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Screening of antiproliferation activity of Thai medicinal plants against human cholangiocarcinoma cells *in vitro*

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Abstract

Cholangiocarcinoma is a serious public health concern in Thailand with increasing incidence and mortality rates. The present study aimed to investigate antiproliferation activity of crude ethanolic extracts of 28 plants and 5 recipes used in Thai folklore medicine against human cholangiocarcinoma (CL-6) and human hepatocarcinoma (HepG2) cell lines *in vitro*. Antiproliferation activity of the plant extracts against the cancerous cell lines compared with normal cell line (renal epithelial cell) was assessed using MTT assay and the IC_{50} (concentration which inhibits cell growth by 50%) were calculated. The extracts from eight plants and one folklore recipe exhibited promising activity against the cholangiocarcinoa CL-6 cell line with percentage cell survival of less than 50% at the concentration of 50 μg/ml. Among these, the extracts from the five plants and one recipe (AtLEt, KaGEt, ZiOEt, PiCEt, MeFEt and PsPyEt) showed potent cytotoxic activity with mean IC_{50} values of 24.09, 37.36, 34.26, 40.74, 48.23 and 44.12 μg/ml, respectively. In contrast, activities against the hepatoma cell HepG2 varied markedly; mean IC_{50} ranged from 9.67 to 115.47 μg/ml and the only promising extract was from ZiOEt (IC_{50} 9.67 μg/ml). The sensitivity of all the three cells to 5-FU also varied according to cell types, particularly with CL-6 cell (IC_{50} 757 μM). In overall, it can be concluded that ethanolic extracts of AtLEt and ZiOEt contained antiproliferation against CL-6 and Hep-G2 cell line, respectively.

Keywords: Thai medicinal plants, cytotoxic activity, cholangiocarcinoma, CL-6, HepG2

Introduction

Cholangiocarcinoma, malignant epithelial cells that arises within bile duct, is a serious public health in Thailand with increasing incidence and mortality rates. It accounts for approximately 15% of liver cancer worldwide (1). Chemotherapeutic treatment of cholangiocarcinoma is largely ineffective. The standard chemotherapeutic agent, 5-fluorouracil (5-FU) always produces low clinical response rate (2). Numerous cancer research studies have been carried out using medicinal plants in an effort to discover new therapeutic agents that lack toxic effects associated with current therapeutic agents. Traditional medicine is commonly used as an alternative treatment for cancer by Thai people (3). Several Thai traditional folklores have been assessed for their anticancer activities in various human cancerous cell lines with some promising candidates (4). In the present study, the ethanolic extracts of a total of 28 plants and 5 recipres used in Thai folklore medicine were investigated for their antiproliferation activity *in vitro* in two human cancerous cell lines (cholangiocarcinoma: CL-6, hepatocarcinoma: HepG2), and one normal human cell line (renal epithelial cell: HRE).

Methods

Plant materials were collected from various parts of Thailand and some were purchased from the city markets. Ethanolic extracts of all plant materials were prepared. CL-6, HepG2 and HRE used for cytotoxic screening of the medicinal plant extracts were maintained in standard culture medium at 37ºC in a 5% CO_2 atmosphere with 95% humidity. The MTT colorimetric assay (5) was used to screen for cytotoxic activity of all the plant extracts and 5-FU (positive control) in a 96-well plates at a cell density of 10^4 per well and 100 µl culture medium. Each extract was screened initially for its cytotoxicity against all cancerous and normal cell lines at the concentration of 50 µg/ml. The potential candidates which resulted in cell survival of less than 50% were further assessed for their IC_{50} (concentration that inhibits cell growth by 50%) at the concentration range of 250-1.95 µg/ml. Following a 3 h incubation with MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] solution (20 µl of 5 mg/ml) at 37ºC, cells were lyzed with DMSO. The yellow MTT dye was reduced by succinic dehydrogenase in the mitochondria of viable cells to purple formazan crystals. Absorbance (OD) was measured at 570 nm using a microplate reader (Varioscan Flash, Thermo, Finland). The percentage of cytotoxicity compared to the untreated cells was determined. The IC_{50} values were calculated with probit analysis software (CalcySyn™, USA).

Results and Discussion

Results from the initial screening showed that the extracts from eight plant species exhibited promising activity against the cholangiocarcinoa CL-6 cell line with percentage cell survival of less than 50% at the concentration of 50 µg/ml (Figure 1). Crude extracts from AtLEt showed the highest promising activity against CL-6 cell line, whereas it showed no activity against HepG2 cell. CuLEt and MaSEt exhibited high activity against HepG2 (% survival of 13.9 and 29.2%, respectively; Figure 1) but relatively low activity against CL-6 cell line (% survival of 59.9 and 51.4, respectively; Figure 1). Among these, six showed potent cytotoxic activity with IC_{50} of less than 50 µg/ml (AtLEt, KeGEt, ZiOEt, PiCEt, MeFEt and PsPyEt). The extracts of the six plants were further investigated for their spectrum of anticancer activity against HepG-2 cell line. Results showed that the activity against hepatoma cell HepG2 varied markedly; mean IC_{50} ranged from 9.67 to 115.47 µg/ml. The most promising extract was from ZiOEt (IC_{50} 9.67 ± 3.91 µg/ml). The extract from AtLEt exhibited the most potent activity against CL-6 but the activity against HepG2 was only moderate. The results are generally in agreement with that shown in the screening test (Figure 1), confirming that HepG2 was more resistant to the tested ethanolic extracts from Thai traditional folklore than CL-6. The extract from AtLEt appears to be the most potent (IC_{50} 24.09 ± 3.40 µg/ml), whereas that from ZiOEt appears to be the most potent against HepG2 (IC_{50} 9.67 ± 3.91 µg/ml). Further investigation of all the six extracts for their cytotoxic activity against cholangiocarcinoma in hamster model is underway to fully assess the anticancer activity *in vivo*.

Conclusion

Six out of a total of 28 plants and 5 recipes used in Thai folklore medicine exhibited promising cytotoxic activity against CL-6 human cholangiocarcinoma cell line. HepG2 appears to be more resistant to the tested extracts. The extract from AtLEt was more potent against cholangiocarcinoma, whereas that from ZiOEt was more potent against HepG2.

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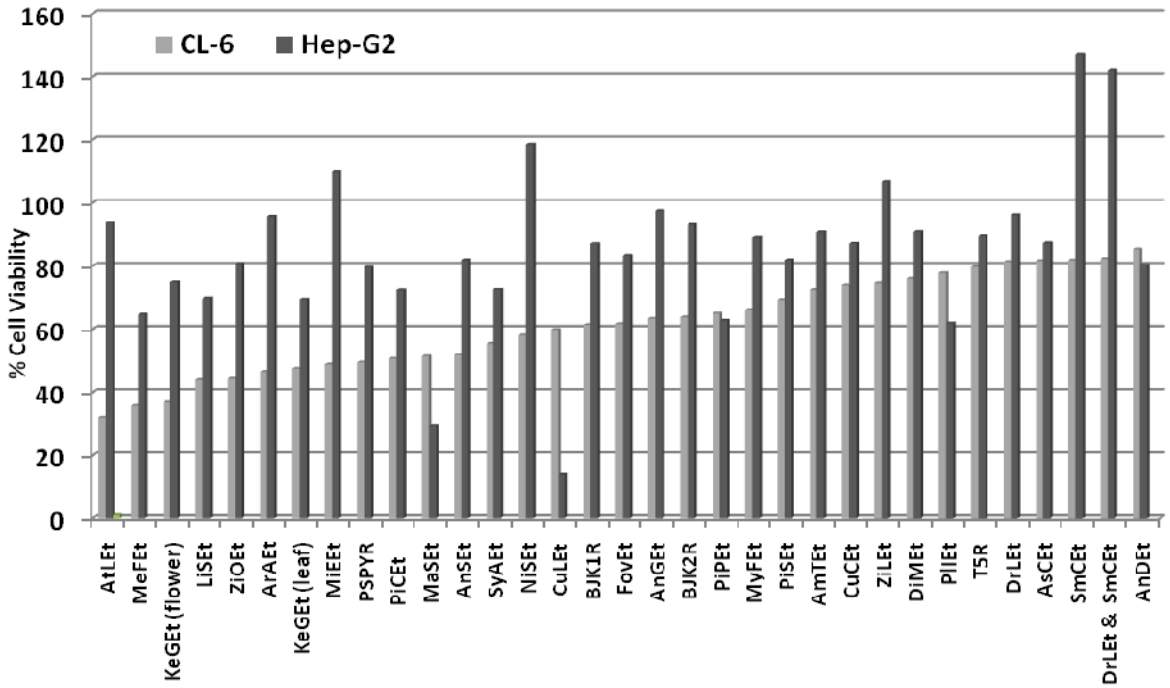

Figure 1. Percentage survival of cancer cell lines (CL-6 and HepG2) treated with ethanolic extract from a total of 28 plants and 5 recipes used in Thai folklore medicine at the concentration of 50 μg/ml.

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