The Selective Cytotoxicity of Ethanolic Extract of *Annona muricata* Leaf on HeLa Cervical Cancer Cells

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Abstract

Cancer is one of threatening diseases that is now increasing in prevalence. The problem in managing this disease is not only due to its expensive cost of therapy but also the side effect following the use of non selective chemotherapeutic agent. This research aimed to evaluate the potential cytotoxic activity of ethanolic extract of Annona muricata *leaves against HeLa cervical cancer cell and its selectivity by comparing the effect on HeLa and Vero cells line. The cytotoxic activity of the extract was evaluated on HeLa and Vero cell line using MTT assay method. The cytotoxic effect of the extract was confirmed by observing the dead cells using Ethidium Bromide-Acridin Double staining method. The 50% inhibition of cell growth (IC_{50}) value of the extract for HeLa cervical cancer cell line was 97 µg/ml and the value for Vero Cell line was 356 µg/ml. Dead cells observed using double staining method for HeLa cells treated with IC_{50} concentration of the extract confirmed the cytotoxic activity of the compound. This cytotoxic effect was not observed in Vero cell treated with the same concentration of the extract. This result indicated the potential of selective anticancer activity of ethanolic extract of* Annona muricata *leaves.*

Key words: Annona muricata*, leaf, Ethanolic extract, HeLa, Vero.*

INTRODUCTION

The incidence of cancer among Indonesian society creates a serious health problem (BPPK, 2008). The burden of the disease emerge as in fact the majority of patients contracting the disease can not afford the high cost of therapy. In addition, the side effect of currently available chemotherapy is rising another problem in the management of the disease. The use of herbal medicine as an alternative way in combating malignancy is quite common in Indonesian community. As a tropical country, Indonesia is rich of plants that have been known empiricaly to possess therapeutic activity in many kind of diseases. *Sirsak*, the local name of *Annona muricata,* is one of easily found plants that is already used in folk remedy in treating cancer. People usualy drink the infusat of the leaves to lessen the symptom of cancer (Sunarjono, 2005; Mao, *et al*., 2011). Moreover, sirsak leaf extract has been available in store now.

The research on anticancer activity of *Annona muricata* has been done worldwide. The active compound of this plant has been figured out. Acetogenin, the anti cancer compound of this plant work through inhibition of NADH production of cancer cells (Taylor, 2002; Pardhasaradhi *et al*, 2005; Liu, *et al*., 2007). Although the activity of this isolate has been proven, the purification process to isolate the compound will end up with the classic problem

of unaffordable cost of therapy for most patient with cancer in Indonesian country. Taking into account to this problem, we are questioning wether the crude extract of the plant can also work as cytotoxic agent effectively. Research in evaluating the anticancer activity of the crude extract of *Annona muricata* leaf is therefore required.

In this research we investigated wether the ethanolic extract of *Annona muricata* leaves show potential cytotoxic activity against cancer cell proliferation. The selective activity of the extract was also evaluated by comparing the antiproliferative effect on HeLa and Vero cell line.

MATERIALS AND METHODS

The *Annona muricata* leaves was obtained from trees grow on around Surakarta. The extraction was conducted using maceration and evaporation method in 70% ethanol. The extract was dissolved in DMSO (dimethyl sulfoxide) (Sigma Chemical Co.,USA) to prepare a stock solution of 5 mg/ml.

The HeLa and Vero cell line used in this research was courtesy of Parasitology Laboratory of Medical Faculty of Gajah Mada University. Cells were cultured in RPMI. MTT assay to investigate the cytotoxyc activity of the extract was conducted for HeLa and Vero cell line according to the standardized protocol. We used serial concentration of the extract (10µg/ml, 20µg/ml, 30µg/ml, 50µg/ml, 100µg/ml, 150µg/ml, 200µg/ml, 300µg/ml, and 500µg/ml). The IC50 value of the extract was calculated with the following formula: $IC_{50} = (X2 - X1) \times (50 - Y1) / (Y2 - Y1)) + X1$, where X1 and X2 are the higher and lower used concentrations, respectively, that borders the concentration that reduces the global cell growth of 50% and Y1 and Y2 are the mean percentages of viable cells at the X1 and X2 concentrations, respectively (Mathieu, *et al.*, 2008).

The cytotoxic activity of the extract was then visualized by double staining using Etidium Bromide-Acridin orange method. For this purpose, HeLa and Vero Cell line was cultured and treated with the IC_{50} concentration of extract.

RESULTS AND DISCUSSION

MTT Assay

The cytotoxicity of ethanolic extract of *Annona muricata* leaf on HeLa and Vero cells is shown in figure 1. The IC_{50} of the extract against HeLa cells was 97 µg/ml, while the value against Vero Cell line was 356 µg/ml.

Etidium Bromide-Acridin Orange Double Staining

The cytotoxic effect of the extract was visualized in figure 2 and 3. The dead cells were stained as orange while the viable cells were stained green. The figure indicate the cytotoxic effect of the extract on HeLa cells. On the other hand, the activity was not observed in Vero cells line.

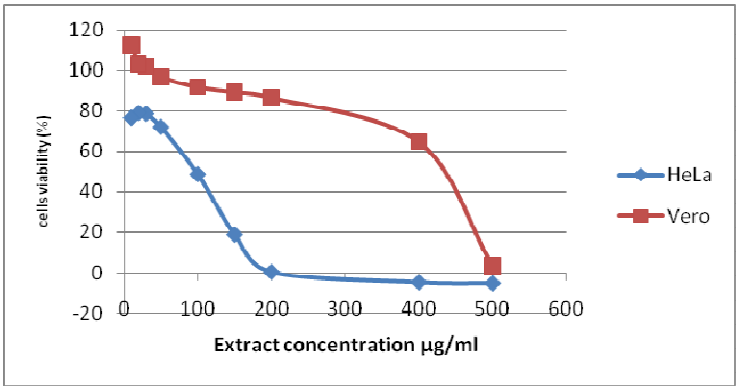

Figure 1. The cytotoxic effect of ethanolic extract of *Annona muricata* leaf was shown. The extract showed its cytotoxic effect on HeLa cells, but this effect was not observed on Vero cells.

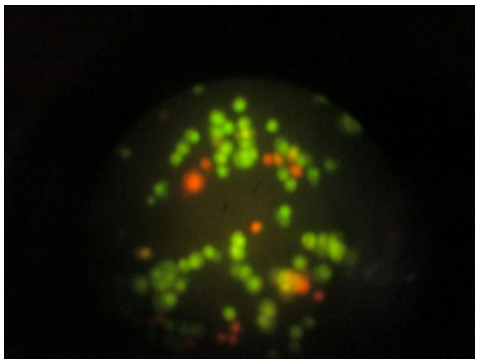

Figure 2. Double staining of HeLa cell treated with IC_{50} concentration of extract (97 µg/ml). The orange stained cells are the dead cells, while the green stain cells alive.

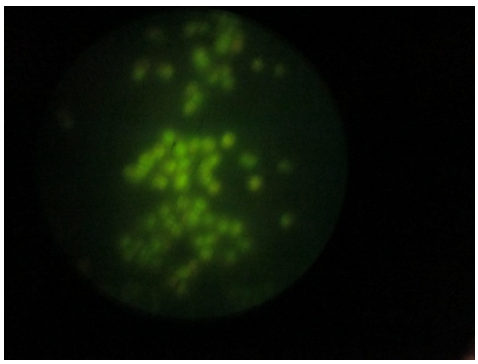

Figure 3. Double staining of HeLa cell treated with IC_{50} concentration of extract. All cells staines green, no dead cells observed in Vero cells treated with the extract.

Discussion

Acetogenin is the active compound of *Annona muricata* leaf that kills the cancer cell through inhibition of NADH production. Previous studies has revealed a potent cytotoxic activity of this isolate on cancer cells line. In this research we observed the cytotoxic activity of the crude extract of *Annona muricata* leaf (Liu, *et al*., 2007; McLaughlin, 2008). Although the IC_{50} value calculated for the extract was quite high compare to the IC_{50} value of acetogenin isolate found in other research, this finding indicated a possibility to use crude extract as an alternative therapy for cervical cancer.

The cytotoxic selectivity of the extract was also shown in our data. We calculate a high IC_{50} value of the extract (356 µg/ml) against Vero cells proliferation. The microscopic evaluation of the dead cells treated with 97 µg/ml of extract using ethidium bromide-acridin double staining method showed numerous dead cells of HeLa cells but none of Vero cells. This data confirmed the selectivity of the extract against cancer cell proliferation.

CONCLUSION

Although the anticancer activity of ethanolic extract of *Annona muricata* leaf in this research was lower than the acetogenin isolate, result of this research bring a hope to develop anticancer regimen based on simple standardized extraction. More research need to be done to optimized the quality of extract and to evaluate the anticancer activity in vivo.

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