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Potential of a Chalcone Derivate Compound as Cancer Chemoprevention in Breast Cancer

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Abstract. Some flavonoid and terpenoid compounds have been known to have antitumor activity. Chalcone (1.3-difenilpropen-1-on), belonging to the flavonoid family has been studied as therapeutic, especially as antitumor drugs. Most literatures have been indicated that the primary target of these compounds affected the cell cycle. A chalcone derivate, mono para hydroxy chalcone (MPHC A) or 3 - (4'-hydroxy-3'-methoxyphenyl)-1-phenyl-2-propene-1-on was synthesized by reaction between vanillin and acetofenon through cross-aldol condensation reaction under acidic condition. The aims of the research were to investigate the character of anti proliferation and the effect of MPHC A on cell cycle arrest in cancer cell lines T47D. The cytotoxic effect on T47D was analyzed by MTT assay, and its activity as anti proliferation was determined with doubling time method. To determine whether MPHC A influenced the regulation of cell cycle arrest, we used flowcytometry method using propidium iodide staining. This compound had cytotoxic effect on T47D with IC_{50} = 48.306 μM. The doubling time test indicated that MPHC A had activity as anti proliferation on T47D by suppressing viability cell or inhibit its growth. At concentration 48μM, this compound caused cell cycle arrest in G2M phase. Anti proliferation properties of these compounds related to its influence in affecting the cell cycle arrest in G2M phase.

Keywords: Anti proliferation, Cell cycle, and MPHC A.

1. Introduction

As we know that cancer is uncontrolled cell growth, followed by the invasion of surrounding tissue and metastasis to other parts of the body. The main properties of cancer cells are characterized by loss of growth control and development of cancer cells [1]. Some methods of cancer treatment have been attempted at this time, including surgery, radiation, immunotherapy, and chemotherapy. However each treatment has weakness, so the level of healing is still low [2]. These problems stimulate to find a new anti-cancer medicine that has more specific and sensitive [3, 4].

Some strategies to discover new anticancer drugs have been conducted, for example by isolating the active compounds from natural ingredients, seeking anti metabolite compounds to inhibit the growth of cancer cells more specific and sensitive, and synthesizing organic compounds are known to have anticancer activity. Some literature suggests that some of the flavonoid and terpenoid compounds have been known to have antitumor activity [5, 6]. Of which, the compound lycopene, a carotenoid in tomatoes, is able to stimulate the release of cytochrome c from mitochondria in prostate cancer cells [7]. Similarly, flavonoid compounds found in plant extracts of *Semecarpus anacardium* (*Anacardiaceae)* has been shown to cause cells arrest in G2M phase followed by induction of apoptosis in cancer cells T47D[5]. Other studies have shown that flavonoids such as flavonols, anthocyanins, flavan-3-ol, and proanthocyanidin contained in cranberry extract

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could inhibit cell growth through the G1 and G2M arrest and was able to induce apoptosis in breast cancer cells MDA-MB-435 [8].

Chalcone (1.3-diphenilpropene-1-on) is a compound included in flavonoid family and widely studied as a therapeutic, especially as antitumor drugs. Most literatures have been indicated that the primary target of these compounds affected cell cycle [9].

Indyah, *et a*l.[10] succeeded in synthesizing several derivatives chalcone, i.e. mono para hydroxy chalcones (MPHC). Based on activity test of inhibition of non-enzymatic lipid peroxidation and cyclooxygenase, these compounds showed very potent as an antioxidant. In some classes of terpenoid compounds, the activity of anti-inflammatory, antimutagenic and antioxidant is expected to stimulate apoptosis and suppress carcinogenesis caused by chemical triggers [11].

We have conducted cytotoxic tests of some chalcone derivates (MPHC) against several cancer cell lines. Those studies indicated that 3- (4'-hydroxy-3'-methoxyphenyl)-1-phenyl-2-propene-1-on or MPHC A had cytotoxic activity in Raji cells [12], T47D cells, but had not activity against normal cells (Vero) [13]. Therefore, further research needs to evaluate the potential of chalcone derivative compounds, especially MPHC A as a cancer chemoprevention in T47D cells by studying mechanisms of action and molecular target of these compounds. In the present work, we have investigated the character of anti proliferation and the effect of MPHC A on inhibiting cell cycle arrest in cancer cell lines T47D.

2. Material and Methods

2.1. Material

MPHC A used in this research had been synthezed at laboratory of Organic Chemistry and Biochemistry, Faculty of Mathematic and Natural Sciences, Yogyakarta State University. Other material are cancer cell line T47D, Rosewell Park Memorial Institute Medium (RPMI) 1640 (GIBCO BRL), grower medium containing growth factor 10% and 20%, FBS (Fetal Bovine Serum) (Sigma Chem. CO. St. Louis. USA), ethidium bromide, RNA-se, DMSO (dimethylsulfoxide), sodium carbonate (E.Merck), 0.2 μm filter paper, fungizon and antibiotics penicillin and streptromycin (Sigma Chem. CO. St. Louis.USA), HEPES and trypsin (Sigma Chem. CO. St. Louis. USA). PBS (phosphate buffer saline), MTT (3 - (4, 5-dimethyl thiazole-2-yl) -2.5 diphenyl-tetrazolium bromide), SDS (Sodium sulphate duodecyl) 10% in HCl 0.01 N, and propidium iodide (PI).

2.2. Cytotoxic and Doubling Time Assay

Cytotoxic test was performed before doubling time test to determine the IC_{50} values. The test was performed by MTT assay method. Cells with a concentration of 1 x 10^4 cells was distributed into 96 wells plate and incubated for 24 hours. The next day the media was taken and then added with 100 ml culture medium containing 0.2% DMSO (as control) or samples (MPHC A) with concentration 125; 62.5; 31.25; 15.625; and 7.8125 μM respectively, and incubation for 0, 24, 48, and 72 hours. At the end of incubation, the culture medium containing sample was removed, and was washed with 100 μL PBS. Then into each well was added 100 μL of culture medium containing MTT 5 mg / ml, and was incubated again for 4 hours at 37°C. Living cells would react with MTT to form formazan purple crystals. After 4 hours, MTT-containing media was removed, then was added a solution of 10% SDS in 0.01 N HCl to dissolve the formazan crystals.

Cells was distarvasi (fasted) for 24 h in culture medium with containing 0.5% FBS. Furthermore as much as 3 x 10^3 cells were grown on the plate (multiple dishes) with the medium that was contained sample with non-lethal concentrations (under IC_{50} values, i.e. 12, 24 and 48 μM). Sampling was conducted at 0, 24, 48 and 72 hours. The living cell in each well was measured using multiwell scanning spectrophotometers (ELISA reader) at wavelength 595 nm. The absorbance is directly proportional to the number of living cells. The curve between the number of living cell and incubation time indicated how the anti proliferation effect. The difference of doubling time was measured from the slope of log the number of cell versus incubation time curve.

2.3. Inhibition of Cell Cycle Arrest Assay by Flowcytometry

As much as 5 x 10^5 T47D cells/wells were grown on a culture plate 6 wells. After incubation for 24 hours the cells were treated with 48 µM MPHC A (IC_{50}) and without MPHC A as control. Cells were harvested at 24 hours after treatment using trypsin/ EDTA 0.25%/0.02%. Then it was centrifuged at 1000 rpm for 5 minute and washed with PBS cold. Cells were incubated with 500 ml propidium iodide (PI) 50 mg/ml in PBS containing 0.1% triton-X). After that they were treated with RNAse-free DNase (20 mg/ml) for 10 minute at 37^0C, and were analyzed with flowcytometer BDFACSCalybur.

3. Results and Discussion

3.1. Anti Proliferation Activity

The cytotoxic test showed that MPHC A decreased of viability cell (Figure 1) and had IC_{50} = 48.306 µM that was included in the category of very active. Doubling time test was performed on concentration of MPHC A at 0, 12, 24, and 48 µM respectively then incubation for 0, 24, 48, and 72 hours. The results of the test (Figure 2) indicated that there was difference between the numbers of control cells with cells that were treated MPHC A. There was a decrease in the number of cells in T47D cells that were treated MPHC A because the media has changed so that the cells do not grow well. The greater the concentration of MPHC A is added, the greater the reduction in cell number that occurred. Furthermore, the doubling time assay was analyzed by creating a curve which was a correlation between time and the logarithm of the number of cells. The results showed that cells were treated with MPHC A had slopes smaller than that control cells (Table 1). As a whole, treatment with MPHC A showed a negative slope value and smaller than control cells. This proved that MPHC A could inhibit the process of signal transduction in cell proliferation and cell cycle progression.

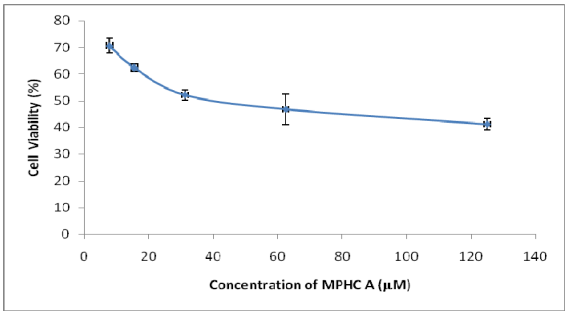

Fig. 1: Effect of MPHC A compound on cell viability of T47D

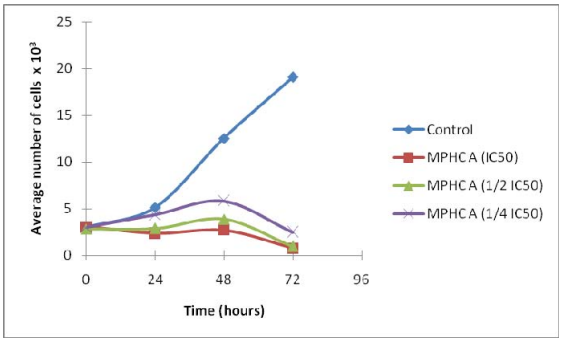

Fig. 2: The growth profile of T47D cells (3X10^3) had been treatment with MPHC A at concentration: 0 µM (Control), 48 µM (IC_{50}); and 24 µM (1/2 IC_{50}), and 12 µM (1/4 IC_{50}). There was a decrease in the number of T47D cells treated by MPHC A

Table 1: Equation of linear regression of log number of T47D cells versus time in various treatments with MPHC A .

Concentration of MPHC A (µM)	Equation of linear Regression	Slope	Doubling Time (Hour)
0 (Control)	y = 0.011x + 3.472	0.001	27.84
12	y = -0.000x + 3.587	0	-
24	y = -0.005x + 3.562	0.005	-
48	y = -0.007x + 3.554	0.007	-

3.2. The influence on Cell Cycle Arrest

Cell cycle analysis using flowcytometer performed to observe the distribution of cells in each phase of the cell cycle. This data is used to support data of cell proliferation test. Analysis of cell cycle based on DNA content in cells. After the cells were incubated with propidium iodide, a red fluorescent of DNA will be captured by the flowcytometer detector. Flowcytometer data of T47D cells without treatment (control cells) and with treatment MPHC A with concentration 48 µM (IC_{50}) are presented in Figure 3 and the morphology of T47D cell are featured in Figure 4.

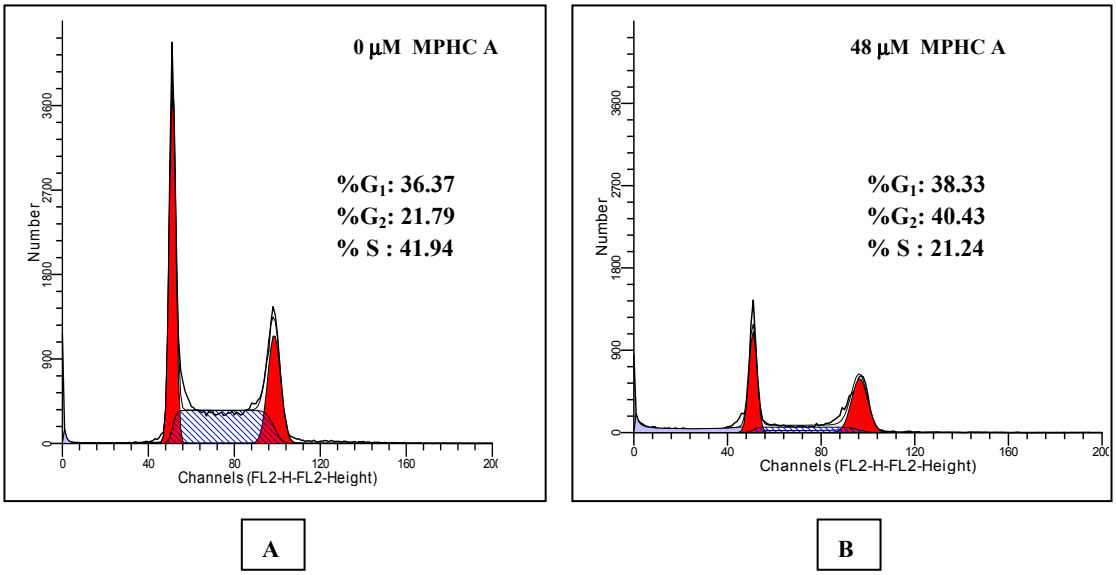

Fig. 3: Cycle cells analyses of T47D had been treatment with MPHC A for 24 h at concentration (A) 0 µM (Control) and (B) 48 µM

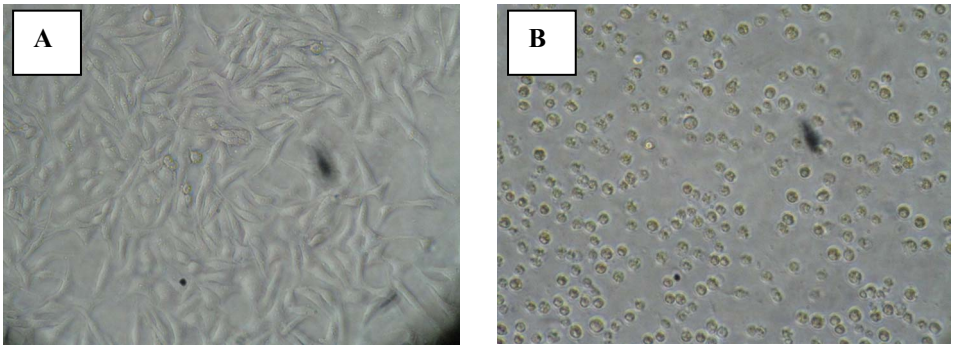

Fig. 4: Morphology of T47D cells had been treatment with MPHC A for 24 h at concentration (A) 0 µM (Control) and (B) 48 µM

Cell proliferation is regulated by molecular checkpoints at each stage of the cell cycle. The cell cycle involves a chain process which resulted in duplication of DNA and cell division. Four major phases in the cell cycle are gap phase 1 (G1), synthesis phase (S), gap phase 2 (G2), and mitosis phase (M). Treatment with MPHC A for 24 hours with concentration 48 µM on T47D cells shows a different distribution pattern compared with the pattern of control cells (Fig. 3). The results show that the treatment with MPHC A influence the cell cycle checkpoint control of T47D by inducing G2 cell cycle arrest. In other words, there was accumulation in the G2M population occurs in T47D cells by treatment with MPHC A, accompanied by a decrease of cells in S phase. Related to the influence of MPHC A induces the G2M phase, it is possible that this compound has the potential to affect apoptosis

4. Conclusion

Based our research, we conclude that MPHC A has activity as anti proliferation against T47D cancer cells lines. This activity related to its influence in affecting the cell cycle by inducing G2M. In further

research, we will evaluate its activity in influence apoptosis mechanism, and protein target that play a role in influence cycle cell.

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