is a plant of the Zingiberaceae family. It is traditionally used in Thailand for treatment of various conditions including hypertension, pectoral and abdominal pains, headaches and toothaches, rheumatism (Othman et al., 2006), and for antinociceptive activity (Ridtitid et al., 2008). Ethyl-p-methoxycinnamate (EPMC) is a major bioactive component of KG rhizome which has been demonstrated for several biological activities such as anti-inflammatory and analgesic (Vittalrao et al., 2011), antibacterial (Arambewela et al., 1999), sedative (Huang et al., 2008), anti-angiogenic (He et al., 2012), anti-Mycobacterium tuberculosis (Lakshmanan et al., 2011), anticancer activity (Liu et al., 2010), and hyperpigmentary (Ko et al., 2014) activities. The aim of the present study is to investigate cytotoxic activities of the ethanolic extract of KG rhizomes and EPMC against the human CCA cell line CL-6 compared to PBMC for finding herbal medicine as drug in cancer treatment.

2. Objectives

The objectives of the study is to investigate cytotoxic activities of the ethanolic extract of KG rhizomes and EPMC comparing with 5-FU as a reference drug against the human CCA cell line CL-6 compared to PBMC.

3. Materials and Methods

3.1 Preparation of plant extract

The dried and powdered rhizomes of KG was obtained from Nakhon Pathom Province, Thailand. Preparation of the ethanolic extracts of the plant materials was according to the previously described method (Sasidharan et al., 2011). The extract was standardized for extraction efficiency and quality control using high performance liquid chromatography (HPLC) to determine the amount of the marker compound ethyl-p-methoxycinnamate (EPMC). The HPLC system consisted of HPLC 1200 Series (Agilent Technologies, CA, USA), Hypersil Gold Column (250 × 4.6 mm ID, 5 µm particle size, reversed phase C18: Thermoscientific, MA, USA); UV-detector (270 nm, Thermoscientific, MA, USA), and an isocratic solvent of methanol and distilled water (54:46% v/v) running at a flow rate of 1 ml/min. The injection volume was 10 µl. The plant extract and standard EPMC were prepared as stock solutions of 20 and 10 µg/ml, respectively.

3.2 Cell culture

Peripheral blood mononuclear cell (PBMC) was separated from healthy donors using Lymphoprep™ solution (Nycomed Pharma AS, Drammensveien, Norway). Human CCA cell line CL-6 was cultured in RPMI1640 medium supplemented with 10% (v/v) heated fetal bovine serum (FBS), and 1% of 100 IU/ml of antibiotic-antimycotic solution (Gibco, Grand Island, NY, USA) under 5% CO_2 atmosphere and 95% humidity (37^{o}C). PBMC was cultured and maintained as CL-6 cells.

3.3 Cytotoxic activity assay

CL-6 and PBMC were exposed to the crude ethanolic extract of KG rhizomes, EPMC, and 5-FU at various concentrations, i.e., 1.95, 3.9, 7.8, 15.6, 31.25, 62.5, 125, 250, 500, and 1,000 µg/ml in a 96-well microtiter plate. Cytotoxic activity was determined using MTT assay. Briefly, CL-6 (10,000 cells) and PBMC (200,000 cells) were plated into each well of the 96-well plate and incubated for 24 hours. The cells were exposed to each test materials for 48 hours. After an 48 hour incubation, 20 µl of 5 mg/ml MTT reagent (Sigma-Aldech, Saint Louis, MO, USA) was added into each well and the cells were further incubated for an additional 4 hours before the addition of dimethyl sulfoxide (DMSO: 100 µl). The plate was left at room temperature (25 $^{\circ}$C) in a dark room for 15 minutes. Then, the absorbance of formazan was measured at 570 nm by Varioskan Flash reader (Thermo Fisher Scientific Inc., Rockford, IL, USA). Finally, the data were calculated by CalcuSyn-Version 1.1 (Biosoft, Cambridge, UK).

4. Results and Discussion

The KG extract was evaluated for the content of the marker compound ethyl-p-methoxycinnamate (EPMC) using HPLC. The standard and marker analysis of EPMC in the test extract was identified at the retention time of 19.99 minutes. Based on the HPLC analysis, EPMC was the major component in the KG extract with a peak area of 94.09% of total content of the extract preparation (Figure 1). From this result confirmed the quality control of KG preparation with standardization using HPLC method.

The IC_{50} values of all test materials for the CL-6 and PBMC cells are presented in Figure 2. The IC_{50} values (mean ± SD) of EPMC, KG extract, and the reference 5-FU were 93.63 ± 22.34, 82.56 ± 24.89 and 19.45 ±12.37 µg/ml, respectively for CL-6 and 96.14 ± 9.82, 227.87± 16.54 and 502.14 ± 11.71µg/ml, respectively for PBMC (Figure 2). The results show the safety profile of KG on PBMC cell with high IC_{50} value and moderate inhibitory activity of KG and EPMC compound on CL-6 cells. The corresponding selectivity index (SI) values for KG, EPMC and 5-FU compounds on CL-6 as compared to control normal cell line, PBMC were 5.36, 1.16, and 11.71 respectively. The summarized IC_{50} and SI values of KG and 5-FU are given in Table 1. The compound was relatively more cytotoxic towards CL-6 with IC_{50} values less than 100 µg/ml, which is quite remarkable for highly drug resistant cancer of this type. However, a previous study using the same cell line (Mahavorasirikul et al., 2010) reported a mean IC_{50} of 37.36 µg/ml and SI of 2.9 with the ethanolic extract of the leaves of KG. In our study, the dried rhizome of KG was selected to investigate and the ethanolic extract of KG rhizomes exhibited moderate cytotoxic activity against the CCA cell line CL-6 with IC_{50} (mean ± SD) of 93.63 ± 22.34 µg/ml and SI of 5.36.

The degree of selectivity of the compounds can be expressed by its Selectivity Index (SI) value. The SI values were calculated as follows: SI = IC_{50} normal cell/IC_{50} cancer cell. High SI value (>2) of a compound gives a selective toxicity towards cancer cells (Badisa et al., 2009). Selectivity of the cytotoxic activity of KG and EPMC compound were determined by comparing the cytotoxic activity (IC_{50}) of KG and EPMC compound against the cancerous CL-6 cell with the normal PBMC cell. Only KG showed significant SI values, which is 5.36. Hence, KG display potential to be further exploited in the discovery and development of new herbal medicine as a drug in cancer treatment.

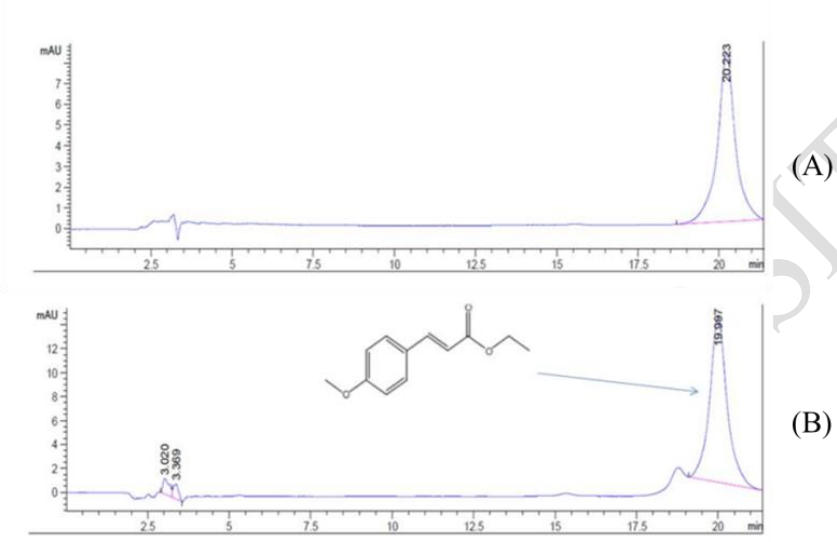

Figure 1 HPLC chromatograms of (A) standard ethyl-p-methoxycinnamate (EPMC) and (B) the ethanolic extract of KG rhizomes. Note: Chromatographic separation condition used was as follows: Thermoscientific™ Hypersil Gold Column 5 µm C18 column; mobile phase: a mixture of water and methanol with isocratic elution (46%: 54% v/v) at follow rate of 1 ml/min; injection volume 0f 10 µl; and UV-detection at 270 nm

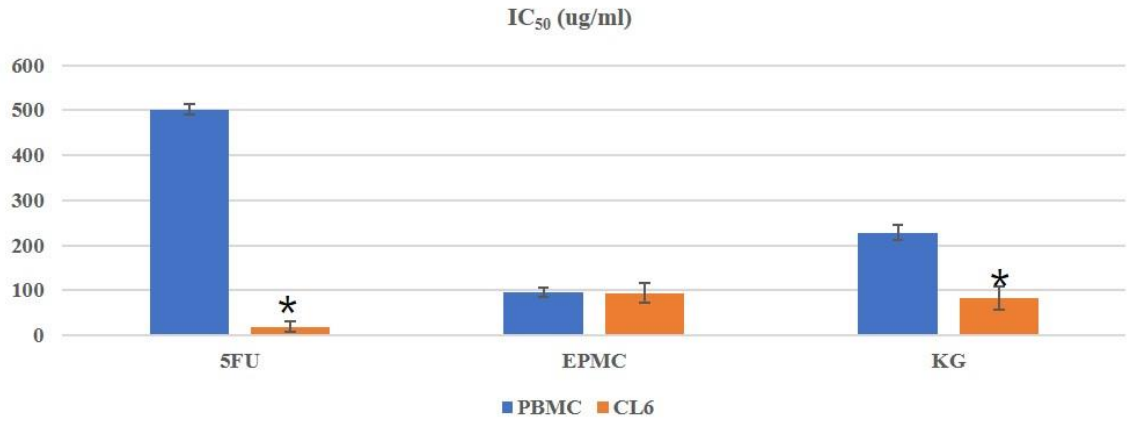

Figure 2 Mean (SD) IC_{50} values of KG, EPMC, and 5-FU for PBMC and CL-6
***** Significant between CL-6 and PBMCs odd

Table 1 Summary of the IC_{50} and SI values for 5-FU, KG and EPMC compound

No.	Cell line	Test compounds	IC_{50} (µg/ml)	Specificity Index (SI)
1	CL-6	KG	93.63 ± 22.34	5.36
		EPMC	82.56 ± 24.89	1.16
		5-FU	19.45 ± 12.37	11.71
2	PBMC	KG	502.14 ± 11.71	1
		EPMC	96.14 ± 9.82	1
		5-FU	227.87 ± 16.54	1

Data are represented as mean ± SD for three independent experiments done in triplicate. Half maximal inhibitory concentration (IC_{50}), specificity index (SI), KG (*Kaempferia galanga* Linn.), EPMC (ethyl-p-methoxycinnamate) and 5-FU (5-fluorouracil)

5. Conclusion

Results from the present study suggests that cytotoxicity activity of KG and EPMC exhibit selective cytotoxicity towards CL-6 cells and had potency of about 4 times less than of 5-FU. The selectivity and comparatively low IC_{50} (<100 µg/ml) of KG and EPMC provides possibilities for further in-depth investigation of these compounds on underlying potential molecular mechanism for observed cytotoxicity in CL-6 cells. Consequently, KG could be considered as a promising anticancer agent due to its high SI value on PBMC cells. It provides as a first-step, screening information on potential anti-CCA and cytotoxic effects of the KG extract and EPMC. Confirmation of anti-CCA activities in animal models is required for their further development as chemotherapeutics for treatment of CCA.

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