RESISTIVITY OF PROTEIN KINASE-B (AKT), NF-κB TRANSDUCTION OBSTACLES, AND APOPTOSIS INDUCTION (CASPACE -3, -9) AS ANTI-PROLIFERATION AND ANTI-CANCER OF BURKITT’S LYMPHOMA USING FLAVONOID FRACTION OF ETHYL ACETATE FROM ANT NEST (MYRMECODIA PENDANS)

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ABSTRACT

Introduction: Burkitt’s lymphoma (LB) is a tumor with high malignancy and rapid cell growth and originates from B-cell lymphoma. LB is usually found in children in endemic areas with dengue fever and HIV-AIDS with low socioeconomic levels. Objective: This research aims to analyze resistivity of protein kinase-B (Akt), NF-κB transduction obstacles, and apoptosis induction (Caspase -3, -9) as anti-proliferation and anti-cancer of burkitt’s lymphoma using flavonoid fraction of ethyl acetate from ant nest (Myrmecodia pendans). 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: Resistivity of protein expression of protein kinase-B (Akt), transcription factor of nuclear factor-kappa B (NF-κB), and apoptosis induction (Caspase -3,-9) showed increased protein expression was significantly obstacles and prove that the ethyl acetate fraction flavonoid inhibits translocation and activation of transcription pathway NF-κB and growth factors that induces the phosphorylation of Akt signal transduction pathway, and apoptosis induction (Caspase -3,-9). Conclusion: Flavonoid fraction of ethyl acetate from ant nest (Myrmecodia pendans) resistivity of protein kinase-B (Akt), NF-κB transduction obstacles, and apoptosis induction (Caspase -3,-9) as anti-proliferation and anti-cancer of burkitt's lymphoma.

Keywords: Ant nest, Burkitt’s lymphoma, Flavonoid fraction of ethyl acetate, protein kinase-B (Akt), NF-κB, Apoptosis
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 or 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

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. 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.2,3 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.4

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.5 Chemical screening tests for ant nest plants indicate that these plants contain flavonoid and tannin class chemicals. Many working mechanisms of flavonoids have been revealed, such as inactivation of carcinogens, antiproliferation, cell cycle inhibition apoptosis, induction Akt’s signal activity, and NF-κB transduction obstacles.6

Signal transduction is a process which begins by the activation of receptors located in the membrane (trans-membrane receptor) by the molecular signals from outside the cell, in turn will trigger a molecule in the cells secrete a particular response. The Akt protein, also known as protein kinase B (PKB) is one of the signal transduction protein kinase that
plays a key role in several cellular processes, such as glucose metabolism, cell proliferation, apoptosis, transcription and cell migration.7

The Akt proteins bind either PIP3 (phosphatidylinositol (3,4,5)-trisphosphate) or PIP2 (phosphatidylinositol (3,4) bisphosphate). This is useful for controlling a mobile signal for dephosphorylation phosphoinositide (PIP2) only phosphorylated by the enzyme family of PI3-kinase (phosphoinositide 3-kinase or PIK3) and after receiving chemical signals, then inform the cell to begin the process of growth. The PI3 kinase can be activated by G protein-coupled receptor pairs of tyrosine kinase receptors, such as insulin receptor. Once activated, the PI3-kinase phosphorylating PIP2 form PIP3-kinase (phosphoinositide 3-kinase or PI3K).8

Protein NF-κB is one member of the family transcription factors, which is important in the regulatory gene expression associated with biological functions as well as the immune response and inflammatory, growth and cell proliferation, as well as defense cell to stress (UV rays, irradiation, oxidants and DNA damage). The mechanism of NF-κB activation (Protein rail and p50) begins when the activation factor stuck to the receptor. After the signal in the receptor, IKK protein (Inhibitory kappa beta kinase) will phosphorylate IκB” protein. While the NF-κB (Protein rail and p50) will be to the cell nucleus for gene transcription.9

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.10 Now, only a few potential anti-proliferation and anti-cancer agents such as flavonoids are known to cause apoptosis. This study was intended to analyze and identify the resistivity of protein kinase-B (Akt), NF-κB transduction obstacles, and apoptosis induction (caspase -3, -9) as anti-proliferation and anti-cancer of cell burkitt's lymphoma using of flavonoid ethyl extract on ant nests (Myrmecodia pendans).
MATERIAL AND METHODS

This study is a purely experimental laboratory study with a post-test control group design (control group post-test only design). Burkitt’s lymphoma cells (Raji) were obtained from the Department of Parasitology, Faculty of Medicine, Gadjah Mada University, with the characteristics of ATCC CCL-86 B lymphocyte cells (United States). Cells and cell cultures, Burkitt’s lymphoma cells (Raji) were cultured in Dulbecco's modified eagle medium (DMEM, Sigma-Aldrich, United States) which were added with 10% serum fetal calf (FCS, Moregate BioTech, Australia), 100 mg/ml streptomycin, and 100 units/ml penicillin (Invitrogen Corp., United States). LB cells were incubated in 95% humidity and 5% CO₂ at 37°C.

Apoptotic induction test was carried out using AO-EB staining, stock solution of Ethidium Bromide-Acridine Orange made from 50 mg of Ethidium Bromide plus 15 mg of Acridine Orange and dissolved in 1 ml 95% ethanol and 49 ml distilled water. Furthermore, from the stock solution 1 ml of solution was taken and diluted in phosphate buffer saline (PBS, Nacalai, Japan) in a ratio of 1:100. Microplate 24 wells were prepared and glass cover was placed on each well. Raji cells (2 x 10⁴ cells/well) from the incubator are cultured in wells containing DMEM 10% fetal calf serum (FCS). Then the cells were incubated for 24 hours at 37°C and 5% CO₂.

After incubation, the media was pipetted and in each well a new 10% FBS DMEM was added which had various concentrations of Doxetaxel hydrate. Cells were incubated for 48 hours. After that the cover glass at the bottom of the well is taken and placed on the slide according to the dosage label of the Doxetaxel hydrate listed. All samples were stained with a 10 µl ethidium bromide-acridine orange combination solution by dripping the solution on a glass cover for 20 minutes. All preparations were then observed using a fluorescent microscope at 40x magnification. The living cells appear bright green, while the yellow-red cells indicate cells that undergo apoptosis. Apoptotic cells were measured by a standard procedure manual (unit:%).

Methods for barriers cell proliferation (MTT assay) are testing the proliferation barriers, put up 3 fruit plate contains 24 wells, testing MTT assay with MTT, 3-(4,5 dimethylthiazol-2-yl)-2,5-diphenyl-tetrazolium bromide (Sigma-Aldrich, USA) on 0, 24,
48 and 72 hours. Then on each plate insert tongue cancer cells SP-C1 as much as $2 \times 10^4$ cells/wells in 100 mL of DMEM (Sigma-Aldrich, USA) according to the concentration of flavonoid compounds. Based on the calculation of the total number of cells required is $128 \times 10^5$ cells for all the wells and the amount of solution DMEM (Sigma-Aldrich, USA) is needed as much as 256 mL. The calculation of the number of cancer cells is determined using a hemocytometer. All cells are then incubated at a temperature of 37EC for 24 hours. Plate 24 wells are measured with Bio-rad Microplate Reader (Bio-Rad Laboratories, Hercules, CA, USA) OD with the wavelength of 540 nm. Testing on 0, 24, 48 and 72 hours.

RESULTS

Protein kinase-B (Akt) and NF-κB expression: On ELISA reader test result obtained protein kinase-B (Akt) and NF-κB expression with the concentration of 5, 10, 25, 75 and 125 showed increased protein expression obstacle based on the concentration of flavonoids fraction of ethyl acetate given (Figure 1 and 2). This is seen in the expression band on protein kinase-B (Akt) and transcription of NF-κB show the same pattern of increased protein expression obstacle based on the concentration of flavonoid fraction of ethyl acetate concentrations ranging from 15-500. The test concentration with "-tubulin as control shows the pattern of bands that do not change in protein expression obstacles.

![Protein Kinase B (Akt) graph](image)  

Figure 1. Akt Protein Expression

Sources: (Primary data, 2019)
Apoptosis induction Caspace -3, Caspace-9: Apoptosis Induction of cell apoptosis by the treatment of Doxetaxel hydrate has been investigated in vitro on oral Burkitt lymphoma cells through the analysis of AO-EB double staining. Doxetaxel hydrate significantly increases apoptotic cells compared with negative controls. Cell apoptosis also occurs in Raji cells induced by IC50 carboplatin which functions as a positive control. Living cells appear bright green, while apoptotic cells appear yellow-orange. Cell apoptotic activity increases dramatically after the treatment of Doxetaxel hydrate. The highest dosage of docsetaxel has the highest apoptotic activity. It was also found that the positive control had higher apoptotic activity than the highest dose of Doxetaxel (5.0 x 10^{-2} M). Cell apoptotic activity starts from a dose of 1.25 x 10^{-2} to 5.0 x 10^{-2} M. Apoptotic cells Raji by administering various concentrations of Doxetaxel hydrate; A. negative control; B. Concentration of 1.25 x 10^{-2} M; C. Concentration of 2.5 x 10^{-2} M; D. Concentration of 5.0 x 10^{-2} M ; E. Positive control of carboplatin IC50 (3.1 x 10^{-6} M). (Green: vivid cells and yellow-orange: apoptotic cells). The relative number of therapeutic cells treated with various concentrations of Doxetaxel hydrate [** p <0.01; compared to control (-), one-way ANOVA, concentration unit: M] (Figure 3,4,5).
Figure 3. Apoptosis Raji cells were treated with various concentrations of: A. negative control; B. Concentration 1.25 x 10^{-2} M.; C. Concentration 2.5 x 10^{-2} M.; D. Concentration 5.0 x 10^{-2} M.; E. Positive control carboplatin IC50 (3.1 x 10^{-6} M). (Green color: viable cell and yellow-orange color: apoptotic cells)

Sources: (Primary data, 2019)

Figure 4. Caspase-3 Expression

Sources: (Primary data, 2019)
DISCUSSION

Nature of cellular transmission is a system that is highly regulated in cancer cells, as well as recognizing that diseased processes are also a process that causes abnormal or abnormal growth. The growth signal circuit does not only regulate growth but also regulates various other cellular processes including differentiation, angiogenesis, migration, apoptosis. Various signaling molecules including the form of "kinase" including its case which holds an important role in cellulite processes.11

Transactions of signals to control growth often depend on the ability of certain proteins to modify other proteins, often referred to as communication between proteins. Communication between lines can always occur through enzymatic reactions, such as the addition or reduction of phosphate phosphate, or the conversion of bound GTP to free GDP which can then be changed back to GTP. In addition, there can also be signs that are not passed on through enzymatic corrections but are always through transient protein contacts, also called protein interactions. Examples of simple protein interactions are the interaction of growth factors with their receptors.12

Figure 5. Caspace-3 Expression

Sources: (Primary data, 2019)
Understanding the protein kinase-B (Akt) is important for getting better therapy for treating cancer. Under various circumstances, Activation is shown to overcome cell cycle interventions in the G1 and G2 phases. Activation of Akt allows the proliferation process and maintains the survival of cells that have a potentially mutagenic ongoing impact, so that it can contribute to mutations in other genes.\footnote{13}

NF-kB in addition to having a role in cell cycle activation for cell proliferation and apoptosis inhibition, NFkB can also play a role in cell cycle inhibition. The core of the activation of the IKK/NFkB pathway is to "cell survival". The activity of IKK/NF-kB removes cycles as well as related to cellular defense systems so that they can survive as long as the responders in this case are DNA damage. Exposure to compounds that damage DNA can induce cells to activate NFkB to stop the cell cycle. It is a mistake to respond to cell phone to maintain the life of the child after continuing through cycles, the possibility of death.\footnote{13}

The pathway of NF-\kappa B transcription plays an important role in growth regulation and proliferation, apoptosis, inflammation, and other physiological processes. Some important molecules such as NF-\kappa B, \kappa light polypeptide gene repair inhibitors in the B-cell (I\kappa B), and I\kappa B-kinase (IKK) are involved in the NF-\kappa B encoding pathway. NF-\kappa B is a key protein in the liver, and has been described as the main target and the therapeutic target of the cell. Activation of NF-\kappa B has been investigated in PCa. Blockade activity of NF-\kappa B in the cell PCaman suppresses the process of angiogenesis and metastasis of the PCa cells. Activation of PI3K/Akt and NF-\kappa B was also seen during the process of advancement of PCs in autochthonous transgenic mouse models, so that it was concluded that Akt and NF-\kappa B were molecular potential targets to prevent or intervene in the poetic Pca.\footnote{12,13}

The most important results shown in the above study are an increase in the activity of apoptosis Raji cells treated with various concentrations of Doxetaxel hydrate. Followed by an increase in proliferation and suppression of cell migration occur along with an increase in Doxetaxel hydrate concentration. These results are thought to involve a variety of complex protein barrier mechanisms including cyclin-dependent protein kinase, cell-cycle arrest, matric-metallo protein (MMP), Akt/PKB signal transduction, NF-kB transcription factors, and proapoptosis proteins.\footnote{14,15}
Docsetaksel is reported to be able to induce apoptosis of KB cells through the mitochondrial intrinsic pathway and increased antitumor activity. Recent studies report that Doxetaxel hydrate causes inhibition of the cell cycle in the G2-M phase and induces apoptosis of kidney cancer cells by inhibiting the mitogen-activated protein kinase (MAPK) pathway. Inhibition of the cell cycle in the G2-M phase is also accompanied by a complex obstacle in the form of CDK-1 and cyclin B. Decreased cell cycle protein expression is known to activate cyclin-dependent kinase inhibitor protein p27Kip1 as a negative regulator of the cell cycle that can increase cell growth resistance and apoptosis induction in oral cancer of squamous carcinoma and salivary gland cancer.14,16

CONCLUSION

Flavonoid fraction of ethyl acetate from ant nest (Myrmecodia pendans) resistivity of protein kinase-B (Akt), NF-κB transduction obstacles, and apoptosis induction (Caspace -3, -9) as anti-proliferation and anti-cancer of Burkitt's lymphoma.

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