ANTICANCER EFFECT OF AFRICAN LEAVES (*VERNONIA AMYGDALINA* DEL.) TO T47D CELL RESISTANT

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ABSTRACT

Objective: African leaves *(Vernonia amygdalina* Del) have medical effect to threat many kind of diseases, and recently, it is suggested have anticancer effect. Therefore, this study is held to testing anticancer activity of extract African leaves to T47D cell resistant.

Method: Cytotoxic activity was determined *in vitro* used 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide method. Analysis of cell cycle and apoptosis were determined with flow cytometry method.

Results: Examination cytotoxicity of extract n-hexana African leaves (ENDA), extract ethyl acetate African leaves (EEADA), and extract ethanol African leaves (EEDA) to T47D cell were showed with IC_{50} values 164.85±1.88 µg/mL for ENDA; 55.50±0.79 µg/mL for EEADA and 311.72±4.15 µg/mL for EEDA. Therefore, EEADA has the most active activity as anticancer. Moleculer evolution assay produced T47D cell resistant. Examination toxicity of EEADA to T47D-cell resistant showed IC_{50} values 59.19±0.55 µg/mL. EEADA might able inhibit cell cycle at G0-G1 phase and increased apoptosis of T47D cell resistant.

Conclusion: This study shows that EEDA can be developed as cochemotheraphy to breast cancer with inhibits cel cycle and increase apoptosis.

Keywords: African leaves, T47D, Resistant, Cytotoxic, 3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide, Molecular evolution assay, Cell cycle, Apoptosis, Flow cytometry.

INTRODUCTION

Cancer is also described as a disease caused by a damage of cell-cycle regulation [1]. Breast cancer is cancer that commonly occurred, especially in women. Data from American cancer society, in 2013; there were 64.640 cases of breast cancer. Approximately 39.620 females were died every year because of breast cancer [2].

Breast cancer occurs when breast cells start to grow uncontrollably. These cells can invade nearby tissues and spread throughout the body [3]. Several advantages have been achieved for breast cancer treatment, including combinatorial treatment of chemotherapy and therapy with antibody and endocrine therapy [4]. Doxorubicin is one of chemotherapy agents that effective to breast cancer [5]; however, chemoresistance is still a major obstacle in breast cancer treatment [4].

Research into plants with anticancer effects is still encouraged with a view to discover any new drugs with less toxic but more potent effects [6]. The study was carried out to investigate the activity of ethyl acetate extract of Zanthoxylum acanthopodium DC., fruit (EEZ) against doxorubicin-resistance T47D cells suggest that EEZ can use for chemoresistance cancer cell [7].

One of the plant materials that uses as an alternative to treat cancer is African leaves (*Vernonia amygdalina* Del.), which use as medical plant to treat various dissease and has efficacy such as antibacterial, antifungal, antiparasitic, antimalaria, antivirus, anticancer, antidiabetic, and antioxidant [8]. The purpose of this study was to investigate the activity of extract African leaves (*V. amygdalina* Del.) against T47D cell resistant.

MATERIALS AND METHODS

Materials

Fresh and old African leaves were taken from Tangkul street, Siduarjo Hillir village, Medan Tembung district, North sumatra, Indonesia. African leaves was identified in Research Centre for Biology, Indonesian Institute of Science, Bogor. *n*-hexane, ethyl acetate and ethanol were purchased from Merck (Darmstadt, Germany), Doxorubicin (Ebewe), DMSO (Sigma), [3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl tetrazolium bromide] (MTT) (Sigma), RPMI media and Phosphate Buffer Saline (FBS) 10% v/v (Gibco, Grand Island, NY, USA).

Preparation of extraction

African leaves were dried in 45°C temperature and powdered. Simplicia powder of African leaves (500 g) was extracted using n-hexana with maceration method. After 3 days of maceration in room temperature, maserati was transferred using rotatory evaporator, dried with freeze dryer [9-13]. Ethyl acetate and ethanol solvent used to maceration subsequently with the same way.

Cytotoxic examination

T47D cell was planted on 96 well-plate with density $1×10^4$/wells in RPMI growth media, maximal volume was 0.1 mL per wells. Incubation was done in incubator at 37°C in 5% CO_2 for 24 h to get well growth. Afterward, media change was done with addition extract n-hexana African leaves (ENDA), extract ethyl acetate African leaves (EEADA), and extract ethanol African leaves (EEDA)/doxorubicyn solution in various concentration. After 24 h incubation, media was changed with 100 µL MTT (sigma) with concentration 5 mg/mL. Cell was incubated again for 4–6 h in incubator at 37°C in 5% CO_2. MTT reaction was stopped with stopper reagent (SDS 10% in HCL 0.01 N), plate was wrapped to avoid light penetration and was silenced in one night. Data of absorbance were changed to percentage of surviving cell and analyzed with SPSS 17 [14]. The lowest IC_{50} values of extract was chosen to testing for cell cycle and apoptosis.

Molecular evolution assay

T47D cell was exposed with doxorubicin 0.02 g/mL for 72 h followed media turnover until 80% cell confluent and was done with six times

repetition of same exposure [15]. Cytotoxic examination was done after second, fourth, and sixth exposure.

Examination of cell cycle and apoptosis

Examination of cell cycle and apoptosis of T47D cell resistant used flow cytometry method. The number of cell that needed was $5×10^5$ cell/wells which was planted in 6-well-plate with RPMI media, then incubated for 24 h. The next day, cell was added with ENDA/EEADA/EEDA solution, and then incubated again for 24 h. After that media in each wells was inserted to each 15 mL conical tube, washed with PBS for one times. Tripsin 250 µL was added and incubated for 3 min at 37°C, adding 1 mL culture media then media was accommodated in the same konikel tube. Centrifugation was done with 600 rpm for 5 min and supernatan was excluded from the study. PBS 1 mL was added, and media were transferred to microtube 1.5 mL, and centrifugated with 2000 rpm for 3 min, after that supranatant was excluded from the study. Examination cell cycle with adding propidium iodide, while apoptosis with adding Annexin V and propidium iodide and then was measured with flow cytometer [16].

RESULT AND DISCUSSION

This study was aimed to know anticancer activity of African leaves extract to breast cancer cell. Cytotoxic trial was an initial parameter to know potential toxicity of experiment material to cancer cell that manifest with IC_{50} parameter. This study used ENDA, EEADA, and EEDA that examined to T47D cell. The purpose of this extract study to obtain which extract was most active and later used as material in advance trial of anticancer activity.

The result of cytotoxic examination extract of African leaves to T47D cell shows IC_{50} values 164.85±1.88 µg/mL for ENDA; 55.50±0.79 µg/mL for EEADA and 311.72±4.15 for EEDA, where at this concentration, each of extract was able to inhibit 50% T47D cell growth. The potential of an extract as anticancer if has IC_{50} value ≤100 µg/mL [17,18]. IC_{50} value from these three extract show EEADA with lowest IC_{50} value and EEDA with highest IC_{50} value. Therefore, EEADA has the most active activity as anti-cancer. In this study, EEADA was chosen to the next anticancer clinical trial.

The main problem associated with the failure of cancer treatment was resistance to chemotherapy agent. At least 30% female who diagnosed with early-stage breast cancer will develop and recurrence to advance stage or metastatic cancer [19]. According that fact, this study was necessary to resolve the resistance and obtain the better outcome of cancer treatment. Regiment of chemotherapy agent clinically administered in sequence in several treatment cycle [20]. Molecular evolution assay was an *in vitro* method as aplication of clinically chemotherapy treatment. T47D cell was exposed with doxorubicin with same concentration repetitively, accompanied with a time of recovery after the exposure. This procedure was done six times [15].

Parameter that used to evaluate T47D cell result from MEA which has been resistant was increasing IC_{50} value of doxorubicin to T47D cell from MEA. In this study, cytotoxic examination of doxorubicin was done to T47D cell before exposed to MEA (R0), second expose (R2), fourth (R4), and sixth (R6). Cytotoxic effect of doxorubicin with concentration serial of 5 µg/mL; 2.5 µg/mL; 1.25 µg/mL; 0.625 µg/mL, and 0.3125 µg/mL to T47D from MEA inversely proportional to experiment compound. The greater of experiment compound then the smaller percentage of T47D that survive. The cell calculation outcomes then analyzed with SPSS 17 and obtained IC_{50} value. IC_{50} value of doxorubicin (R0) was 0.20±0.02 µg mL, forth exposure (R4) was 2.41±0.04 µg/mL, and sixth exposure (R6) was 2.59±0.02 µg/mL (Fig. 1). Results of this study show increasing of IC_{50} value of doxorubicin on T47D cell from MEA compared to IC_{50} value T47D cell (R0) before exposed to MEA and can be concluded that T47D cell from MEA was T47D that has been resistant to doxorubicin.

The next step was examination toxicity of EEADA to T47D cell resistant, and the result was compared to IC_{50} value of EEADA to T47D cell

nonresistant to identified effect administration of EEADA to T47D cell which has been resistant. The value of IC_{50} for EEADA to T47D cell was 55.50±0.79 µg/mL and to T47D cell resistant was 59.19±0.55 µg/mL. There was increasing IC_{50} value of EEADA to T47D resistant but still able to consider as an anticancer because these extracts have potential to inhibit cancer cell growth if IC_{50} value ≤100 µg/mL [17,18].

Characteristic toxicity of EEADA to T47D cell because there was flavonoid compound that able to inhibit cancer cell proliferation [21], triterpenoid/steroid that able induced apoptosis [22] and saponin that able to identified cancer cell and altered permeability membrane of cancer cell [23].

The one way to resolve resistance of chemotherapy was inhibiting activity of P-gp. The content of flavonoid that lied in EEADA may able inhibit P-gp. The plant that contain flavonoid was ideal inhibitor for P-gp because typically not toxic and the safety was guaranteed generationally for long time. Flavonoid from essential material was considered as P-gp inhibitor that able to increase drugs bioavaibility, penetration to the tissue, and decreasing drug excretion [24-26]. Other than direct interaction with P-gp, flavonoid may able decrease gen MDR (multidrug resistance) expression [27]. The content of triterpenoid/steroid in EEADA has capability to block Nuclear Factor-kappa B (NF-κB) where excess expression of P-gp was regulated by transcription factor of NF-κB [28].

The further examination to identified capability of EEADA to increasing cell cancer death with modulating cell cycle programs with flow cytometry methods. Examination of cell cycle was done to sel T47D resistance. The outcome of flow cytometry EEADA to T47D resistance (Fig. 2) was compared to controls (Fig. 3).

Data were analyzed descriptively with compared between exposed and control cell resistant. The greatest cell accumulation was held in G0-G1 phase from EEADA exposure to T47D cell resistant in value 46.18% compared to control was 40.62%, that can be considered mechanism of inhibition cell cycle in cell T47D resistant that expose with EEADA in G0-G1 phase. Higher percentage that compared to control show that the cell experience inhibition in preparation DNA materials that will be synthesized, other than that it gives chance to repair the damaged DNA and if this mechanism was failed, this process will be continued by cell apoptosis. Therefore, regulation process of cell cycle plays roles in preventing of cancer [29].

Apoptosis examination was done to T47D cell resistant with flow cytometry method. The result of flow cytometry of EEADA to T47D cell resistant (Fig. 4) compared to controls (Fig. 5).

The T47D cell resistant that exposed with EEADA has apoptosis cell percentage greater 4.47% than controls 3.37% and cell percentage that experienced apoptosis at the end was greater 12.05% than control 6.34%. It shows that mechanism of cell death with administration EEADA may able increase cell apoptosis and decrease necrotic cell.
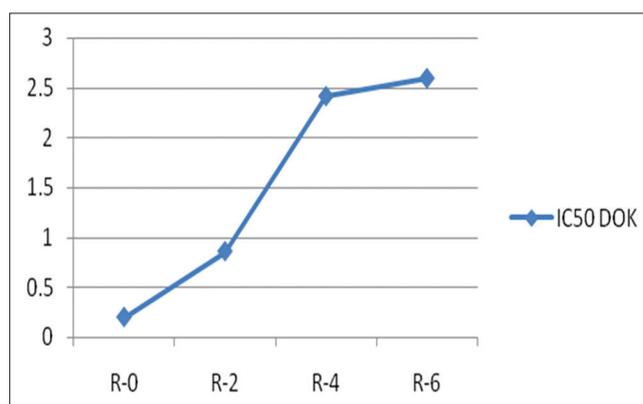

Fig. 1: Increasing IC_{50} value doxorubicin MEA chart

The capability of EEADA is to increase cancer cell death, inhibit cel cycle, and induced cell apoptosis. It is caused by flavonoid and triterpenoid/steroid that have efficacy as anticancer agents [30].
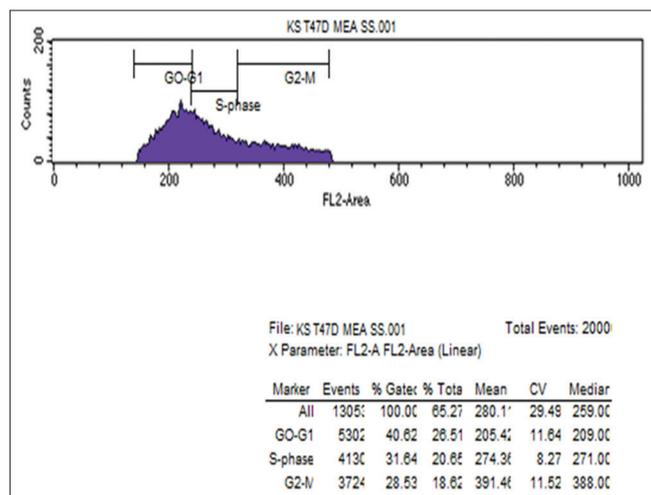

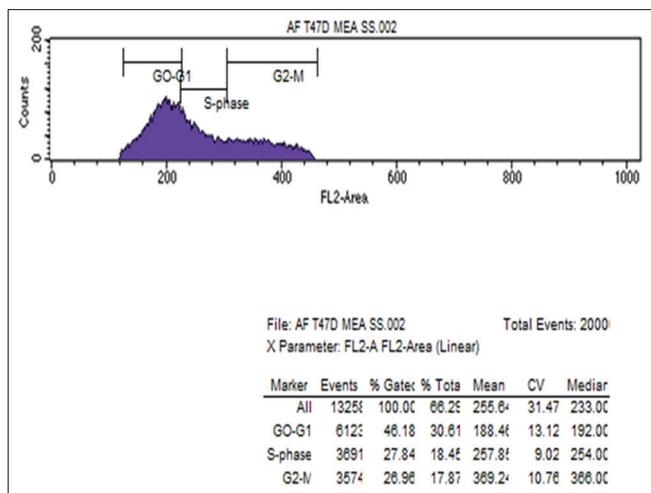Fig. 2: Illustration cell cycle in T47D cell resistance controls

Fig. 3: Illustration cell cycle in T47D cell resistance that exposed to extract ethyl acetate African leaves
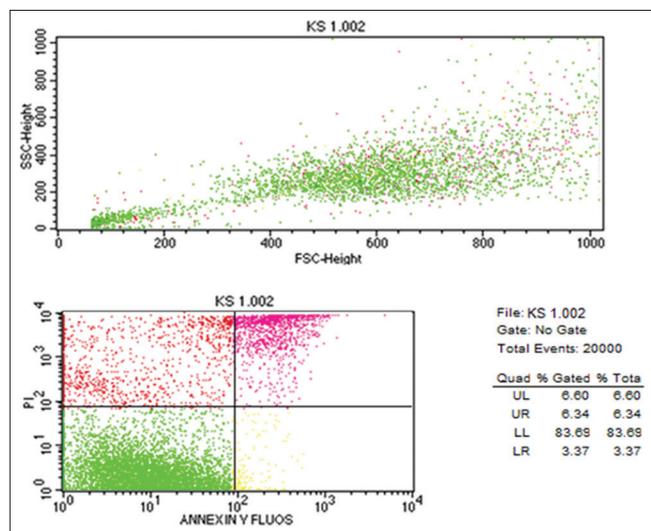

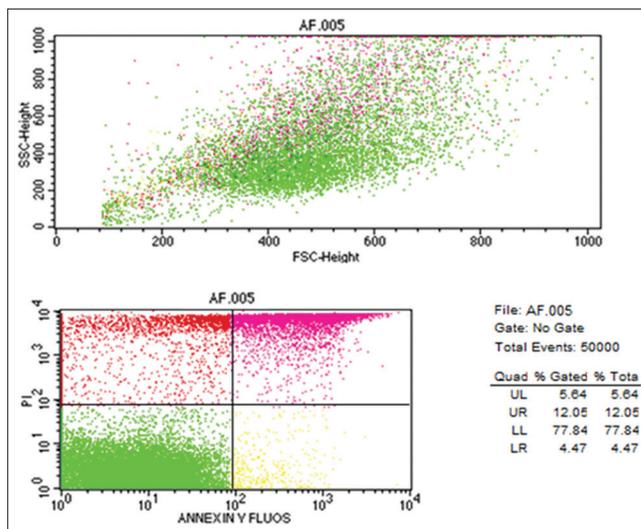Fig. 4: Illustration percentage control condition of sel T47D resistant

Fig. 5 Illustration percentage condition of sel T47D resistant that exposed with extract ethyl acetate African leaves

CONCLUSION

The result of this study show that EEADA was the most potent as anticancer agent to T47D cell compared to ENDA and EEDA and have toxic effect to T47D cell resistant. The capability of EEADA to inhibit T47D cancer cell resistant growths with inhibiting cell cycle in G0-G1 phase and able to induce apoptosis.

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