http://www.pjbs.org

PJBS

ISSN 1028-8880

Pakistan Journal of Biological Sciences

ANSInet
Asian Network for Scientific Information
308 Lasani Town, Sargodha Road, Faisalabad - Pakistan

OPEN ACCESS Pakistan Journal of Biological Sciences

ISSN 1028-8880
DOI: 10.3923/pjbs.2016.323.330

Research Article

Akt Signal Transduction Pathways and Nuclear Factor-kappa B (NF-κB) Transcription as a Molecular Target of Oral Tongue Squamous Cell Carcinoma (SP-C1) Using Papua's Anthill Plant (*Myrmecodia pendans*)

[1]Harun Achmad, [2]Supriatno, [1]Marhamah F. Singgih and [1]H. Hendrastuti

[1]Department of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Indonesia
[2]Department of Oncology and Oral Medicine, Faculty of Dentistry, Gadjah Mada University, Indonesia

Abstract

Background and Objective: Squamous cell carcinoma is a malignant tumor derived from epithelial tissue with cell structure group, capable to infiltrate through the bloodstream and lymphatic tissue, spreading throughout the body. This study aim to complete theoretical foundation of flavonoid compound from anthill plant (*Myrmecodia pendans*) which contribute in growing cell line oral tongue squamous cell carcinoma through proliferation inhibition, inhibition mechanism transduction Akt signal and NF-κB in tongue cancer cell Supri's-clone (SP-C1). Application benefit to explore potential fractionation anthill plant use herbal ingredients for chemo protective therapy. **Materials and Methods:** This whole study conducted with experiment laboratorium method utilized tongue cancer human cell SP-C1. This study consist 2 steps, first to determinate, extraction and fractionation anthill plant and carry out tonicity test to get flavonoid fraction from anthill plant which has anticancer potential against tongue cancer cell SP-C1. The second stage held with invasion inhibition test, proliferation and inhibition test against protein Akt expression and NF-κB in tongue cancer cell SP-C1. The barriers to proliferation through the test of MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay, barriers to invasion through Boyden chamber assay and the membrane polycarbonate, ELISA analysis and Western blotting analysis on the obstacle Akt signal transduction pathways and transcription factor of nuclear factor-kappa B (NF-κB). Data analysis conducted with 2 way ANOVA followed with LSD *post hoc* test with significance is set on 95%. Pearson correlation conduct to find strong relationship intervariable. **Results:** This study showed that the average cell growth inhibition SP-C1 based on the time and concentration using the MTT [3-(4,5- dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] assay. The resistance of cancer cell *in vitro* SP-C1. On ELISA testing and Western blotting analysis, inhibiton of protein expression of Akt signal transduction and transcription factor of nuclear factor-kappa B (NF-κB) 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. **Conclusion:** Ethyl acetate fraction flavonoid anthill has antitumor activity in multiple molecular targets transduction pathway including Akt and nuclear factor-kappa B (NF-κB) squamous cell carcinoma of the tongue.

Key words: Akt signal transduction, nuclear factor-kappa B (NF-κB), SP-C1 cells, anthill plants

Received: July 21, 2016 Accepted: August 02, 2016 Published: August 15, 2016

Citation: Harun Achmad, Supriatno, Marhamah F. Singgih and H. Hendrastuti, 2016. Akt signal transduction pathways and nuclear factor-kappa B (NF-κB) transcription as a molecular target of oral tongue squamous cell carcinoma (SP-C1) using papua's anthill plant (*Myrmecodia pendans*). Pak. J. Biol. Sci., 19: 323-330.

Corresponding Author: Harun Achmad, Department of Pediatric Dentistry, Faculty of Dentistry, Hasanuddin University, Indonesia

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

In developing countries, cancer is the leading cause of death caused by disease in children over the age of 6 months. Riskesdas 2007 Indonesia each year found about 4.100 new patienst with childhood cancer. Although still rare cancer occurs in age groups of children, about 2-6%, but cancer is a degenerative disease that causes 10% death in children[1]. The etiology of cancer in children remains unclear, the cause is suspected because of irregularities of cell growth due to genetic defects in utero. The trigger is suspected by environmental factors that are not healthy, the food was consumed not adequate, the radiation, as well as viral infections[2]. The occurence of cancer (oncogenesis) in children the same as adults in terms of aspects of biomolecular, is a fundamental difference in the course of their illness[3].

Squamous cell carcinoma of the tongue characterized by a high degree of local invasion and metastasis to the cervical lymph nodes. Tongue cancer frequently shows local recurrences after initial treatment, due microinvasion or micrometastases of cancer cells primer[4]. The ferocity of the tongue is one of the biggest challenges in oncology for the treatment of adverse effect on the functioning of the oral cavity, the final quality of life and the poor prognosis of the disease. Unique behavior of these tumors requires vigilance and aggressive management to minimize the risk of invasive spreading[5].

High cell proliferation and uncontrolloed nature due to interruption balance protooncogene factor and tumor supressor genes resulting in increased production of growth factors and the number of cell surface receptors that can stimulate signal transduction intercelluler to increase the production of transcription factors. The DNA damage causes the cessation of the cell cycle at the G1 phase and subsequent repair process will occur, if the DNA damage can not be repaired then these cells will undergo apoptosis[6].

Supri's cell-clone (SP-C1) has been studied to obtain anticancer compounds from medicinal plants (herbs) and synthetic drugs effectiveness against cancer cell growth. The SP-C1 is a tongue cancer cells isolated from patients with tongue cancer lymph nodes, derived from aquamous cell carcinoma differentiate moderate and has not experienced the invasion to the muscle tissue. The SP-C1 cells have characteristics of rapid growth, fast invasion and metastasis ability, refractory disease, recurrence is very high despite radical surgery and the short average of patient's life duration[7].

Anthill plant (*Myrmecodia pendans*) contains flavonoids, tocopherols, polyphenols and tannins. Flavonoids are very diverse class of plant secondary metabolites with approximately 9.000 structures. Flavonoids are polyphenolic compounds derived from 2-phenylchromane commonly found in many plants, vegetables and flowers. In the medical filed flavonoids act as antioxidants, anticancer, antibacterial, antiinflammatory, antiallergic and antivirus[8,9].

Potential anticarcinogenic flavonoids is interesting as a chemoprotective agent. Flavonoids have an important role in inhibiting the biological activity of another protein, such as Akt and NF-κB. The Akt plays an important role in the regulation of cell cycle defense and proliferation of cancer cells by affecting excessive phosphorylation status of Akt. Blockade of these signals causes growth inhibition with the termination of the cell cycle and apoptosis of cancer cells. The antioxidant content of flavonoids have the ability to modulate cellular transmissions of important processes such as cell growth, cell differentiation and actiovation of NF-κB. Different types of flavonoids can disrupt the flow of signal transduction that regulates cell growth, cell cycle and apoptosis[10].

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. Trans-membrane receptor located on the membrane, same receptors are outside and partially inside the cells, signaling receptors bind to parts that are outside the cells change shape and deliver the signal into the cell[11].

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[12,13]. The Akt protein has a protein domain known as plckstrin homology domain. This domain with high affinitybinding phosphoinositides. The Akt proteins bind either PIP3 (phosphatidylinositol (3,4,5)-trisphosphate) or PIP2 (phosphatidylinositol (3,4)-biphosphate). This is useful for controlling a mobile signal for dephosphorylation phosphoinositide (PIP2) only phosphorylated by the enzime 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)[11,14].

Once positioned on the basalis mebrane through PIP3 binding, Akt transduction factor then phosphorylated by the kinase that activated it, which kinase that depend on phosphoinositide i (PDK1 in the threonine 308) and

mTORC2 (in the sering 473). Once activated Akt then continue to enable or disable a myriad of the substrate (e.g., mTOR) through the kinase activity. In addition to be a downstream effector PI3-kinase, Akt also enabled in PI3-kinase-independent. Activation Akt through the PI3K can be set through the tumor supressor PTEN. The PTEN act as a phosphatase to dephosphorylate PIP 3 (3, 4, 5) back to PIP 2 (4, 5) in a state of inactive[14-16].

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). Transcription factor is located in the cell cytoplasm in the form of the bond with specific protein IkB (Inhibitory Kappa B) in the form of inactive. Transcription factor has a role in cell transformation, tumor growth, apoptosis, differentiation of immune cells. The NF-κB can be activated by several factors, which is TNF-α (Tumor necrois factor α), IL-1 (Interleukin 1), lipopolysaccharide, nitric oxide and ROS. These factor will activate the receptor-sensitive to stress, then activate signal transduction pathways NF-κB through a variety of protein kinase[17]. 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 IkBα protein. While the NF-κB (Protein rail and p50) will be to the cell nucleus for gene transcription[18].

MATERIALS AND METHODS

Cell line tongue cancer SP-C1 cell activation: Tongue cancer cell SP-C1 drawn from the tank of liquid nitrogen an then thawed in a water bath with temperature 37°C until it melted, then sprayed with 70% alcohol. Cell is inserted into centrifuge tubes containing 10 mL DMEM medium (Sigma-Aldrich, USA) plus 10% FBS, penicillin, streptomycin 3% (Penstrep: Gibco, USA) and 0.5% in the space Fungizone laminary airflow (Thermo scientific, USA) and centrifuged at a speed of 1200 rpm for 5 min. The supernatant was discarded, the precipitate is added with DMEM (Sigma-Aldrich, USA) serum. After settling 20 min cells were centrifuged at a speed of 1200 rpm for 5 min. The supernatant was discarded. The cell suspension is put into TFC (Tissue culture flask) with grower media containing 10% FBS (Penstrep: Gibco, USA) and viewed under an inverted microscope (Olympus, Japan).

Tongue cancer cell SP-C1 culturing: Tongue cancer cells stored in a flask, then centrifuged at 2000 rpm for 5 min. The supernatant was discarded and left about 1 mL^{-1} for resuspension. After a homogenous cell suspension, added DMEM medium (Sigma-Aldrich, USA) containing 10% FBS (Penstrep: Gibco, USA) and then the cancer cells are distributed into several TFC (Tissue culture flask). In laminary airflow (Thermo scientific, USA) discarded the old media and the cells attached sprayed slowly with new media. The cell suspension obtained were distributed into several flask, stored in an incubator at a temperature of 37°C and CO_2 5%.

Cytotoxicity test: Cytotoxicity test was performed by incubating the cells with the number of 2×10^4 cells for 24 h with series concentrations of flavonoid anthill plant. The analysis used is the MTT (3-(4,5 dimethyl thiazol-2-yl)-2.5-diphenyltetrazolium bromide) assays, a tetrazolium salt commonly used in the quantitative determination of living mammalian cells or proliferation by colorimetric method *in vitro*, concentrations in anthill plant flavonoid cytotoxicity assay is the interval number upper limit 1000 μg mL^{-1} and lower limit 7,812 μg mL^{-1} at successive interavals of 7812, 18625, 3125, 625, 125, 250, 500, 1000 and 0 μg mL^{-1} as a control.

Barriers cell proliferation (MTT assay): In 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 h. Then on each plate insert tongue cancer cells SP-C1 as much as 2×10^4 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 128×10^5 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 detemined using a hemocytometer. All cells are then incubated at a temperature of 37°C for 24 h. Plate 24 wells are measured with Bio-rad Microplate Reader (Bio-Rad Laboratories, Hercules, CA, USA) OD withe wavelength of 540 nm. Testing on 0, 24, 48 and 72 h.

Barriers to SP-C1 cell invasion: Cancer cell SP-C1 invasion barrier test is done by using a Boyden chamber assay (Cell Biolabs, Inc.) and a membrane polycarbonate. Layer polycarbonate membrane disc is placed on cell culture (Petri dish), treated with a solution mill-Q and incubated at a temperature of 37°C. In the lower chamber inserted media experimetn/control (ethyl acetate fraction flavonoid) with concentrations of 5, 10, 25, 75 and 125. Polycarbonate membrane mounted on the lower chamber (ethyl acetate fraction flavonoid). Replacing the upper chamber with the pressure evenly. Cancer cells inserted into the upper wells of

Boyden chamber as each 50 mL. Third Boyden chamber for 24, 48 and 72 h put back into the incubator with wet tissue to get the humidity at a temperature of 37°C. Prepare a 4 cell culture discs, each containing a solution of Mill-Q, Ethanol solution, PNS solution and a solution of hematoxylin as a staining. Cell discs are then stored at room temperature for 24 h. Furthermore, do calculations on a light microscope with a magnification 0f 40x.

Western blotting analysis: Western blotting as analytical technique used to detect specific proteins in a sample of tissue homogenate or cell culture lysate. Western blotting analysis performed by administering oligonucleotide for 48 h. The initial formation of cell lysis step using a solution of SDS (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) and acrylamide 10%. Measure the protein concentration by Bradford protein assay method. Furthermore, forming gel SDS-PAGE (sodium dodecyl sulfate-polyacrylamide gel electrophoresis) to separate proteins by size in the presence of an electric current (SDS-PAGE study principle denature polypeptides discharged after first polypeptide secondary and tertiary structures). Next, transfer gel using polyvinylidene difluoride membrane (PVDF). The PVDF membranes were placed on top of the gel and a pile of absorbent study placed on top of it. Buffer solution then will propagate upward through capillary reaction with the protein. Transfer of proteins was done by using electroblotting (electric current) to draw proteins from gel to membrane. Membrane protein samples were incubated with antibodies. After the transfer, the membrane is placed in blocking solution (5% skin milk in 1x TBST) and shaking for 1 h at room temperature. Last detection is done with antibodies which have been modified by an enzyme (reporter enzyme). Results obtained in the form to see the thickness of the band.

RESULTS

Cytotoxicity test: Cytotoxicity assay result obtained LC_{50} of each fraction consisting of a fraction of ethyl acetate, ethanol fraction, the fraction of hexane and water fractions in a row is 452.059, 938.003, 2691.535 and 12302.69 μg mL^{-1}. These results were obtained from the equation of the curve that connected from log probit levels. This study refers to the standard of Meyer *et al.*[19] stated if <1000 pg mL^{-1} for extract and ≤30 μg mL^{-1} of a compound. The toxicity level of interpretation of potential antitumor. The LC_{50} is used as a parameter to identify potential cytotoxicity anthill plant flavonoid fraction against cancer cells tongue SP-C1 (Fig. 1).

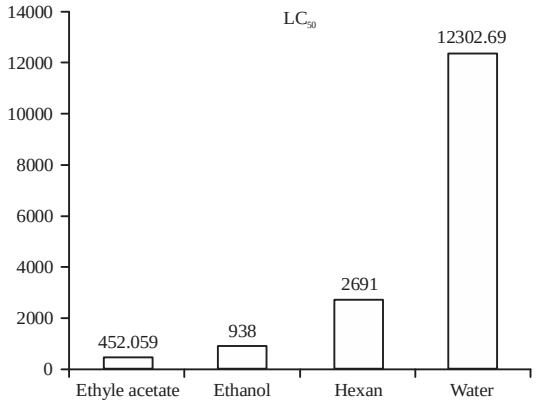

Fig. 1: Results of cytotoxicity assay LC_{50} in each flavonoid fraction[9]

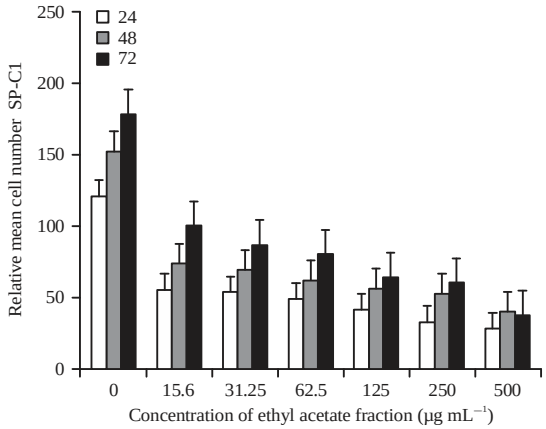

Fig. 2: Tongue cancer cell SP-C1 growth profile proliferation assay results from the ant nests ethyl acetate fraction at 24, 48 and 72 h[9]

Barriers cell proliferation (MTT assay): The test result indicate that there are barriers to the proliferation of cell growth inhibition by a given concentration ranging from the lowest concentration of 15.625 μg mL^{-1} up to the highest concentration of 500 μg mL^{-1}. Similarly, the incubation time factor 24, 48 and 72 h showed that incubation, respectively. The increase of cell growth inhibition shown in Fig. 2 indicated that the ethyl acetate fraction flavonoid having cell growth inhibitory activity of SP-C1 as shown on the measurement of SP-C1 cells using ELISA reader. Visible effect of fractions of some greater concentration of growth constraints compared with controls. The greater the concentration of the sample the smaller the number of cell SP-C1 vibrant, growth inhibition evident at a concentration of 500 μg mL^{-1}, by inhibiting the activity of SP-C1 cell proliferation.

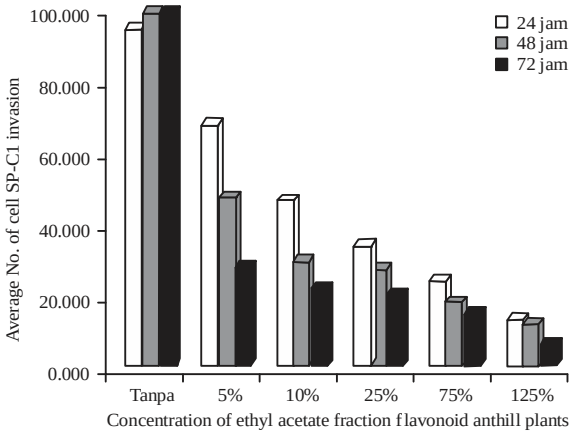

Fig. 3: Average barriers to invasion by the concentration and time

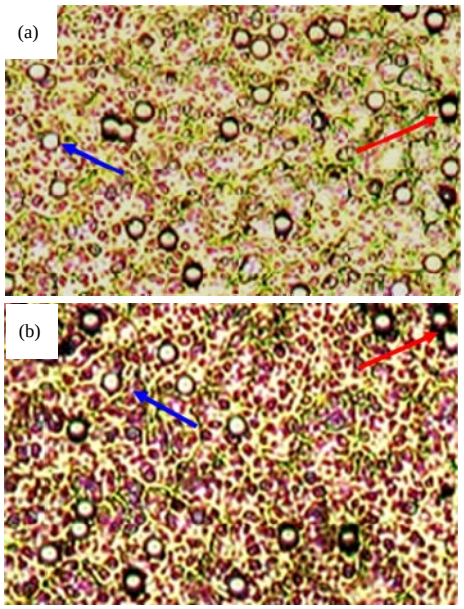

Fig. 4(a-b): Test results of cell SP-C1 invasion with the treatment of ethyl acetate fraction anthill plant. Blue arrow: Alive cancer cell tongue SP-C1 (magnification 40x). Red arrow: Mute cancer cell tonguse SP-C1 (lysis) (magnification 40x)

Barriers to SP-C1 cell invasion: The observation and calculation of the number of cancer cells tongue SP-C1 conducted in a span of 24, 48 and 72 h on the membrane polycarbonate under a light microscope (Fig. 3), appear to have changed significantly for blocking cancer cell SP-C1, ranging from concentrations of 5 to at concentrations of 25 (Fig. 4). In addition, comparing the control group 24, 48 and 72 h indicates the number of cancer cells tongue SP-C1 are still experiencing an increasing No. of invasions after the recalculation.

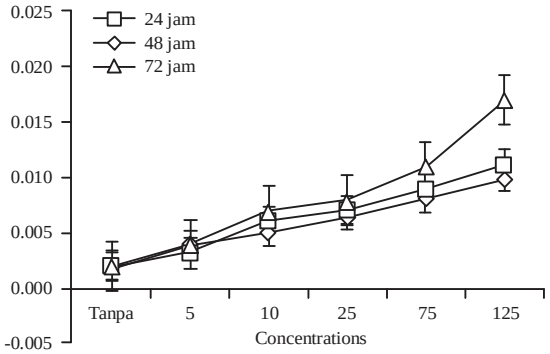

Fig. 5: Emphasis Akt protein expression based on concentrations and time

Akt protein and NF-κB expression: On ELISA reader test result obtained obstacles Akt protein 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 fracion of ethyl acetate given (Fig. 5).

In the Western blotting analysis of the test results obtained barriers Akt protein and NF-κB expression with concentration of 7, 31.25, 62.5, 125, 250 and 500 showed suppression of protein expression based on the concentration of flavonoids fraction of ethyl acetate given.

This is seen in the expression band on Akt signal transduction and transcription of NF-κB show the same pattern of increased protein expression obstacle based on the concentration of flavonoid fraction of ethyl acetate concentratiorns ranging from 15-500. The test concentration with α-tubulin as control shows the pattern of bands that do not changes in protein expression barriers.

DISCUSSION

Effects barriers proliferation of cancer cells tongue SP-C1 based on the results obtained, shows that flavonoids ethyl acetate fraction has inhibitory activity cell growth SP-C1. It is shown in the measurement of cancer cells tongue SP-C1 using ELISA reader. Flavonoid fraction anthill plant inhibit the process of cancer cell proliferation human tongue (SP-C1) with the biggest obstacle cell growth tongue cancer SP-C1 for 57.90%. Statistical analysis of research results flavonoid fraction of ethyl acetate anthill on the difference incubation time seen affect the percentage inhibition of cancer cells tongue SP-C1. The longer incubation time the increase the percentage inhibitione of the cell. The results were statistically significant ($p = 0.00$)[9]. The results of the above line with the research has been done before the extract the anthill plants that contain flavonoids and tannins by showing the inhibition

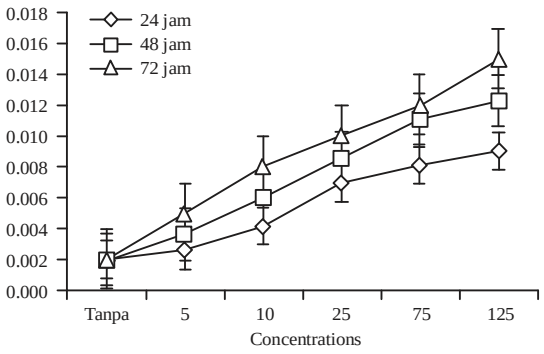

Fig. 6: Emphasis protein NF-κB expression based on concentration and time

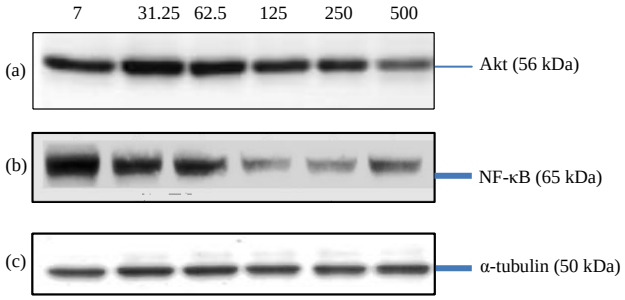

Fig. 7(a-c): Western blotting analysis using anthill plants ethyl acetate fraction on tongue cancer cell SP-C1, (a) Akt protein expression (56 kDa), (b) NF-κB protein expression (65 kDa), (c) α-tubulin protein expression (50 kDa)

of cell growth HeLa cells and MCM-B2[20,21]. In the testing barriers invasion, obtained barriers cancer cell invasion by the concentration given between 5-125 μM experienced significant changes, shown in the decline in the average number of cancer cells on the calculation of boyden chamber assay barriers cancer cell invasion, both on the range 24, 48 and 72 h. The greater concentration of ethyl acetate fraction of flavonoids anthill plants given will give effect more effective also to the barriers cell invasion tongue cancer SP-C1. The results are confirmed that the flavonoid fraction of the anthill plants has barrier firmly on the cell proliferatiorn and barriers cell invasion tongue cancer SP-C1. Flavonoids can inhibit the performance of all CDK which is the regulator cell cycle. The mechanism of proliferation and barriers cell invasion occurs through the cell cycle regulation[22,23].

Western blotting analysis in this study showed that provision of flavonoids ethyl acetate fraction of flavonoids in cancer cells tongue SP-C1 shows the emphasis expression of protein Akt transduction signal and NF-κB transcription (Fig. 6, 7). Western blotting analysis showed that flavonoid ethyl acetate fraction pressing the level of Akt protein phosphorylation in cancer cells SP-C1. The results in line with the Gibbs[24] on study by using ethanol extract stem bark B gymnorhiza, which states that point catch molecular natural compounds, such as flavonoids so that effect as anti-cancer caused the possibility of the 1st, natural compounds are able to inhibit signal transduction on the cell membrane. Signal transduction started in the presence of stimuli from outside the cells form of growth factor which was arrested by the receptors. Receptors will convey signals proliferative to protein in the cytoplasm. Activation signal transduction with the process of phosphorylation by involving ATP and protein involved are generally is a type of protein kinase. The process signal transduction cascades can be inhibited by some natural compounds containing substances kinase inhibitors so it can inhibit signal transduction cascades including flavonoids. Second possibility, affect the program cell cycle, which inhibit cell cycle progression and induce cell cycle arrest. Test compound can inhibit phosphorylation pRb or induce INK4 family proteins which is a protein inhibitor of CDK. Barriers cell cycle progression is also can occur in S phase, G2/M transition which is usually form of cell cycle arrest[25,26].

The study also confirmed that ethyl acetate fraction of flavonoids inhibit the translocation and activation NF-κB and growth factor which induces phosphorylation of Akt. Stimulation pro-inflammation encourage the phosphorylation of a fast and decreased protein inhibition I κB, free NF-κB to do the translocation to the nucleus in roder to activate transcription of gene targets. Effect antiangiogenic of ethyl acetate fraction of flavonoids in touch with effect of barriers activation of NF-κB and I κB phosphorilation, leading to translocation of NF-κB to the nucleus[27,28].

Circuit signal growth is not only set the cell growth but also set the various cellular processes including differentiation, angiogenesis, migration and apoptosis. A variety of molecular signals, such as the form of kinase including its cascade that holds an important role in the cellular processes such as the path Race/MAPK, pTEN/PI3K/Akt and others[29,30].

Signal transduction to control the growth depends on the ability of ceratin proteins to modify the another protein, often referred to as communication between proteins. Communication between cells can occur through the reaction-enzymatic reactions, such as the addition or reduction of phosphate residues, or conversion GTP bound to be GDP free which can then be changed back to be GTP. In addition can also occur signal is not be passed through enzymatic reaction but through contact a protein is temporary (transient) also called the interaction between protein. These are also indicated from some studies related to the interaction of growth factor with its receptor[26,29,30].

Understanding on the Akt transduction pathways is important to get better therapies to treat cancer[31,32]. In various circumstances, activation of Akt shown to overcome the intervention cell cycle in G1 and G2 pahase. Activation of Akt allows the occurence of the process of proliferation and keep the survival of cells that have an impact sustainable potentially mutagenic, so, it may contribute to the occurence of mutations in the other genes[16,32,33].

The NF-κB have a role in the activation of the cell cycle for induce cell proliferation and inhibition of apoptosis. The NF-κB can also instrumental in the inhibition of the cell cycle. The point of the IKK/NF-κB pathway activation is to cell survival. The IKK/NF-κB activities inhibit cell cycle also associated with the defense system cells in order to stay can survive in response to stress in this case is DNA damage. Cells with the activity proliferation of a high, such as cancer cells, will enter the cell cycle. Exposure to a compound that damage DNA can induce cell to activate NF-κB for termination of the cell cycle. One of the response emergency of cells to maintain its life because if the cell is still continue to the cell cycle, the possibility of cells will die[27,28].

CONCLUSION

In conclusion that the ethyl acetate fraction of flavonoids anthill plants (*Myrmecodia pendans*) have antitumor activity on several molecular target of them Akt and nuclear factor-kappa B (NF-κB) pathway transduction squamous cell carcinoma tonguem including barriers invasion.

SIGNIFICANT STATEMENTS

- Exploring medical herbs as demand of future chemopreventive agent that potentially application on cancer therapies. Myrmecodiapendans is available in large quantity, cheap, easily obtained and nontoxic. Contrary, this epiphytes plant using limited as traditional techniques
- A developmental study of biology molecular about carcinogenesis inhibition using Akt signal transduction pathways and transcription factor of nuclear factor-kappa B (NF-κB) as molecular targets on oral tounge squamous cell carcinoma, a malignant tumor that capable to infiltrate through the bloddstream and lymphatic, spreading throughout the body
- Not enough data is available to scientists so it complete theoritical foundation of flavonoids compound from anthill plant which help other scientists, public health workers, practitioners and even people in general hence boarden and improving its function and usefullness widely

ACKNOWLEDGMENTS

The author heartfully thanks and appreciated to Mr. Supriatno, Department of Oral Medicine, Faculty of Dentistry Gadjah Mada University, Yogyakarta, Indonesia. Thank to all the staff members LPPT-Gadjah Mada University, Yogyakarta, Indonesia.

REFERENCES

1. Soendoro, T., 2008. Laporan Riskesdas 2007. Health Ministry of Republic of Indonesian.
2. Cho, S.Y., A.C. Cheng and M.C. Cheng, 2000. Oral care for children with leukaemia. Hong Kong Med. J., 6: 203-208.
3. Rubnitz, J.E. and W.M. Crist, 1997. Molecular genetics of childhood cancer: Implications for pathogenesis, diagnosis and treatment. Pediatrics, 100: 101-108.
4. Hasibuan, S., 2004. Early detection procedures and oral cavity cancer diagnosis. Digitized by USU Digital Library, pp: 1-7.
5. Supriatno, 2008. [Decetaxel hidrat inhibits proliferation and metastatic cancer cells oral SP-C1 melalui induksi protein maspin]. Indonesian J. Dentist., 15: 2-7.
6. Williams, H.K., 2000. Molecular pathogenesis of oral squamous carcinoma. Mol. Pathol., 53: 165-172.
7. Supriatno and S. Yuletnawati, 2006. [Cepharanthine anticancer activity *in vitro* human tongue cancer (Review proliferation, invasion and metastasis of cells). Magazine Dentistry, UGM., Jogyakarta, pp: 141-145.
8. Achmad, H., M.H. Chandha, S. Ramadhany, H. Handayani and R. Samad, 2014. The role of sarang semut (*Myrmecodia pendans*) flavonoid's fraction in proliferation and angiogenesis inhibition of human tongue squamous cell carcinoma. J. Biol. Agric. Healthcare, 4: 65-69.
9. Achmad, H., S.A. Armyn, Supriatno and M.F. Singgih, 2014. Anti-cancer activity and anti-proliferation ant nests flavonoid fraction test (Myrmecodya Pendans) human tongue cancer cells in SP-C1. IOSR J. Dental Med. Sci., 13: 1-5.
10. Ding, Y., H. Yao, Y. Yao, L.Y. Fai and Z. Zhang, 2013. Protection of dietary polyphenols against oral cancer. Nutrients, 5: 2173-2191.
11. Christoffersen, T., T.K. Guren, K.L.G. Spindler, O. Dahl, P.E. Lonning and B.T. Gjertsen, 2009. Cancer therapy targeted at cellular signal transduction mechanisms: strategies, clinical results and unresolved issues. Eur. J. Pharmacol., 625: 6-22.
12. Zhou, B.P., M.C.T. Hu, S.A. Miller, Z. Yu, W. Xia, S.Y. Lin and M.C. Hung, 2000. HER-2/neu blocks tumor necrosis factor-induced apoptosis via the Akt/NF-κB pathway. J. Biol. Chem., 275: 8027-8031.

13. Zhang, S. and D. Yu, 2010. PI(3) king apart PTEN's role in cancer. Clin. Cancer Res., 16: 4325-4330.
14. Vimalanathan, A.B., A.K. Raja and M.G. Tyagi, 2009. The role of PI-3 kinase in cancer biology and approaches to the therapeutics of cancer. Biol. Med., Vol. 1.
15. She, Q.B., D. Solit, A. Basso and M.M. Moasser, 2003. Resistance to gefitinib in PTEN-null HER-overexpressing tumor cells can be overcome through restoration of PTEN function or pharmacologic modulation of constitutive phosphatidylinositol 3'-kinase/Akt pathway signaling. Clin. Cancer Res., 9: 4340-4346.
16. Chun, K.S. and Y.J. Surh, 2004. Signal transduction pathways regulating cyclooxygenase-2 expression: Potential molecular targets for chemoprevention. Biochem. Pharmacol., 68: 1089-1100.
17. Pande, V. and M.J. Ramos, 2005. NF-κB in human disease: Current inhibitors and prospects for de novo structure based design of inhibitors. Curr. Med. Chem., 12: 357-374.
18. Gilmore, T.D., 2006. Introduction to NF-jB: Players, pathways, perspectives. Oncogene, 25: 6680-6684.
19. Meyer, B.N., N.R. Ferrigni, J.E. Putnam, L.B. Jacobsen, D.E. Nichols and J.L. McLaughlin, 1982. Brine shrimp: A convenient general bioassay for active plant constituents. Planta Medica, 45: 31-34.
20. Soeksmanto, A., M.A. Subroto, H. Wijaya and P. Simanjuntak, 2010. Anticancer activity test for extracts of sarang semut plant (*Myrmecodya pendens*) to HeLa and MCM-B2 cells. Pak. J. Biol. Sci., 13: 148-151.
21. Simanjuntak, P., Fanny and M.A. Subroto, 2010. [Isolation of active compounds from the extracts of hypocotyl anthill (*Myrmecodia pendans*) as inhibitors xantinoksidase]. J. Pharmaceut. Sci. Indonesia, 8: 49-54.
22. Kandaswami, C., L.T. Lee, P.P. Lee, J.J. Hwang, F.C. Ke, Y.T. Huang and M.T. Lee, 2005. The antitumor activities of flavonoids. *In vivo*, 19: 895-909.
23. Wang, H.K., 2000. The therapeutic potential of flavonoids. Expert Opin. Invest. Drugs, 9: 2103-2119.
24. Gibbs, J.B., 2000. Anticancer drug targets: Growth factors and growth factor signaling. J. Clin. Invest., 105: 9-13.
25. Shapiro, G.I. and J.W. Harper, 1999. Anticancer drug targets: Cell cycle and checkpoint control. J. Clin. Invest., 104: 1645-1653.
26. Kulp, S.K., Y.T. Yang, C.C. Hung, K.F. Chen and J.P. Lai *et al*., 2004. 3-phosphoinositide-dependent protein kinase-1/Akt signaling represents a major cyclooxygenase-2-independent target for celecoxib in prostate cancer cells. Cancer Res., 64: 1444-1451.
27. Araki, K., K. Kawauchi and N. Tanaka, 2008. IKK/NF-κB signaling pathway inhibits cell-cycle progression by a novel Rb-independent suppression system for E2F transcription factors. Oncogene, 27: 5696-5705.
28. Knowles, M.A., F.M. Platt, R.L. Ross and C.D. Hurst, 2009. Phosphatidylinositol 3-kinase (PI3K) pathway activation in bladder cancer. Cancer Metastasis Rev., 28: 305-316.
29. Kumar, V., R.S. Cotran and S.L. Robbins, 2007. Robbins Basic Pathologic. 7th Edn., Book Medical Publishers EGC., Jakarta, pp: 185-224.
30. Kumar, V., A.K. Abbas and N. Fausto, 2005. Robbins and Cotran Pathologic Basis of Disease. 7th Edn., WB Saunders Co., New York, ISBN-10: 0721601871. Pages: 1552.
31. Brachmann, S., C. Fritsch, S.M. Maira and C. Garcia-Echeverria, 2009. PI3K and mTOR inhibitors-a new generation of targeted anticancer agents. Curr. Opin. Cell Biol., 21: 194-198.
32. Assinder, S.J., Q. Dong, Z. Kovacevic and D.R. Richardson, 2009. The TGF-β, PI3K/Akt and PTEN pathways: Established and proposed biochemical integration in prostate cancer. Biochem. J., 417: 411-421.
33. Supriatno, 2008. [Cis-Platinum increase apoptosis and barriers human tongue cancer cell in vasion *in vitro*]. Majalah Ilmu Kedokteran Gigi, 10: 73-78.