

Cytotoxic effects of the ethanolic extract from Benjakul formula and its compounds on human lung cancer cells

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Abstract

Benjakul have also been used for cancer therapy by folk medicine. The ethanolic extracts, five fractions and three pure compounds from *Benjakul* were investigated for cytotoxic activities against two types of human lung cancer cell lines (COR-L23 and A549) and one normal human lung myofibroblasts (MRC-5). The cytotoxic activities of the BEN-3 fraction which fractionated with vacuum liquid chromatography method by chloroform solvent showed stronger cytotoxic activities against COR-L23 and A549 cells than the ethanolic extracts and other fractions (IC₅₀ values 28.09 and 34.43 µg/ml, respectively). Its pure compounds, plumbagin exhibited the greatest cytotoxic activity against COR-L23, A549 and MRC5 with IC₅₀ values 0.36, 0.59 and 2.17 µg/ml, respectively. Piperine and gingerol exhibited cytotoxic activities against COR-L23 more than A549. In conclusion, all *Benjakul* extracts shown high cytotoxic activities against COR-L23 but less activities against other cells.

Keywords: cytotoxic activity; Benjakul; COR-L23; A549; MRC-5

Introduction

Benjakul is Thai traditional medicine formula that composed of 5 plants, root of *Plumbago indica* Linn. (Plumbaginaceae), root of *Piper sarmentosum* Roxb. (Piperaceae), stem of *Piper interruptum* Opiz. (Piperaceae), fruit of *Piper longum* Linn. (Piperaceae) and rhizome of *Zingiber officinale* Roscoe. (Zingiberaceae). In Southern folk medicine of Thailand, it was used as an adaptogenic drug for cancer patients^[1]. Previous work, the ethanolic extract, fractions and three pure compounds that isolated from Benjakul; plumbagin, piperine and 6-gingerol shown cytotoxic activities against human large cell breast carcinoma cell line, MCF-7^[2]. In the present study, we investigated cytotoxic activities of them against human large cell lung carcinoma cell line, COR-L23, human lung adenocarcinoma epithelial cell line, A549 and normal human lung myofibroblasts, MRC-5.

Method

Plant material and preparation of extracts

Five plants of Benjakul formula were purchased from folk doctor (August, 2009). All plants were cleaned immediately of extraneous material and were dried in at 50 °C. The dried powdered plant material of Benjakul (1 kg each) was extracted by maceration with 95% ethanol. The extracts was concentrated by rotary evaporator then was freeze-dried.

Fractionation of ethanol extract of Benjakul

Sixty gram of the ethanolic extract of Benjakul were separated to be fractions using vacuum liquid chromatography by ordering increase polarity of solvents. Five fractions being denoted as BEN-1 (hexane), BEN-2 (hexane:chloroform 1:1), BEN-3 (chloroform), BEN-4 (chloroform:methanol 1:1) and BEN-5 (methanol). Plumbagin, gingerol and piperin were isolated from the ethanolic extract of this preparation followed the method of Sakpakdeejaroen

thesis [2]. All compounds and extracts were dissolved in DMSO before testing cytotoxic assay.

Cytotoxicity testing

Two types of lung cancer cells and one normal human lung cell lines were used in the initial screening of the extract, fractions and pure compounds. The lung cancer cell lines were A549 (human small cell lung carcinoma cell line) and COR-L23 (human large cell lung carcinoma cell line), while the normal cell line used was MRC-5 (normal human lung myofibroblasts). The culturing of the cancer cells was as described by Keawpradub *et al.* (1999) [3] while the MRC-5 cells were cultured as described by Itharat *et al.* (2004) [4].

The cells were seeded in 96-well microtiter plates for all the experiments and 100 μ l of cell suspension used in each well. The plates were incubated at 37 °C to allow for cell attachment. After 24 h the cells were treated with the extract, fractions or pure compounds. All of them were initially dissolved in either DMSO. 100 μ l/well of each concentration was added to the plates in four replicates to obtain final concentrations of 1, 10, 50, 100 μ g/ml in the wells for the extract and fractions and 0.05 - 20 μ g/ml for pure compounds. 2% of DMSO being used in the solvent control wells. The plates were incubated for 72 h. After that, the medium was removed and the wells were then washed with PBS, and 200 μ l of fresh medium were then added. The plates were incubated at 37 °C for a recovery period of 72 h and cell growth was then analysed using the SRB assay [5]. It was carried out as previously described Itharat *et al.* (2004) [4].

The plates were fixed with 100 μ l of ice-cold 40% TCA per well and incubated at 4 °C for 1 h and were washed with cold water (five times). SRB stain (50 μ l; 0.4 in 1% acetic acid) was added to each well and left in contact with the cells for 30 min and were washed with 1% acetic acid (five times). The fixed plates were dried at room temperature and 100 μ l of 10 mM Tris base pH 10.5 were added to each well to solubilise the dye. The plates were shaken and the absorbance (OD) of each well was read on a Power Wave X plate reader at 492 nm. Cell survival was measured as the percentage absorbance compared to the control (non-treated cells). The IC₅₀ values were calculated from the Prism program obtained by plotting the percentage of survival versus the concentrations. All experiments were done in three replicates on each plate.

Results and discussion

The results of cytotoxic activity of ethanolic extract, fractions and three pure compounds of Benjakul against three human cell lines (COR-L23, A549 and MRC-5) are shown in table 1.

Table 1. Cytotoxic activity of ethanolic extract, fractions and three pure compounds of Benjakul on three human cell lines.

Cell lines	% yield	IC ₅₀ (μ g/ml) ^a		
		COR-L23	A549	MRC-5
Ethanolic extract	9.11	43.64 \pm 4.34	48.96 \pm 3.68	60.83 \pm 3.59
BEN-1	2.02	38.33 \pm 1.66	72.85 \pm 2.83	74.67 \pm 4.08
BEN-2	2.22	43.69 \pm 1.88	76.33 \pm 4.37	44.54 \pm 2.06
BEN-3	20.73	28.09 \pm 0.86	34.43 \pm 2.68	32.16 \pm 2.43
BEN-4	53.25	43.04 \pm 3.10	56.26 \pm 5.23	78.26 \pm 7.47
BEN-5	16.69	>100	>100	>100

Cell lines	% yield	IC ₅₀ (µg/ml) ^a		
		COR-L23	A549	MRC-5
Plumbagin	4.18	0.36±0.02	0.59±0.09	2.17±0.77
Piperine	7.81	17.00±0.14	>20	>20
6-Gingerol	0.54	15.91±0.91	>20	>20

^a IC₅₀ values were expressed as the mean ± S.D., determined from the results of SRB assay in three replicates.

Following the criteria for cytotoxic activity of extracts and pure compounds by the National Cancer Institute guidelines (NCI) [6] with IC₅₀ values <30 µg/ml and <4 µg/ml, respectively. The ethanolic extract showed cytotoxic activity against two lung cancer cells (COR-L23 and A549) more than normal lung cells with IC₅₀ = 43.64, 48.96 and 60.83 µg/ml, respectively. The cytotoxic activity of the BEN-3 fraction against two human lung cancer cell exhibited higher activity than the ethanolic extract and the other fractions however it showed active against only COR-L23 but it showed IC₅₀ less 30 µg/ml (IC₅₀=28.09 µg/ml). For the pure compounds, plumbagin exhibited the greatest cytotoxic activity against COR-L23 and A549 with IC₅₀ = 0.36 µg/ml and 0.59 µg/ml, respectively, whereas piperine and 6-gingerol showed cytotoxic activity against COR-L23 stronger than A549, however the effect of them were less than NCI criteria. All s of samples showed less cytotoxic activity against normal cells (MRC-5).

Conclusion

In conclusion, the present study supports using of Benjakul formula for cancer treatment as to be claimed by folk doctors. Plumbagin as its active cytotoxic showed the most activity, so it may develop for chemotherapeutic drug in the future.

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