Anticancer Activity of Bruceine A Isolated from The Seeds of Brucea javanica on Hela Cell

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Abstract— **Brucea javanica seeds has been used traditionally to treat cancer, even though the scientific base of this has not been completely investigated. Bruceine A from Brucea javanica exhibit biological activities as a antimalarial, antitumor, antiviral and antiamoebic activities. This study is to examine the anticancer activity of Bruceine A from Brucea javanica seeds include cytotoxic activity and induced p53 protein expression on Hela cell. Cytotoxicity test of Bruceine A from Brucea javanica seeds has been performed by counting method of the cell directly with trypan blue exclusion dye. Analysis of induced p53 protein expression on Hela cell was detected by using immunocytochemistry technique. Cell that stained positive p53 protein showed that there was nucleus or cytoplasm stained with brown color, while the one stained negative p53 protein would be purple. From cytotoxicity test results of Bruceine A from Bruceine javanica seeds against Hela cell, value IC_{50} obtained as big as 76.4 μg/ml. From observation of p53 protein expression on Hela cells by immunocytochemistry staining, it can be seen that Bruceine A from Brucea javanica seeds can increase the expression of p53 protein Hela cells by 44.3%. From the result of this study, it can be concluded that Bruceine A from Brucea javanica seeds have anticancer activity through inhibition mechanism of Hela cell proliferation, by activating the tumor suppressor gene such as p53.**

Keywords—Brucein A; Brucea javanica; anticancer; cytotoxic; p53 protein expression (key words)

I. INTRODUCTION

Brucea javanica belongs to the family of Simaroubaceae is a shrub which is distributed in Souteast Asia including Indonesia [1]. The bioactive component of quassinoid from Brucea javanica has an interesting biological effect [2]. There were javanicosides, flazin, bruceocides, bruceantine, bruceine, brusatol and terpeniod blumenol A. Some with glycone or aglycone derivates. The mode of action based on inhibition of protein synthesis ([2],[3]). These quassinoid were reported for thheir potential to induce human promyelocytic leukemia (HL-60) cell differentiationand to inhibit cyclooxygenase-1 (COX-1), cyclooxygenase-2 (COX-2) and 7,12 dimethyl benz[a]antracence (DMBA)-induced lessions in a mouse mammary organ culture model. Bruceantinols and bruceines have been shown to posses as antibabesial activity with IC_{50} at 5 ng/mL [4]. Bruceantine and brusatol have antileukemic effects induced down-regulate c-myc protein levels [5]. Some quassinoid have also been found to exhibit antiplasmodial activity againts Plasmodium falciparum [6]. In this study was conducted to evaluate cytotoxic activity and induced p53 protein expression of Bruceine A isolated from the seeds of Brucea javanica on Hela cell.

II. METHODOLOGY

This study is a purely experimental study with *by post-test only control group design* approach.

The seeds of Brucea javanica fruits were purchased and collected from Merapi Farma Herbal & Co., Yogyakarta, Indonesia. This sample was identified by Department of Biology, Faculty of Pharmacy UGM, Yogyakarta.

The cytotoxic activity was tested by assessing their inhibition of growth of cells derived from human cancer [7]. The cell lines tested was Hela cell. Cells are adapted for growth in RPMI 1640 media supplemented with 10% bovine serum and 60 mg/ml amikacin and maintened 150 cm^2 flasks at 37ºC and 5% CO_2. Into 96 *micro plate* wells , contains 100 µl experimental cells with a density of 2 x 10^4 cells / *well,* added 100µl of Bruceine A at various concentrations levels (1000, 500, 250, 100, 50, 10µg / ml) in triplicate. As a control, culture media considered to have growth of 100% is used. Culture contained the experiment material was incubated in an incubator with 5% CO_2 stream at 37 ºC for 24 hours. At the end of incubation, each well calculated the number of living cells by using *trypan blue*. Cytotoxic activity was analyzed by probit analysis, determined the value of IC_{50} concentration required to inhibit tumor cells up to 50%.

Detection of p53 protein on Hela cell is done using immunohistochemistry test. Cells stained positive p53 protein showed that there was nucleus or cytoplasm with brown color.

III. RESULT AND DISCUSSION

A. Bruceine A cytotoxicity on Hela Cells

In this cytotoxicity test, method of counting cells directly using the aid of trypan blue staining was used, which is based the principle that the dead cell has lost its membrane integrity, so that trypan blue can enter the cell. Thus, the dead cell will absorb the blue color, while the living cell does not absorb blue [8] . The average number of living Hela cells after Bruceine A addition at various concentrations is seen in Table I. From the Table I can be seen that the higher the concentration, the greater Bruceine A percentage of Hela cell's death. In this cytotoxicity test, parameter value IC_{50} is determined. IC_{50} value indicates the toxicity potential of a compound against Hela cell. The smaller the value of IC_{50} means that the compound is more toxic. Toxicity potential of Bruceine A against Hela cell shown by IC_{50} value of 76.4 µg / ml.

B. Analysis of p53 protein Expression on Hela Cells

Results of p53 protein detection by immunocytochemistry, data were obtained on the number of p53 protein expression per 100 cells as presented in Table II. The Table II shows the dispose of expression increase of p53 protein in the treated group compared to the control. The picture of p53 protein expression in Hela cells control and treatment with Bruceine A can be seen in Fig. 1and 2.

In the Fig.1 and 2 above can be seen that most of the Hela cells control showed positive expression of p53 protein. Thus in Hela cells by adding Bruceine A concentration of 10µg/ml an increase in the expression of p53 protein positive is seen. This case indicates that the Hela cell's death through the mechanism of proliferate inhibition, by activating the *tumor suppressor gene* such as p53. Any stress or DNA damage can stimulate the expression of p53 protein in Hela cells [9].

In Hela cells control or Bruceine A treatment, most of p53 protein expression is located at the cell nucleus, although some located at the cytoplasm (Table III). The existence of positive expression of p53 protein in the cytoplasm shows that inhibition of Hela cell growth occurs in the G1 phase of the cell cycle. Quassinoid Bruceine A is estimated can inhibit cell division in the G1 phase of the cell cycle by increasing the expression of p53 protein in the cytoplasm. According to [9], p53 gene uses its function during the G1 phase of the cell cycle. Most of the p53 gene acts as: '*the guardian of the genome*', i.e. : (1) level of p53 rapidly increased in response to DNA damage, (2) lead to inhibition of *cell cycle* during the G1 phase, (3) provide time for cells to repair DNA damage, (4) if the damage cannot be repaired, p53 will induce *programmed cell death* (apoptosis).

In this study, an antibody that can detect both *wild-type* p53 protein and mutant in cancer cells is used. So in this study the positive p53 protein expression indicated with brown color in the nucleus or cytoplasm that cannot be distinguished between the expression of p53 from *wild* or mutant type.

Both wild-type p53 protein *(wild type)* and mutant migrated in the cell nucleus are known as the *Nuclear Localization Signals* (NLS) attached to their primary sequence [10]. According to [11] and [12], wild-type p53 causes growth inhibition in G1 phase, so that means in order cell to enter S phase, p53 should be inactivated..

Overall, it can be concluded that quassinoid Brucein A can cause death to Hela cells. The Hela cell's death mechanism is through the mechanism of inhibition of Hela cell proliferation, as evidenced by an increase in p53 protein expression in Hela cells. The expression of the p53 protein cannot be distinguished whether *wild* or mutant type p53, but seeing the expression of p53 protein in the cytoplasm shows that inhibition of Hela cell proliferation through *cell cycle progression* inhibition that occurs in G1 phase. This provides an opportunity for the genes that control DNA repair to repair the DNA damage that exists.

TABLE I. THE AVERAGE NUMBER OF LIVING HELA CELLS AND HELA CELL'S DEATH PERCENTAGE AFTER BRUCEINE A ADDITION AT VARIOUS CONCENTRATIONS

Cons. (µg / ml)	Number of living cells x 10^4								% Mortality
	Bruceine A				Control				
	I	II	III	Average	I	II	III	Average	
1000	7.5	7.5	6.0	7.0	45.0	46.5	43.5	45.0	84.4
500	14.0	15.0	12.0	13.5	43.5	64.5	45.0	51.0	73.5
250	17.0	18.0	15.0	16.5	45.0	48.0	42.0	45.0	63.3
100	18.0	19.5	19.5	19.0	46.5	37.5	39.0	41.0	53.7
5 0	29.0	30.0	28.5	29.0	51.0	52.5	55.5	53.0	45.2
10	38.0	36.0	37.5	37.0	63.0	63.0	52.5	59.5	37.8

TABLE III. EXPRESSION OF P53 PROTEIN ON HELA CELLS CONTROL AND TREATMENT BRUCEIN A CONCENTRATION OF 10 µG / ML PER 100 CELLS

Replication	Expression of p53 Protein Hela Cells			
	Control		Bruceine A	
	Positive	Negative	Positive	Negative
I	29.0	74.0	43.0	62.0
II	19.0	83.0	50.0	56.0
III	17.0	85.0	49.0	60.0
Total	65.0	242.0	142.0	178.0
Percentage (%)	21.1	78.8	44.3	55.6

TABLE III. LOCATION POSITIVE P53 PROTEIN EXPRESSION IN HELA CELLS CONTROL AND BRUCEIN A TREATMENT

Location of p53 positive	**Control**					**Brucein A**				
	I	II	III	Total	%	I	II	III	Total	%
Nucleus	23.0	12.0	15.0	50.0	92.6	30.0	35.0	41.0	106.0	89.1
Cytoplasm	1.0	2.0	1.0	4.0	7.4	5.0	4.0	4.0	13.0	10.9

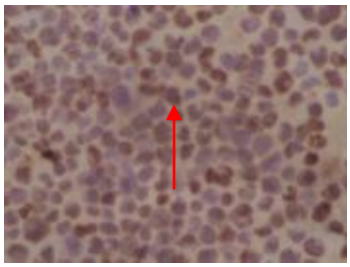

Fig. 1. Microscopic picture of Hela cells control with immunocytochemistry staining (magnification 400x).

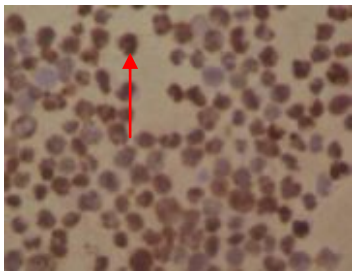

Fig. 2. Microscopic picture of Hela cells after Bruceine A treatment with immunocytochemistry staining (magnification 400x).

IV. CONCLUSION

In conclusion that Bruceine A from Brucea javanica seeds have anticancer activity through inhibition mechanism of Hela cell proliferation, by activating the tumor suppressor gene such as p53. Anticancer activity test toward Hela cells showed the IC_{50} value of Bruceine A was 76.4 µg/ml. Bruceine A can stimulate the expression of p53 protein in Hela cells by 44.3%.

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