RESEARCH ARTICLE

Cytotoxicity of *Alpinia galanga* Rhizome Crude Extract on NIH-3T3 Cells

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Abstract

BACKGROUND: *Alpinia galanga* (*A. galanga*) was reported as a potential medicinal source due to its wide effect. *A. galanga* rhizome crude extract (ARCE) was reported to have high cytotoxic effect in cancer cells, but low in normal cells. However half maximal inhibitory concentration (IC_{50}) of ARCE is not clearly known yet. Hence, current study was conducted to investigate the IC_{50} of ARCE in normal standard fibroblast cell line, NIH-3T3 cells.

METHODS: Rhizomes of *A. galanga* were collected, peeled, dried, milled and weighed. Extraction was performed using maceration method, then filtered and evaporated. ARCE with various concentrations were applied in NIH-3T3 cells for 24 or 48 hours. Cells were documented and counted with 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) assay.

RESULTS: Five hundreds grams of simplicia were macerated with ethanol and evaporated, 1 mg/mL crude extract with total volume of 114 mL was obtained. By addition of ARCE in NIH-3T3 cell culture, number of NIH-3T3 cells were shown less when treated with higher concentration of ARCE. Cell numbers of 0, 3.125, 6.25, 12.5, 25 and 50% ARCE treatment for 24 hours are 11,531, 11,352, 10,920, 10,365, 9,471, 8,360, respectively, meanwhile for 48 hours are 13,219, 12,686, 12,278, 11,390, 10,279, 8,390, respectively.

CONCLUSION: IC_{50} of ARCE in 24 hours treatment was 620.5 µg/mL, while in 48 hours treatment was 666.6 µg/mL. Hence, ARCE is suggested to have low cytotoxic effect in NIH-3T3 cells.

KEYWORDS: *Alpinia galanga*, ginger, extract, cytotoxic, MTT, NIH-3T3

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Introduction

Plants have been used as one of the most important sources for medicines. Indonesia with its wide biodiversities has plenty of resourceful natural materials those should be explored. Previously our team reported several potential extracts derived from plants including *Ipomea batatas* (1-3), *Artocarpus altilis* (4), *Piper crocatum* (5), *Kleinhovia hospita* (6), *Artocarpus heterophyllus* (7), *Catharanthus roseus* (8), *Piper betle* (9), *Catharanthus roseus* (9), *Dendrophtoe petandra* (9), *Curcuma mangga*(9), *Curcuma longa* (10), *Ananas comosus*(11), *Brucea javanica* (12,13), *Nephelium lappaceum* (14), *Cucumis melo* (15,16), *Caesalpinia sappan* (17) and *Artocarpus elasticus* (18). These extracts shows various activities, including osteoclastogenesis inhibition (1-3), apoptosis induction or proliferation

inhibition on breast cancer cells (4,5,7-9), antioxidant (6,14,16,18), proliferation and differentiation induction on stem cell (10,15), and apoptosis induction on human oral squamous cell carcinoma (12,13,16,17).

In addition, *Alpinia galanga* (*A. galanga*) was reported as a potential medicinal source due to its wide effect as anti-inflammatory, analgesic, anti-cancer, anti-melanogenic, hepato-protective, anti-microbial and anti-fungal agent.(19) In neuroprotective report against alzheimer disease, *A. galanga* was suggested to play the neuroprotective effect on Met-Leu-Gly-Ile-Ile-Ala-Gly-Lys-Asn-Ser-Gly Amyloid-β (Aβ)-induced amnesia in mice via the increment in Na^+, K^+-adenosine triphosphate (ATP)-ase, the improvement of antioxidant activity and the decrease of acetylcholinesterase level to improve the cognition by enhancing cholinergic transmission.(20) *A. galanga* was reported to contain, among other components, essential oils, tannins, phenol, glycosides, monoterpenes, carbohydrates, gallic acid, galangoisoflavonoid, β-sitosterol, galangin, alpinin, zerumbone, kampferide, pinocembrin, hydroxycinnamaldehyde (HCA) and acetoxychavicol acetate.(19,21-23) It is mostly cultivated in Egypt, India, Indonesia, Malaysia, Saudi Arabia, Sri Lanka and Thailand.(19)

It has been reported that 4'-HCA isolated from *A. galanga* was cytotoxic to human leukemic HL60 and U937 cell lines in a dose-dependent manner.(19,22) Meanwhile in normal cell, *A. galanga* rhizome crude extract (ARCE) at a dose of 100 µg/mL caused 15% apoptosis of MCF-12A cells, a normal epithelial cell derived from breast in 24 hours treatment, and at a dose of 1,000 µg/mL caused 5% apoptosis of CRL2522 cells, a normal fibroblast cell, in 24 hours treatment.(24) Therefore, half maximal inhibitory concentration concentration (IC_{50}) of ARCE is not clearly known yet. Hence, current study was conducted to investigate the IC_{50} of ARCE in normal standard fibroblast cell line, NIH-3T3 cells.

Methods

Extraction of *A. galanga* Rhizome

Rhizomes of *A. galanga* were collected, peeled, dried at 40°C for 3 days, milled and weighed. Extraction was performed using maceration method with 70% ethanol solvent in ratio of 1:10 for 5 days at 4°C. The solvent was added gradually, then filtered and evaporated with a rotary evaporator (Buchi Rotavapor R-124, Buchi, Flawil, Switzerland) at 40°C. Maceration results were stored in a refrigerator at 4°C.

NIH-3T3 Cell Culture

NIH-3T3 cells, a cell line derived from desegregated NIH Swiss mouse embryo fibroblasts which has become a standard fibroblast cell line (25), were cultured using Dulbecco's Modified Eagle Medium containing 10% fetal bovine serum (FBS), penicillin, streptomycin, and amphotericin B in a humidified, 37°C, 5% CO_2 incubator. Upon reaching 80% confluency, cells were subcultured and propagated.

Cytotoxic Test

Cytotoxic test was performed using 3-(4,5-dimethylthiazol-2-yl)-2,5-Diphenyltetrazolium bromide (MTT) assay (Sigma). The MTT assay provides a quantitative measurement of viable cells by determining the amount of formazan crystals produced by metabolically active cells. Briefly, 1 x 10^4 cells were seeded into each well of 96-well plates in medium containing active agent, 1:1,000 diluted hydrogen peroxide or medium merely. Cells were incubated for 24 or 48 hours. Ten µl of 5 mg/mL MTT in phosphate buffer saline (PBS) was added to each well. The plate was then incubated for 4 hours, and then the medium was discarded and formazan crystals were dissolved in 100 µL of 0.1N HCl. The absorbance of cells was measured at 570 nm by a microplate reader. Untreated cells were counted with a hemacytometer and used for interpolating the absorbance.

Results

ARCE Production

Eight kilograms of *A. galanga* rhizome were collected. After skin peeling and drying, 1 kg of simplicia was obtained. Five hundreds grams of simplicia were macerated with ethanol and evaporated, 1 mg/mL crude extract with total volume of 114 mL was obtained.

Effect of ARCE on NIH-3T3 Cell

Results showed that by addition of ARCE in NIH-3T3 cell culture, number of NIH-3T3 cells were shown less when treated with higher concentration of ARCE (Figure 1). After MTT assay, absorbances were correlated with cell densities

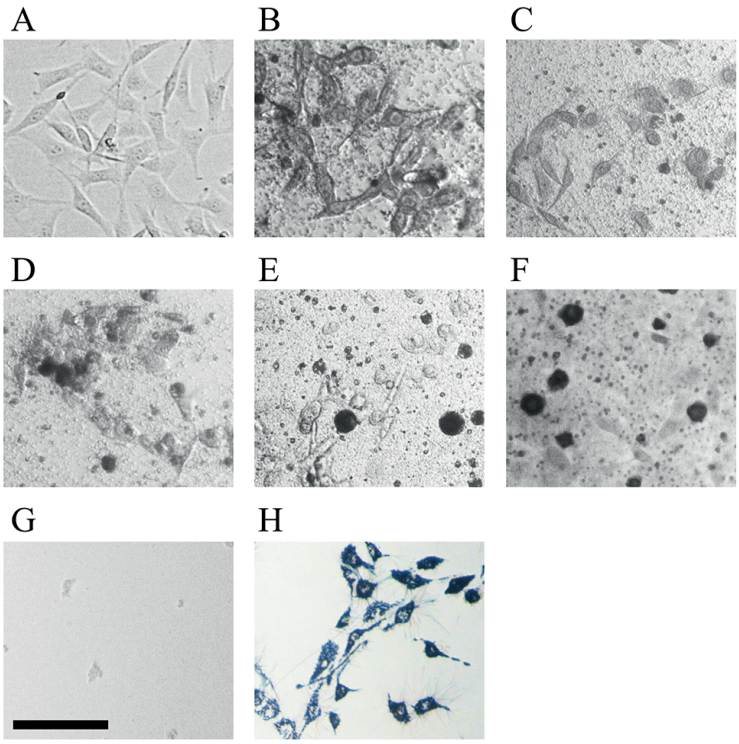

Figure 1. Expression of ARCE-treated NIH-3T3 cells. NIH-3T3 cells were seeded into 96-well plates. NIH-3T3 cells were treated with 0 (A), 3.125 (B), 6.25 (C), 12.5 (D), 25 (E) and 50% (F) ARCE in DMEM containing 2% FBS for 48 hours. Cells were documented with an inverted light microscope in the same magnification. (G): NIH-3T3 cells were treated with H_2O_2. (H): NIH-3T3 cells were incorporated with MTT, formazan crystals were formed in cells. Black bar: 50 µm.

for 24 hours (y=0.0623x+0.0065) (Figure 2A) and 48 hours (y=0.0595x+0.0114) (Figure 3A) treatments, cell numbers were gained after interpolation. Highest cell numbers was observed in 0% ARCE, followed by 3.125% ARCE, 6.25% ARCE, 12.5% ARCE, 25% ARCE and 50% ARCE. By interpolating the cell number for 24 hours treatment, the number for the group treated with 0% ARCE was 11,531, while 3.125% ARCE was 11,352; 6.25% ARCE was 10,920; 12.5% ARCE was 10,365; 25% ARCE was 9,471; 50% ARCE was 8,360 (Figure 2B). Meanwhile, for cell number for 48 hours treatment, number for the group treated with 0% ARCE was 13,219, while 3.125% ARCE was 12,686; 6.25% ARCE was 12,278; 12.5% ARCE was 11,390; 25% ARCE was 10,279; 50% was 8,390 (Figure 3B).

IC_{50} of ARCE on NIH-3T3 Cells

Based on calculation with formula of y=-63.977x+11366, y=half number of untreated cells (1/2 x 11,531) and x= IC_{50}, we found that 24-hours-treatment IC_{50} of ARCE was 87.54%. This equaled to 62.05% x 1 mg/mL = 0.6205 mg/mL or 620.5 µg/mL. Meanwhile based on calculation with formula of y=-94.325x+12897, y=half number of untreated cells (1/2 x 13,219), we found that 48-hours-incubation IC_{50} of ARCE was 66.66%. This equaled to 66.66% x 1 g/mL = 0.6666 mg/mL or 666.6 µg/mL.

Discussion

Based on our current results, we found that ARCE decreased viable NIH-3T3 cells in a dose dependent manner. The decrease of ARCE-induced viable NIH-3T3 cells could be found in both 24 and 48 hours treatments. Based on calculation, IC_{50} of ARCE in 24 hours treatment was 620.5 µg/mL, while in 48 hours treatment was 666.6 µg/mL. These results suggested that ARCE cytotoxic effect was slightly impaired after 48 hours. Previous study in normal cell for 24 hours, 100 µg/mL ARCE was reported to induce 15% apoptosis of MCF-12A cells, and 1,000 µg/mL caused 5% apoptosis of CRL2522 cells.(24) In addition, treatment of 750 µg/mL ARCE on MRC-5 cells, a human fetal lung fibroblast cell line, for 48 hours, did not induce the cell into apoptosis.(26) Hence, ARCE showed low cytotoxic effect in normal fibroblasts, including MCF-12A, CRL2522, MRC-5 and NIH-3T3 cells.

Unlike in normal fibroblasts, IC_{50} of ARCE in cancer cells is relatively low. For example, IC_{50} of ARCE in COR-L23 cells, a lung cancer cell line, was 5.4-13.3 µg/mL and IC_{50} of ARCE in MCF7 cells, a breast cancer cell line, was 5.4-170 µg/mL.(26,27) ARCE was also reported to induce apoptosis in PC-3 cells, a prostate cancer cell line, in concentration of 100 µg/mL, 69% of total tested PC-3 cells

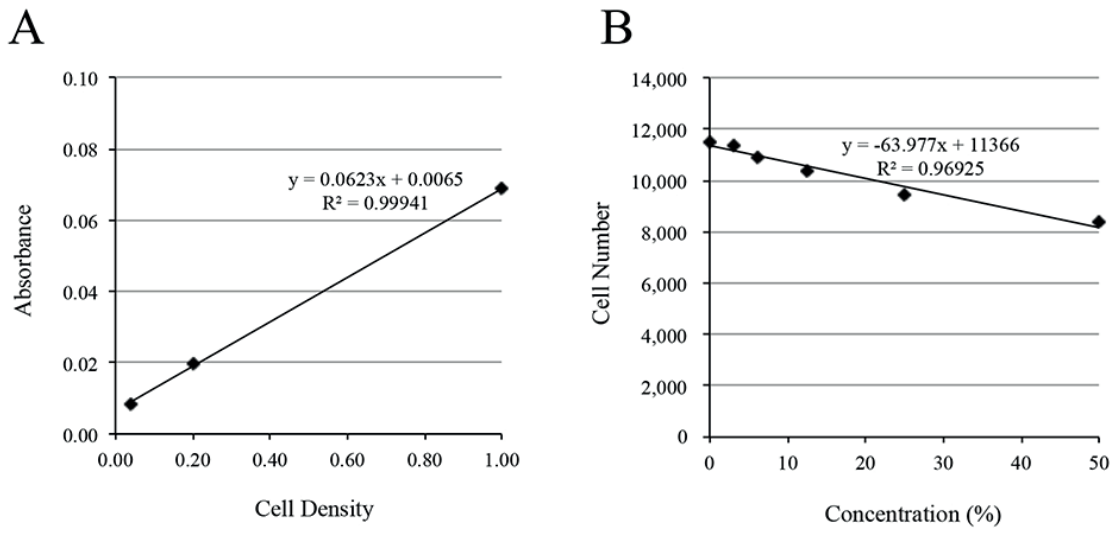

Figure 2. Effect of ARCE on viability of NIH-3T3 cells for 24 hours. Ten thousand NIH-3T3 cells were seeded into each well of 96-well plates. NIH-3T3 cells were treated with 0, 3.125, 6.25, 12.5, 25 and 50% ARCE in DMEM containing 2% FBS for 24 hours. Cell viabilities were measured with MTT assay as described in Methods. A: Obtained absorbances were correlated with cell density (1.0 cell density equaled to 11,531 cells). B: Interpolated number of viable NIH-3T3 cells after treated with ARCE in various concentrations for 24 hours.

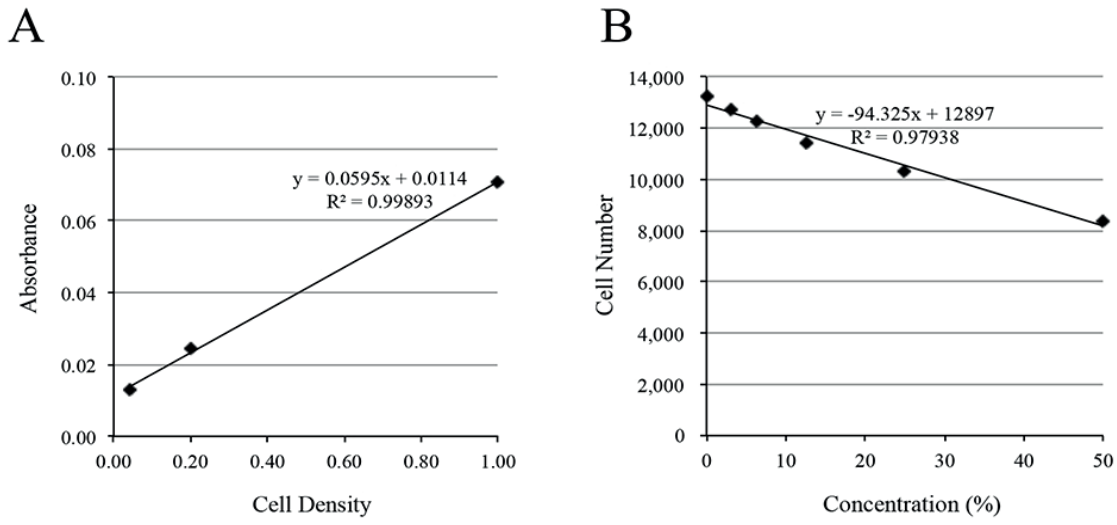

Figure 3. Effect of ARCE on viability of NIH-3T3 cells for 48 hours. Ten thousand NIH-3T3 cells were seeded into each well of 96-well plates. NIH-3T3 cells were treated with 0, 3.125, 6.25, 12.5, 25 and 50% ARCE in DMEM containing 2% FBS for 48 hours. Cell viabilities were measured with MTT assay as described in Methods. A: Obtained absorbances were correlated with cell density (1.0 cell density equaled to 13,219 cells). B: Interpolated number of viable NIH-3T3 cells after treated with ARCE in various concentrations for 48 hours.

were induced into apoptosis.(28) This suggested that ARCE has higher cytotoxic activity in cancer cells.

A. galanga was reported to contain, among other components, essential oils, tannins, phenol, glycosides, monoterpenes, carbohydrates, gallic acid, galangoisoflavonoid, β-sitosterol, galangin, alpinin, zerumbone, kampferide, pinocembrin, HCA and acetoxychavicol acetate.(19,21-23) Pinocembrin induced loss of mitochondrial membrane potential, followed by release of cytochrome c and activation of caspase-9 and -3 in HCT 116 cells, a colon cancer cell line.(21) The initial trigger for pinocembrin-induced mitochondrial apoptosis appears to be by the translocation of cytosolic Bax protein to mitochondria.(21) Similar to pinocembrin, another active

component of *A. galanga*, 4'-HCA, induced apoptosis in HL-60 cells, a leukemic cell line and U937 cells, a lyphoma cell line through intrinsic apoptotic pathway.(22) The 4'-HCA was reported to induce apoptosis by increasing reactive oxygen species production lead to endoplasmic reticulum stress pathway.(22) Meanwhile in an animal model system, galangin, a component of *A. galanga* as well, has inhibited the tumor growth by 73.51%±4.742 in Ehrlich ascites carcinoma (EAC) cells-induced Swiss Albino mice with no evidences of mortality as compared to standard drug, 5-fluorouracil. *A. galanga* and its components could possibly selectively induce apoptosis in cancer cells, but not in normal cells.

Conclusion

IC_{50} of ARCE in 24 hours treatment was 620.5 µg/mL, while in 48 hours treatment was 666.6 µg/mL. Hence, ARCE is suggested to have low cytotoxic effect in NIH-3T3 cells.

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