Induction of Apoptosis in Human Breast Cancer (MCF7) Cells by n-Hexane Extract of Plectranthus amboinicus (Lour.) Spreng.

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Abstract

The n-hexane extract of Plectranthus amboinicus, (Lour.) Spreng. reduced the proliferation of MCF7 cells. The present study was carried out to evaluate the effect of the extract on human breast cancer cells viability and apoptosis. To detect apoptotic cells, MCF7 cells were stained with etydium bromide-acrydine orange (double staining method). Quantitative detectin of apoptotic cells was performed by fluorescens microscope. The growth of MCF7 was inhibited by treatment with n-hexane extract of Plectranthus amboinicus, (Lour.) Spreng. The cell death induced by n-hexane extract Plectranthus amboinicus, (Lour.) Spreng. Was characterized by orange fluorescent. The percentage of cell death (apoptosis), necrosis and viable cell consecutively 39.237%; 12.543%; and 48.229%.

The result suggest that n-hexane extract of Plectranthus amboinicus, (Lour.) Spreng. may play the role in tumor growth suppression by inducing apoptosis in human breast cancer cells.

Keywords: Plectranthus amboinicus, (Lour.) Spreng. apoptosis, MCF7

Background

Cancer is one of the major causes of death in developed countries, together with cardiac and cardiovascular diseases. radioactive rays and most anticancer drugs damage DNA or suppress DNA duplication to kill tumor cells growing rapidly. At the same time, they also affect normal cells to cause serious adverse effects, such as bone marrow function inhibition, nausea, vomiting, and alopecia (Ueda, et al., 2002).

Indonesia has many medicinal resources. One of that is ‘daun bangun-bangun’ Plectranthus amboinicus, (Lour.) Spreng. which have been used as lactagogue and inflammation. The recent study was done to examine the inhibitory effect of Plectranthus amboinicus, (Lour.) Spreng. leaves n-hexane extract on proliferating breast cancer cell, MCF7. To determine the selectivity of its activities, the antiproliferative activities againts MCF7 have examined and showed potent antiproliferative activities based on IC_{50} value. This activity was concluded to be due to the induction of apoptosis, based on characteristic morphological changes and DNA fragmentation. The n-hexane extract of Plectranthus amboinicus, (Lour.) Spreng. reduced the
proliferation of MCF7 cells. The present study was carried out to evaluate the effect of the extract on human breast cancer cells viability and apoptosis.

Material and Methods

- Detection of treatment-induced apoptosis
to assess the degree of treatment-induced apoptosis and or necrosis in MCF7 cells, the cells were exposed to *Plectranthus amboinicus*, (Lour.) Spreng. extracts and cultured. Apoptosis was determined by acridine orange/ethidium bromide nuclear stain. Quantitative detection of apoptotic cells was performed by fluorescens microscope.
- Acridine orange/ethidium bromide
briefly, 10 µL of the reaction mixture (1:1 acridine orange-ethidium bromide) was added to 250 µL of cell suspension. This was kept in the dark for 20 minutes, after which about 5 µL was dispensed onto microscope slides and examined under a fluorescence microscope. Detection of apoptosis was based on morphological and fluorescent characteristics of the stained cells. Viable cells were indicated by bright green, apoptotic cells by orange/brown, and necrotic cells by red. Quantitative assessment were made by determining the percentage of apoptotic cells by counting in five to seven fields of view.

Results and Discussion

The growth of MCF7 was inhibited by treatment with *n*-hexane extract of *Plectranthus amboinicus*, (Lour.) Spreng. As shown on the Figure 1, the cell death induced by *n*-hexane extract of *Plectranthus amboinicus*, (Lour.) Spreng. was characterized by orange fluorescent, meanwhile the viable cells were characterized by bright green fluorescent. The percentage of cell death (apoptosis), necrosis and viable cell consecutively 39.237%; 12.543%; and 48.229%. The apoptotic induction effect of *Plectranthus amboinicus*, (Lour.) Spreng. was suspected by the secondary metabolite contain in the *n*-hexane extract such as steroid or triterpenoids. The study before showed that ursolic acid isolated from many traditional medicine herbs could inhibit proliferation and induced apoptosis of colon carcinoma cells by activating caspase 3 and caspase 9 and suppressed the EGFR (epidermal growth factor hormon) phosphorylation, by MAPK (mitogen-activated protein kinase) pathway (Shan, et al., 2009). One of the secondary metabolite contained in The *Plectranthus amboinicus*, (Lour.) Spreng. is ursolic acid (Kaliappan dan Viswanathan, 2010). There might be the positive correlation between the ursolic acid contained in the *n*-hexane extract with the effect of induction. It can be suspected that ursolic acid dissolved in *n*-hexane could induced apoptotic of the MCF7 cells line, but it still need further study. The morphological of the MCF7 cell could be see on Figure 1. As shown on Figure 1, the *n*-hexane extract of *Plectranthus amboinicus*, (Lour.) Spreng. could induce apoptosis characterized by orange fluorescent, but it is not much enough comparing with the viable cells. But, the extract could induced apoptosis, indeed.
Figure 1 The morphological of the MCF7 cells before and after treatment by n-hexane extract of *Plectranthus amboinicus*, (Lour.) Spreng. And stained by etydium bromide-acrydine orange

**Conclusion**

The result suggest that n-hexane extract of *Plectranthus amboinicus*, (Lour.) Spreng. may play the role in tumor growth suppression by inducing apoptosis in human breast cancer cells.

**References:**


