Cytotoxic Effect of Etanolic Extract of *Ageratum conyzoides*, L Against HeLa Cell Line

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Abstract

In developed countries, cancer represents the second death cause after cardiovascular disease. In Indonesia, cervix cancer represents the most cancer case in women. Continuous efforts to find the effective cancer drugs with less side effects are still being conducted. One of the ways for getting that purpose is by digging the source of nature vegetation owning potency as anticancer. The aim of this research was to investigate anticancer activity from Ageratum conyzoides*, L leaves. The method used was cytotoxicity assay to HeLa cell line, and the cytotoxicity was determined by cell viability method. The number of viable and nonviable cell was counted by direct counting method using trypan blue exclusion. The cytotoxicity test revealed that the LC_{50} value was 855 µg/ml.*

Key words : Ageratum conyzoides*, L, cytotoxicity, direct counting, HeLa cell line*

INTRODUCTION

Cancer is one of the world's deadliest diseases. World Health Organization (WHO) data presents as many as 13 percent of the 58 million deaths, or 7.6 million people died were caused by cancer in 2005. Cancer incidence in Indonesia is estimated at 1 to 10 per 100,000 people per year. With current methods of treatment, half of the patients were helped with surgery and radiation therapy. Healing exclusively occurs almost in patients whose disease has not spread at the time of surgery. Early diagnosis can improve the healing of 50% cancer cases.[1] Theoretically, a cancer drug can be generated, but the current drugs are not qualified yet, especially in terms of effectiveness and safety. Recent studies are conducted to gain selective cancer drugs which do not disturb normal cells. In general, anti-neoplastics suppress the growth and proliferation both normal and cancer cells. At surgery and radiation, it can not be avoided that healthy tissue will go wasted or illuminated. The new paradigm, back to nature, supports the development of traditional medicine and exploration of natural compounds as a source of modern medicine. *Ageratum conyzoides*, L is one of the nutritious plants as medicines and easily obtainable in Indonesia. Empirically this plant is used as a drug for fever, sore throat, malaria, pneumonia, uterine bleeding, diarrhea, dysentery, ear inflammation, preventing pregnancy, diuretic and cervix cancer.[2] Preliminary research showed that the methanol extract of *Ageratum conyzoides*, L herbs have no cytotoxic effect on myeloma cells, shown by the LC_{50} value of 3292.46 µg/ml.[3] Therefore, it is needed to conduct such studies on other cancer cells.

The aim of this research is to determine the cytotoxic effect of ethanolic extract against HeLa cell line.

METHODS

Ageratum conyzoides L. leaves were washed, dried (not in direct sunlight), crushed and sieved to obtain a fine powder which subsequently extracted with Soxhlet. The obtained extract was evaporated to obtain a viscous extract. The viscous extract was dissolved into ethanol then added by medium and shaken till homogenous. Extract solution obtained was filtered using sterile membrane filter and was used for preparing sample solution by being added by cell suspension at cencentration series of 2 mg/ml; 1 mg/ml; 0,5 mg/ml; 0,25 mg/ml; 0,125 mg/ml; 0,0625 mg/ml; 0,0312 mg/ml dan 0,016 mg/ml.

One hundred ml of HeLa cell suspension were distributed into the 96 micro plate wells, added by 100 ml of each concentration series of extract. For control group, 100 ml cell suspension and 100 ml of blank media and 200 ml cell suspension with no media were used. Furthermore, the plate was incubated for 24 h at 37°C. After that each sinks was resuspended, then added by 50 ml of trypan blue, where the dead cells will appear cloudy blue. Counting of the number of living cells was conducted using a hemocytometer by inverted microscope. The number of dead cells was calculated by subtracting the number of living cells in control with the number of living cells in treatment group of a given sample (see equation below).[4]

The LC_{50} value was calculated using probit analysis from percentage of death cell value. Linear regression equation was derived from logarithm of concentration series and probit value, and the LC_{50} value was represented by probit value of 5.

RESULT

From this study, it was obtained that the living cell average number in control group is 81.5.

$$\text{Percentage of death cell} = \frac{\sum \text{living cells in control} - \sum \text{living cells in treatment}}{\sum \text{living cells in control}}$$

DISCUSSION

The result of this study shows that the higher the sample concentration is, the greater the HeLa cell death becomes, which is indicated by the small number of living cells on treatment. The smallest concentration of sample, which was 0.016 mg/ml, resulted in the lowest death cell percentage, i.e. 23.93%. Then, the increasing of sample concentration resulted in the increasing of death cell percentage. The highest percent of death cell by 57.06% was caused by the administration of sample concentration of 2 mg/ml. These results indicated that *Ageratum conyzoides,* L leaves ethanolic extracts could induce cell death of HeLa cell lines. Visually, cell death was shown by the formation of cloudy blue color in the cell nucleus of HeLa cell lines due to the cell membrane lysis, so that the solution of trypan blue entered the cell nucleus.

The LC_{50} value of 855 µg/ml obtained was the sample concentration that could kill 50% of HeLa cell lines. The smaller the sample concentration that can kill 50% of the cell, the more toxic the compound.

Previous study conducted by Kuswandi M *et al* showed that chloroform and gubal extract of *Ageratum conyzoides,* L leaves had LC_{50} value of 16.33 µg/ml and 20.70 µg/ml against myeloma cells respectively.[5] Ethanolic leaves extract can be considered to have more potential toxicity. In addition, the ethanolic leaves extract of *Erytrina fusca* Lour had LC_{50} value of 14 µg/ml against HeLa cell lines,[6] while the ethanolic extract of *Erytrina fusca* Lour seeds had LC_{50} value of 1350µg/ml.[7] This means, *Ageratum conyzoides,* L leaves extract (LC_{50}= 855 µg/ml) is relatively less toxic to HeLa cell line compared to *Erytrina fusca* Lour

Table 1. Correlation between concentration series of sample and percentage death of cells

Concentration (mg/ml)	Number of living cell		% death	Probit number
	I	II		
0.016	61	63	23.93	4.29
0.031	59	54	30.67	4.50
0.063	53	51	36.20	4.64
0.125	50	49	39.26	4.72
0.25	46	46	43.56	4.85
0.5	43	45	46.01	4.90
1	44	42	47.24	4.92
2	37	33	57.06	5.18

leaves extract, but relatively more toxic compared to *Erytrina fusca* Lour seed extract. National Cancer Institute (NCI) states that if a compound generates LC_{50} values <20 µg/ml, the compound is potential to be developed as an anticancer agent.[8] The LC_{50} (855 µg/ml) obtained from this study was bigger than what NCI stated, so it did not have chance to be developed as an anticancer agent espe-cially against HeLa cell lines.

CONCLUSION

Ethanolic extract of *Ageratum conyzoides*, L leaves shown no cytotoxicity activity against HeLa cell lines indicated by LC_{50} value of 855 µg/ml.

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