Cell Cycle Analysis of Plectranthus amboinicus, (Lour.) Spreng. Leaves Ethanol Extract Nanoparticles on T47D Cell Lines

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Keywords: Cell Cycle, Plectranthus Amboinicus, Nanoparticle, T47D Cell Line.

Abstract: The development of molecular targeted therapy in cancer is necessary to reduce the occurrence of cell resistance and toxicity towards normal cell. The aim of this study is to evaluate the effect of *Plectranthus amboinicus* (Lour.) Spreng. leaves ethanol extract nanoparticle on T47D cell cycle. The dried leaves powder of *Plectranthus amboinicus* (Lour.) Spreng. was extracted with ethanol by maceration method. Ionic gelation method was implemented for the preparation of *Plectranthus amboinicus* leaves ethanolic extract nanoparticles. The cell cycle of T47D treated with ethanol extract nanoparticle of *Plectranthus amboinicus* (Lour.) Spreng. leaves were analyzed using FACScan flow cytometer. Treatment of PAEEN with IC_{50} concentration, ½ IC_{50} (44.582 µg/mL, and ¼ IC_{50} (22.291 µg/mL) caused cell accumulation at $G_0 – G_1$ phase. At S phase, the percentage of accumulation at ¼ IC_{50} (22.291 µg/mL) higher than control. The study showed that cells underwent apoptosis indicated by occurrence of inhibition of cell cycle on G0-G1 phase and S phase.

1 INTRODUCTION

Several advantages have been achieved for breast cancer treatment, including combination treatment of chemotherapy, antibody therapy and endocrine therapy. However, the resistance of cancer cells being one of a major problem in breast cancer treatment (Lifiani, 2018).

The development of molecular targeted therapy in cancer is necessary to reduce the occurrence of cell resistance and toxicity towards normal cell. Therapeutic targets may involve many proteins and mechanisms, including the inhibition of protein in the signaling process which regulates the growth and the development of cancer cells and of proteins which cause the resistance of cancer treatment (Hasibuan, 2016).

The previous studies have demonstrated the activity of *Plectranthus amboinicus* (Lour.) Spreng. leaves extracts on cancer cell could be due to the inhibition of cell cycle. The study showed that ethylacetate extracts of *Plectranthus amboinicus* (Lour.) Spreng. leaves changed the accumulation of cell cycle phase from G0-G1 phase (54.61%) to sub G1 phase (69.73%) (Hasibuan, 2014). In this study, we aimed to investigate the effect of *Plectranthus amboinicus* (Lour.) Spreng. leaves ethanol extract nanoparticle on T47D cell cycle.

2 METHODS

The extraction was conducted by maceration method. Dried leaves powder of *Plectranthus amboinicus* (Lour.) Spreng. was extracted with ethanol for 3 days at room temperature. The extract then concentrated using rotary evaporator, and was dried by freeze-dryer.

2.1 The Preparation of Nanoparticles of *Plectranthus amboinicus* (Lour.) Spreng. Leaves Ethanolic Extract (PAEEN)

Ionic gelation method was implemented for the preparation of *Plectranthus amboinicus* leaves ethanolic extract nanoparticles. 0.3% PAEEN (*Plectranthus amboinicus* (Lour.) Spreng. leaves ethanolic extract nanoparticles) was diluted in 1.5%

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acetic acid. The sodium tripolyphosphate (1mg/ml) was added to the extract with dropwise under magnetic stirring for an hour. The mixture result of PAEEN nanoparticles was separated by centrifuge at the speed of 15000 rpm for 20 minutes. The pellet was collected and used for characterization (Raj, 2015).

1 gram of the ethanolic extract of *Plectranthus amboinicus* leaves was diluted into 35 mL ethanol p.a, added by 15 mL distilled water, chitosan in 100 mL glacial acetic acid 1% and 350 mL NaTPP solution. Stirred by using magnetic stirrer for ± 2 hours. Then the colloid of nanoparticle chitosan-NaTPP of *Plectranthus amboinicus* leaves ethanolic extract were separated by centrifugation. The result was put in to the freezer (± -4ºC) for ± 2 days. Then, it was moved to refrigerator (± 3°C) to dry. The resulting nanoparticles were characterized using PSA (Particle Size Analyzer). The formed solids are characterized using TEM (Transmission Electron Microscope) to determine the morphological form in its solid form.

2.2 Cell Line and Culture Condition

T47D cell lines were obtained from Parasitology Laboratory, Faculty of Medicine, Gadjah Mada University, Indonesia. The cell line was maintained in RPMI 1,640 suplemented with 10% Foetal Bovine Serum v/v (Gibco). Cells were cultured in the presence of 1% penicillin-streptomycine (Gibco), and 0.5% fungizone (Gibco) and incubated at 37ºC in humidified atmosphere containing 5% CO_2.

2.3 Cell Cycle Analysis

The cell cycle analysis was carried out according to our previous study (Hasibuan, 2014). T47D cells (5×10^5 cells/well) were treated with 89.166 µg/mL (IC_{50} concentration), 44.582 µg/mL (½ IC_{50}), and 22.291 µg/mL (¼ IC_{50}) PAEEN for 24 hrs. Cells then washed, harvest and fixed with 70% ice-cold ethanol. Cells were washed 3 times with ice-cold PBS, resuspended and centrifuged at 3000 rpm for 3 minutes. Cells were treated with RNAse 100 µg/mL containing PI 40 µg/mL incubated at 37°C for 30 minutes. Cell cycle distribution was then were analyzed using FACScan flow cytometer. Data were calculated using ModFit Lt. 3.0.s; (Satria, 2017).

3 RESULTS AND DISCUSSIONS

The cell cycle involves mainly four steps which lead to cell growth and cell division in order to produce 2 daughter cells. The phases are G1, S, G2 and M (Dalimunthe, 2017). Recent study has showed that IC_{50} of PAEEN was 89.166 µg/mL which is potential as anticancer. Next, we explore the mechanism of PAEEN by analyzing cell cycle distribution using propidium iodide staining and flowcytometry. Propidium iodide is a fluorogenic dye wich binds to DNA, allowing the DNA content of the stained cells to be anayzed by flow cytometry. Cells can be classified according to phases of the cell cycle ((G0/G1, S, and G2/M) based on DNA content (Xuereb and Blundell, 2008). Treatment of PAEEN with IC_{50} concentration, ½ IC_{50} (44.582 µg/mL, and ¼ IC_{50} (22.291 µg/mL) caused cell accumulation at $G_0 - G_1$ phase. At S phase, the percentage of accumulation at ¼ IC_{50} (22.291 µg/mL) higher than control which mean that PAEEN could inhibit on S phase as well. During the G1/S phase checkpoint, DNA damage is sensed and the cell cycle is paused until the DNA is thoroughly repaired. This ensure that the S phase is embarked only when the DNA damage accumulated throughout the entire cycle has been eliminated. If the damage is so severe, apoptosis can be induced.
The cell cycle accumulations were showed on Figure 1-4 as follow:

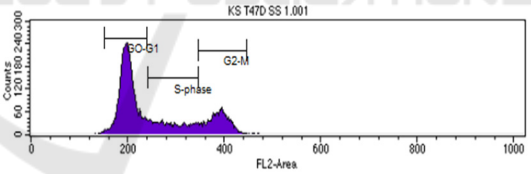

Figure 1: Control cells T47D.

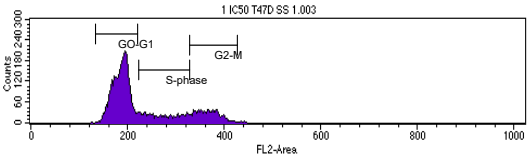

Figure 2: cell cycle inhibition by Plectranthus amboinicus ethnolic extract nanoparticles with IC_{50}.

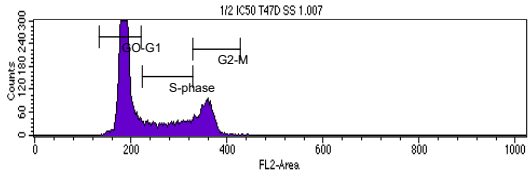

Figure 3: cell cycle inhibition by Plectranthus amboinicus ethnolic extract nanoparticles with ½ IC_{50} .

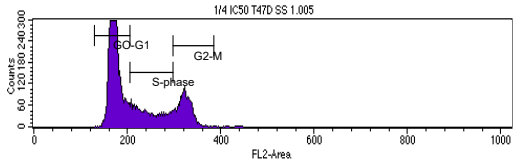

Figure 4: cell cycle inhibition by *Plectranthus amboinicus* ethnolic extract nanoparticles with ¼ IC_{50}.

The percentage of cell accumulation on every phase showed that there is a cell cycle arrest on G0-G1 phase because the percentage was higher than control as shown on table 1.

Table 1: The percentage of cell accumulation.

Treatments	concentrations	Phase (%)		
		$G_0 – G_1$	S	G_2 - M
Control		59.07	18.93	21.36
PAEEN	IC_{50}	63.92	17.30	18.32
	½ IC_{50}	66.89	16.19	16.74
	¼ IC_{50}	60.94	20.46	19.05

We have previously reported that PAEEN can prevent proliferation and induce apoptosis on T47D breast cancer cell lines. On this study, we showed that cells underwent apoptosis indicated by occurrence of inhibition of cell cycle on G0-G1 phase and S phase. Cell cycle control is deregulated in cancer cells in spite of defects in their genome, they can easily pass cell cycle protein such as cyclin D1 and drive the cycle to proliferation without any interruption (Xuereb and Blundell, 2008).

4 CONCLUSIONS

PAEEN could inhibit the cell cycle T47D cell lines at G0-G1 phase and S phase. The data provide the promising candidate for further studies.

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