Anti-Cancer and Anti-Proliferation Activity of Ethyl Asetat Extract From Ant Nest (Myrmecodia pendans) in Burkitt’s Lymphoma Cancer Cells

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Abstract

Objective: To determine the activity of anti-cancer and anti-proliferation of ethyl acetate fraction of ant nest plants (Myrmecodia pendans) in Burkitt’s Lymphoma cancer cells. Material and Methods: The study was conducted in a pure laboratory experimental method using Burkitt’s Lymphoma cancer cell culture. Gradual research begins with the determination, extraction and fractionation of ant nest plants, to test for proliferation barriers. Data analysis using two-way ANOVA followed by Post Hoc LSD test with a significance level of 95%. Pearson correlation test was conducted. Results: The results of testing the inhibition of Burkitt’s Lymphoma cell proliferation with ethyl acetate extract treatment showed that there was inhibition of cell growth based on the concentration given, starting from the lowest concentration of 15.625 µg/mL. Likewise, the incubation time factor of 24, 48, and 72 hours showed that the longer the incubation time, the greater the inhibition of cell growth. Antiproliferation analysis of flavonoid ethyl acetate extract based on concentration and incubation time on absorption of optical density Burkitt’s Lymphoma was statistically significant (p = 0.00). Conclusion: Ant nest ethyl acetate extract has the effect of proliferation inhibition on Burkitt’s lymphoma cells.

Keywords: Neoplasms; Burkitt Lymphoma; Cell Proliferation; Plant Preparations.
Introduction

Cancer is a deadly disease and cause of death in industrialized countries and the second cause of death in developing countries. Cancer is a non-communicable disease, which is characterized by abnormal/persistent, and uncontrolled cell growth, which can damage the surrounding tissues and can spread to places far from their origin called metastasis. According to WHO data in 2013, the incidence of cancer increased from 12.7 million cases in 2008 to 14.1 million cases in 2012, with the number of deaths increasing from 7.6 million people in 2008 to 8.2 million in 2012 [1,2].

Cancer that can cause enlarged lymph nodes is called lymphoma. Lymphoma is a general term for various types of blood cancers that appear in the lymphatic system. According to the 2012 GLOBOCAN (IARC) data, lymphoma is one of the ten most cancers in the world in 2012. Lymphoma is divided into two types, namely Hodgkin's Lymphoma and Non-Hodgkin's Lymphoma. About 90% of lymphoma patients are patients with Non-Hodgkin Lymphoma, and the rest are Hodgkin's Lymphoma [3].

Non-Hodgkin's lymphoma occurs due to mutations that occur in the immune system caused by infectious agents, carcinogenic substances and a history of other diseases suffered by a person [4]. Burkitt's Lymphoma is a type of Non-Hodgkin Lymphoma. Burkitt's Lymphoma is a mature neoplasm of B lymphocyte cells and includes aggressive lymphoma. Burkitt's Lymphoma is a malignancy of B-lymphocytes that can be cured and is first known to be associated with HIV.

Burkitt's Lymphoma is also one of the lymphoid tumors characterized by chromosomal translocation, especially translocation on the MYC, which is characteristic of Burkitt Lymphoma. Burkitt's Lymphoma plays an important role in the mechanism of carcinogenesis and lymphomagenesis. Several studies have found that Burkitt's Lymphoma is most likely derived from B-lymphocytes in the germinal flashlight (GC) [5].

Clinically, Burkitt's Lymphoma generally occurs in children. The incidence occurs at the age of 3 to 8 years, where men are more susceptible to 2x than women. Lesions are commonly found in the maxilla, mandible and abdomen. The most typical sign in the oral cavity of this disease is the presence of local tumors and tooth mobility. Symptoms are localized, blunt and paresthesia pain. Clinical symptoms of Burkitt's Lymphoma are initially only recognized by enlarged lymph nodes without pain and grow rapidly on the neck, thighs, under the jaw or it can also be under the hand. In the sporadic type, a lump begins in the central or abdominal area [6].

The results of the study have shown that ant nest plants are one of the medicinal plants that are believed to have potential effects in the world of health. Although modern therapies such as chemotherapy give positive results in the treatment of cancer, on the other hand many cause side effects. Therefore, herbal treatment is often a cancer treatment option. In addition to the low cost, the side effects produced are also minimal compared to modern therapies [7,8].

Chemical screening tests for ant nest plants indicate that these plants contain flavonoid and tannin class chemicals. Based on the results of this research, this plant also contains active compounds of tocopherols, phenols, and is rich in various useful minerals [9,10]. Many working
mechanisms of flavonoids have been revealed, such as inactivation of carcinogens, antiproliferation, cell cycle inhibition, apoptosis induction and differentiation, and inhibition of angiogenesis [11].

Flavonoids in the human body function as antioxidants so it is very good for cancer prevention. Flavonoids have a carbon base frame consisting of 15 carbon atoms, where two benzene rings (C6) are bound to a propane chain (C3) so that they form a C6-C3-C6 arrangement. This arrangement can produce three types of structures, namely 1,3-diarylpropane or flavonoid, 1,2-diarylpropane or isoflavonoids, and 1,1-diarylpropane or neoflavonoids [12]. In some cases, flavonoids can act directly as antibiotics by disrupting the function of microorganisms such as bacteria or viruses.

In vivo research shows that the influence of flavonoid diets on cancer development is: First, it inhibits protein kinase activity. Before the enzyme activity is excessive, the role of flavonoids is needed to prevent the formation of cancer cells, namely by preventing the joining of carcinogen compounds generated by the kinase enzyme with DNA, so that the DNA does not experience damage (cancer). Second, have anti-proliferation activity. Third, it induces apoptosis. Now, only a few potential anti-cancer agents such as flavonoids are known to cause apoptosis. Fourth, it inhibits metastasis/migration/angiogenesis [13,14].

This study was intended to analyze and identify the effect of flavonoid ethyl extract on ant nests (Myrmecodia pendans) as an anticancer against the inhibition of proliferation in Burkitt’s Lymphoma cells.

Material and Methods

Research on anti-cancer activity and anti-proliferation of ant nest plants (Myrmecodia pendans) was carried out in April - June 2018. This research was a pure laboratory experimental study using Burkitt’s Lymphoma cancer cell culture conducted at the Integrated Research and Testing Laboratory (LPPT) Gadjah Mada University in March - April 2018, and at an Integrated Research Laboratory, Faculty of Dentistry, Gadjah Mada University, Yogyakarta, in May - June 2018.

This study uses raw materials for ant nest plants (Myrmecodya pendans) obtained and imported from the Papua region and then processed in the LPPT laboratory to get the fraction of ant nest extracts needed for research.

The procedure of this study is through two stages. The first phase of the study consisted of 6 phases. The first phase is the determination of ant nests. This procedure aims to find out the type of ant nest used and to avoid errors in the plants used in the research test. The second phase is extraction. Extraction was carried out by maceration to obtain ant nest ethyl acetate extract. Ant nests obtained from Papua are cleaned from dirt. Then chopped into small pieces and dried in the open air. Bulbs that have been dried are ground into simplicia powder. The simplicia powder of the ant nest is put into the maserator whose bottom has been coated with cotton. Then into the maserator ethyl acetate - water was added with a ratio of 9: 1. The maceration process was allowed to
stand for 24 hours, while stirring occasionally. After 24 hours, the mass is removed and accommodated. All the results of the solvent collection are mixed and then the extraction process is concentrated using a rotary evaporator until all the solvents evaporate and a concentrated ethyl acetate extract is obtained.

The next phase is fractionation, which is the process of separating compounds based on the level of polarity they have. Next, activation and breeding of Burkitt’s Lymphoma cancer cells are performed. In the second phase of the study carried out proliferation resistance test with MTT Assay.

Proliferation inhibition testing with flavonoid treatment of ethyl acetate fraction was carried out based on absorbance data of viable living cells in the measurement of optical density 550 nm ELISA reader. The results of measurements of cell proliferation inhibition by flavonoid treatment of ethyl acetate fraction were then carried out by making a relations table of the relative relative number of Burkitt’s Lymphoma cell counts with concentrations, ie from 500, 250, 125, 62.5, 31.25, and 15,625 µg/mL and control cell.

Ethical Aspects

Ethical clearance conducted at the Gadjah Mada University Faculty of Dentistry Ethics and Advocacy Unit No.00761/KKEP/FKG-UGM/EC 2016.

Data Analysis

Data analysis using two-way ANOVA followed by Post Hoc LSD (Least Significant Difference) test with a significance level of 95%. Pearson correlation test was conducted to see a strong relations between variables. Statistical analysis was carried out using IBM SPSS Statistics for Windows Software, version 21 (IBM Corp., Armonk, NY, USA).

Results

The results showed a significant relations between how much concentration of flavonoid ethyl acetate extract from ant nest plants, incubation time, and how many cells that can survive after being treated with anthyl acetate extracts of ant nests in certain concentrations and times. There was a significant decrease in the number of Burkitt’s Lymphoma cells after being treated. For example, at 24 hours of incubation time, the concentration of ethyl acetate extract of ant nests was 15,625µg/mL, while the number of cells that survived was less than 60 cells. The number of cells that survive will decrease with increasing ethyl acetate concentration (Table 1).

Based on the results obtained (Figure 1), it is seen that in general flavonoid ethyl acetate extract has Burkitt’s Lymphoma cell growth inhibitory activity. This is shown in cell measurements using an ELISA reader.

The effect of giving some of the larger concentrations of growth inhibition was seen compared to the control. The greater the sample concentration, the smaller the number of living
cells. Growth barriers were clearly seen at a concentration of 500 µg/mL, by inhibiting Burkitt’s Lymphoma cell activity.

Figure 1. Absorbance data for measurement of Burkitt’s Lymphoma cancer cells with ethyl acetate extract treatment in ELISA reader within 24, 48, and 72 hours.

Table 1. Absorbance data measurement of Burkitt’s Lymphoma cancer cells with ethyl acetate extract treatment on ELISA reader and calculation of standard deviation.

| Time (Hours) | 0   | 15.625 | 31.25 | 62.50 | 125  | 250  | 500  |
|--------------|-----|--------|-------|-------|------|------|------|
| 24           | 1.397 | 0.854 | 0.854 | 0.801 | 0.792 | 0.773 | 0.636 |
| 48           | 1.452 | 0.804 | 0.840 | 0.795 | 0.780 | 0.742 | 0.682 |
| 72           | 1.369 | 0.839 | 0.830 | 0.751 | 0.751 | 0.756 | 0.643 |
| SD           | 0.09857 | 0.01552 | 0.02107 | 0.02730 | 0.02500 | 0.02565 | 0.02478 |

Discussion

The treatment of flavonoids ethyl acetate extract on Burkitt’s Lymphoma cells based on the given concentration showed a decrease in the number of cells seen starting from a concentration of 15,625 µg/mL to a concentration of 500 µg/mL, which means that there is an inhibition of cell growth. However, flavonoid treatment of ethyl acetate extract on Burkitt’s Lymphoma cells based on incubation time was the opposite, namely an increase in the number of cells from 24 hours to 48 hours until the 72nd hour. This increase in cell numbers is not as high as control cell growth. In the graph, a flavonoid treatment of ethyl acetate extract, a concentration of 500 µg/mL based on the incubation time appeared to begin with an increase in the number of cells from 0.76 at 24 hours to
0.92 at 48 hours. But in the 72nd hour there was a decrease in the number of cells to 0.81, which means cells experience saturation. This death may occur through an arrest mechanism, namely cell cycle arrest that usually occurs in the G1/S phase.

The results of this study are in line with previous research that ant nests have anti-cancer activity. This study has been tested through cancer cells obtained from cervical cancer and breast cancer, called HeLa cells and MCM-B2. The results showed that ant nest extracts can inhibit the growth of cancer cells themselves. This effect is obtained from phenol compounds, especially flavonoids. Based on several studies, there are molecular targets that can cause antiproliferation of flavonoids, namely inhibition of the Akt signal and NF-κB. Akt plays an important role in the regulation of cell cycle defense and proliferation of cancer cells by affecting excessive phosphorylation status of Akt, so that the signal blockade causes growth inhibition with the termination of the cell cycle and apoptosis of cancer cells [11,15,16].

The results of this study confirmed that flavonoid ethyl acetate extract of ant nest can inhibit Burkitt’s Lymphoma cell proliferation supported theoretically, that flavonoids can inhibit the overall performance of cyclin dependent kinase (Cdk) which is a cell cycle regulator. The working point of flavonoids lies in the work resistance of Cdk-activating kinase (CAK), which inhibits the formation of an active Cdk-cyclin complex. Flavonoids can bind to protein kinase at the ATP-binding site. Check points on G1/S and in G2/M are interrupted by the presence of flavonoids that inhibit the signal transduction process from growth factors. Flavonoids are able to inactivate proteins that play a role in signal transduction, for example tyrosine kinase. These statements explain the possibility of cell cycle arrest induced by the role of flavonoids [15,17].

Research also shows that quercetin, a flavonoid compound, can increase cyclic AMP levels and can also reduce DNA, RNA, and protein synthesis in tumor cells Ehrlich ascites. Quercetin was also found to inhibit aerobic glycosis in tumor cells. Quercetin can also inhibit the activity of specific protein kinases – tyrosine, which play an important role in malignant fibroblast transformation in sarcoma cells. Enzyme inhibitory activity by flavonoids is an anti-proliferative effect on malignant cells, for example in cases of gastritis in humans and colon cancer cells, found inhibition of cancer cell growth by quercetin [18].

**Conclusion**

Ant nest ethyl acetate extract has anti-cancer activity and anti-proliferation in Burkitt’s Lymphoma Cells. Ethyl acetate extract at a concentration of 15.625 mg/mL to 500 pg/mL showed an inhibitory effect Burkitt’s Lymphoma Cell growth. Large concentrations of the extract and the duration of incubation determines the inhibition of Burkitt’s Lymphoma Cell growth.

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