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The effect of ethanol extract of mangostene (*Garcinia Mangostana* Linn.) peel on tongue cancer cells Supri´s clone-1 apoptosis, in vitro

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ABSTRACT

Apoptosis or programmed cell death serves to regulate physiological and pathological conditions. *Garcinia mangostana* Linn. is one of the medical herbs that is widely used to supress human tongue cancer growth by inducing apoptosis. The research aimed to analyze the effect of ethanol extract of *Garcinia mangostana* Linn. on tongue cancer cells apoptosis *Supri's Clone-1* (SP-C1). The type of the research was experimental laboratory. Tongue cancer cells SP-C1 were treated by various ethanol extract concentrations (0, 300, 400, 500, 600, 700 µg/ml) of *Garcinia mangostana* Linn. to detect the apoptosis, which was done by acridine orange and ethidium bromide coloring tests. The number of tongue cancer cells SP-C1 $2x10^4$ cells/dish. Observation on cells apoptosis was observed by fluorescent microscope with 40 x magnification. The data was analyzed using one-way Anova and was followed by Post Hoc test (Tukey-test) with 95% significancy level. The result showed that tongue cancer cells SP-C1 apoptosis treated by various ethanol extracts of *Garcinia mangostana* Linn. increase significantly. The highest effect of apoptosis was detected at the 700 µg/ml concentration that has an effect on the percentage of tongue cancer cells apoptosis by 65%.

Key words: Ethanol extract of *Garcinia mangostana* Linn, apoptosis, tongue cancer cells *Supri Clone-1* (SP-C1)

ABSTRAK

Apoptosis atau program kematian sel berfungsi untuk mengatur kondisi fisiologis maupun patologis. Garcinia mangostana Linn. adalah salah satu herbal medik yang banyak digunakan untuk menekan pertumbuhan kanker lidah manusia dengan menginduksi apoptosis. Tujuan penelitian ini adalah untuk menganalisis efek ekstrak etanol Garcinia mangostana Linn terhadap apoptosis sel kanker lidah Supri's Clone-1 (SP-C1). Jenis penelitian adalah eksperimen laboratoris. Sel kanker lidah SP-C1 diberi perlakuan dengan berbagai konsentrasi (0, 300, 400, 500, 600, 700 µg/ml) ekstrak etanol Garcinia mangostana Linn untuk mendeteksi apoptosis, yang dilakukan dengan uji pewarnaan akridin oranye dan etidium bromida. Jumlah sel kanker lidah SP-C1 2x104 sel/cawan petri. Pengamatan apoptosis sel diamati dengan mikroskop fluoresen dengan perbesaran 40x. Data dianalisis dengan one-way *Anava dilanjutkan dengan uji Post Hoc (Tukey-test) dengan taraf signifikan 95%. Hasil penelitian menunjukkan bahwa apoptosis sel kanker lidah SP-C1 diberi perlakuan dengan berbagai konsentrasi ekstrak etanol Garcinia mangostana*

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Linn. meningkat secara signifikan. Efek yang paling tinggi terjadi apoptosis terdeteksi pada konsentrasi 700 µg/ml (65%). Simpulan penelitian ini adalah ekstrak etanol kulit manggis pada konsentrasi 700 µg/ml mempunyai efek terhadap persentase apoptosis sel kanker lidah SP-C1sebesar 65%.

Kata kunci: *Ekstrak etanol Garcinia mangostana Linn, apoptosis, sel kanker lidah Supri Clone-1 (SP-C1)*

INTRODUCTION

The World Health Organization (WHO) data estimates that the number of cancer patients in the world increase to 6.25 million people each year. In the next ten years to come, it is estimated that 9 million people worldwide will die from cancer every year. Two-thirds of cancer patients in the world are in developing countries. In Indonesia, it is estimated there will be 100 new cancer patients each year out of 100.000 inhabitants. Deaths due to cancer increase each year with the incidence of head and neck cancer for about 3% from all cancers in human body. Cancer of oral cavity is approximately 30% from all head and neck cancers. More than 90% oral cavity and pharyngeal cancers are from squamose cells. One of the cancers in royal cavity is tongue cancer with 1% incidence from all cancers in human body, while tongue cancer incidence in children is about 1-6%.[1-4]

Cancer is a disease characterized by the uncontrolled growth of tissue cells, the damage in the surrounding tissue, and then spread to other parts through blood vessels or lymph vessels.[5] Even though the incidence of tongue cancer in children is small, still it is a deadly disease. An intensive treatment is needed to treat tongue cancer in children and may increase curing possibility.[3]

Tongue cancer therapy consists of surgery, radiotherapy and chemotherapy. Radiotherapy is the most painful treatment for children, yet it is the most effective therapy, and it takes a long period of time until the entire series of radiation resolved. Combination with chemotherapy can often reduce this complaint.[4] Generally, the therapy will reduce the immunity of patients and it has many side effects. In order to reduce those side effects and also enhancing its effectiveness, adjuvant is given to the cancer therapy. Cancer therapy is combined with adjuvant therapy in order to strengthen the effect of anti-cancer drugs.[4,6]

Chemo preventive using conventional drugs is now switching to the use of natural materials (herbal). One of the natural materials used is mangosteen due to its ability to induce apoptosis. Mangosteen (*Garcinia mangostana* Linn.) contains active compounds such as α-mangostin, β-mangostin, γ-mangostin, mangostinone, garcinone E dan 2-Isoprenyl-1, 7-dihydroxy-3-methoxy-xanthon.[7,8]

Apoptosis is caused by the presence of a signal to begin a series of program (cascade) which is induced by a molecular level that involves the activation of cystein aspartate protease (caspase). There are two main pathways in apoptosis, receptor pathway (extrinsic) and mithocondrial pathway (intrinsic).[9-11]

The research result of Akao et al.[12] showed that active compounds in mangosteen peel can induce apoptosis through intrinsic pathway. The mechanism of those active compounds will inhibit Akt which contributes cells proliferation inhibition and inducing apoptosis as well as being a protein phosphorilated by PI-3 kinase. Alfa-mangosteen modulates the signal from Akt that causes inhibition of cells proliferation and apoptosis induction. Akt inhibition will repair the regulation mechanism of apoptosis so that it can take place by activating caspase-9.[12] The benefits of mangosteen peel have been shown in Suanto's[13] research result which states that ethanol extract posseses inhibitory power on tongue cancer cells *Supri's* Clone-1 (SP-C1) at mangosteen peel ethanol extract concentration of 500 µg/ml after incubation and 24 hour observation reaches IC_{50}. The balance between apoptosis and cells proliferation is an important factor in homeostasis tissue. Apoptosis is also known as programmed cell death. It is an important process in regulating normal homeostasis cells development. The process produces a balance in the number of certain cells tissue through the elimination of damaged cells and physiological proliferation. The growth of cell groups, including cancer cells is determined by the balance of proliferation and apoptosis. Apoptosis serves to maintain tissue's function becomes normal and the existence of apoptosis deregulation resulting

pathological condition.[9,10,14,15]

Based on this, the research was carried out to find out the existence of the effect of ethanol extract of mangosteen peel on tongue cancer cells *Supri's* Clone-1 (SP-C1) apoptosis *in vitro*.

METHODS

The type of the research was experimental laboratory by examining the effects of ethanol extract of mangosteen peel (*Garcinia Mangostana* Linn.) on tongue cancer cells *Supri's* Clone 1 (SP-C1) apoptosis using acridine orange and ethidium bromide coloring tests comprise 15 mg acridine orange and 50 mg ethidium bromide which are dissolved in 1 ml 95% ethanol, then some aquades was added. The subject of the research was ethanol extract of mangosteen peel (*Garcinia Mangostana* Linn.) with various concentrations; 0 (without ethanol extract of mangosteen peel as a control), 300, 400, 500, 600, and 700 µg/ml.

The research procedure was: the confluent (full) tongue cancer cells SP-C1 were harvested using EDTA 0.25%. As many as $2x10^4$ cells were bred in 6 dishes with diameter 60 mm, then each was given a coverslip. Cells breeding was carried out according to the number of extract concentration used, the cells were then incubated for 24 hours. After 24 hours, the fluid in the dish was aspirated by a pipette, and was washed using PBS, and then reaspirated. It was then fixed with 70% alcohol for 10 minutes, then the coverslips were taken and are put on object glasses. The cells in the coverslips were then dyed with acridine orange and ethidium bromide solutions. Preparations are observed by a fluorescent microscope with 40x magnification. An intact bright green nucleus cell was a living cell, and yellow to orange nucleus was the cell undergoing apoptosis. Then counted the cells undergoing apoptosis in two visual fields and the number of cells in each field was of the average 100 cells.

RESULTS

The research was conducted by counting the number of tongue cancer cells *Supri's* Clone (SP-C1) which had been given the treatment of ethanol extract of mangosteen peel in several concentrations comprise control, 300, 400, 500, 600, 700 µg/ml in 24 hour span time. The cells that had been incubated in object glasses were then dropped by acridine orange and ethidium bromide. By using those kinds of coloring, the number of cells undergoing apoptosis was observed using a fluorescent microscope.

The average of cells apoptosis with 24 hour incubation time could be seen in Table 1. The calculation was carried out for counting the number of cells undergoing apoptosis. The calculation result based on 6 different groups of ethanol extract concentration, and then the percentage of cells apoptosis was calculated. The first group was not given the ethanol extract of mangosteen peel. It was the control group and aimed to see the number of cells undergoing apoptosis with 24 hour incubation time without given any ethanol extract of mangosteen peel.

The second group, there was cells apoptosis in 300 µg/ml concentration with apoptosis average percentage by 8%. The third group, there was an increase in the number of cells apoptosis in 400 µg/ml concentration with apoptosis average percentage by 30%. The fourth group, there was less increase in the number of cells apoptosis in 500 µg/ml concentration with apoptosis average

Table 1. SP-C1 cells apoptosis average percentage

Concentration (µg/ml)	Numbers of apoptosis cells	Apoptosis average percentage
Control (0)	0	0%
300	8	85%
400	30	30%
500	45	45%
600	61	61%
700	65	65%

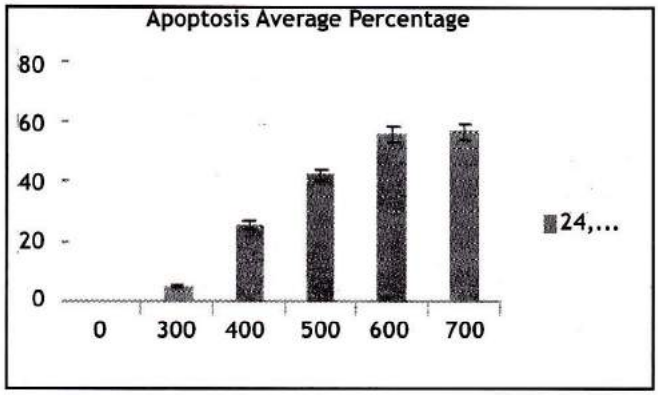

Figure 1. Apoptosis average percentage of tongue cancer cells SP-C1.

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